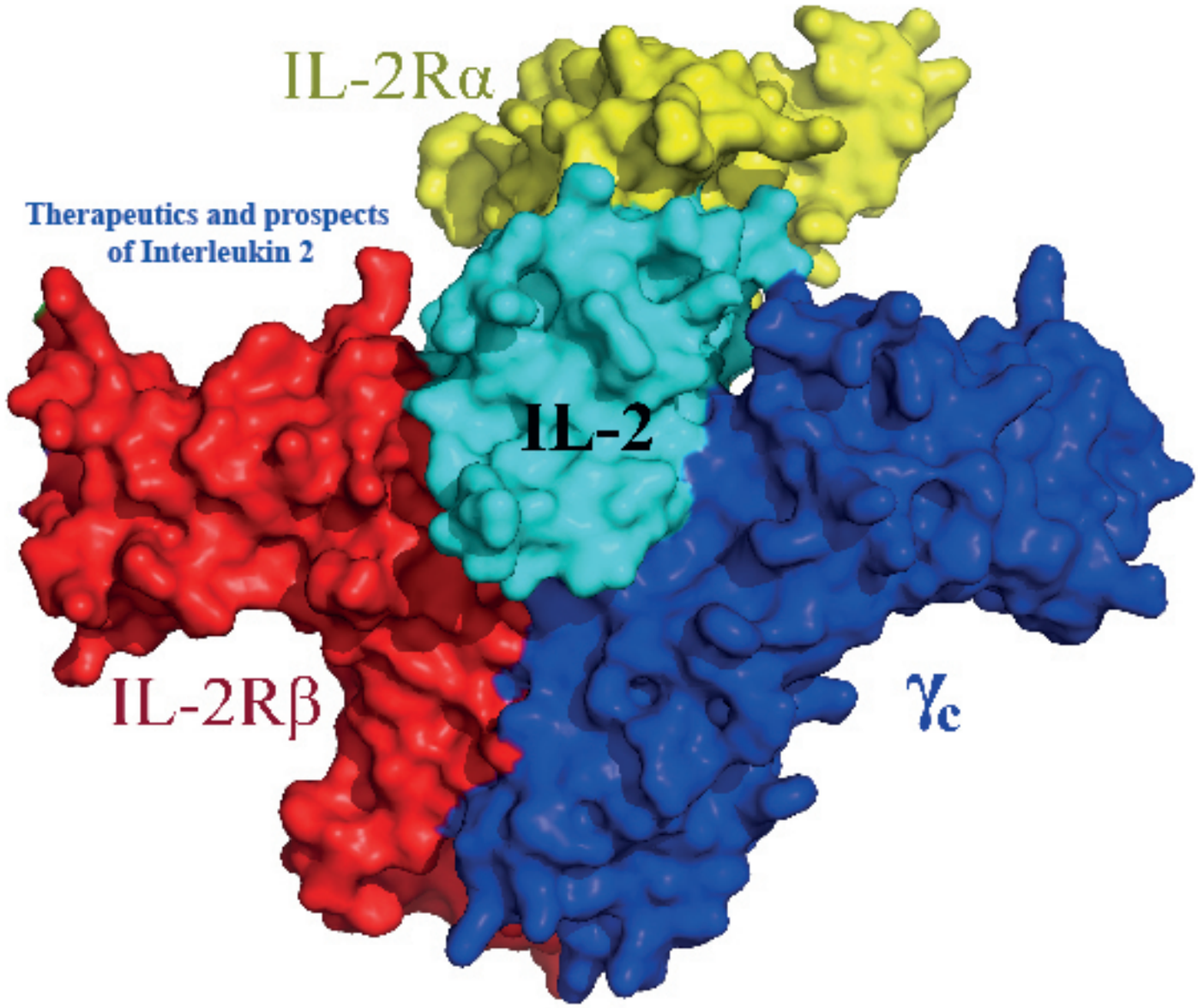
Vol. 4 No. 4 2019
ISSN:1390-9347

Bionatura

Latin American journal of Biotechnology and Life Sciences

Cancer and Cytotoxic T- cells



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Vol. 4 No. 4 2019

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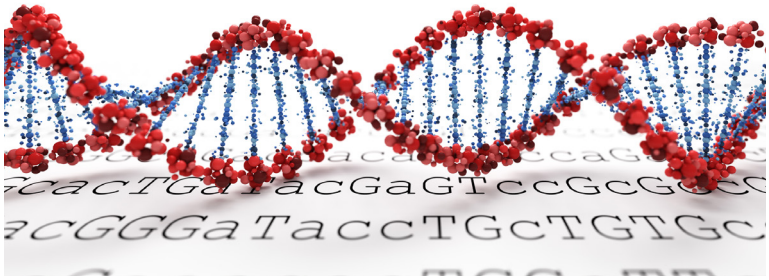

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ISSN: 1390-9347 (Versión impresa)
Formato: 21 x 29,7 cm
ISSN: 1390-9355 (Versión electrónica)
Sitio web: **http://www.revistabionatura.com**

Publicación periódica trimestral
Esta revista utiliza el sistema peer review para la evaluación de los manuscritos enviados.

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EDITORIAL

Bionatura journal enters scopus database.

Frank Alexis.

DOI. 10.21931/RB/2019.04.04.1

Bionatura, a new Ecuadorean journal focused on Life Sciences, was accepted for inclusion in Scopus, the most an excellent bibliographical database of scientific papers and journals worldwide.

Bionatura was born in 2016, under the guidance of Dr. Nelson Santiago Vispo, professor/researcher of the School of Biological Sciences and Engineering of Yachay Tech. Santiago Vispo, with over 20 years of experience in scientific publishing, brought together other universities and institutions, national and foreign, to participate in this project.

The Journal covers topics that include: Biotechnology, Immunology, Biochemistry, Biomedical, Clinical Trials, among others. At the moment, Bionatura counts , ith the support of the following institutions: Universidad Católica de Oriente (Colombia), Universidad Yachay Tech and Clinicalbiotec (Ecuador), and Centro de Biotecnología y Biomedicina (Chile).

In addition, Bionatura has a very well-known editorial board that includes researchers from scientific research institutions such as the University of KwaZulu-Natal (South Africa), University of Montreal (Canada), Universidad de Concepción (Chile), Frauenhofer Institut (Germany), Instituto de investigación biomédica La Paz (Spain), Clemson University (USA), Universidad Central (Ecuador), Universidad San Francisco de Quito (Ecuador), among others. The diverse and international editorial team of Bionatura receives manuscripts from researchers around the world.

To eenters the Scopus databases, Bionatura had to meet rigorous parameters that were thoroughly reviewed by an Evaluation Board. Some of these requirements included: publicationrecord of at least two years; publication of academic research articles; peer review process with easy access; relevant content focused on an international audience, including abstracts and titles in the English language. The journal is evaluated every year to ensure rigorousness and excellence. The Scopus Evaluation Board highlighted the following qualities of the journal: a strong editorial team, the rigorousness of the review process and an attractive website (www.revistabionatura.com). Bionatura publications will uploaded to Scopus (www.scopus.com) within the next three months.

For Yachay Tech, the importance of Bionatura being indexed in Scopus lies in the fact that by being part of the most important database worldwide, its impact factor, or H index, can increase considerably, allowing it to gain more visibility, higher reputation and access to a global audience of researchers and experts.

The acceptance of Bionatura into Scopus, aside from strenghtening the scientific community of Ecuador, embodies a great achievement for Yachay Tech: it is the first indexed journal of the University, something considered by the accreditation process of the University before the Higher Education Quality Assurance Council (CACES, in Spanish). It is also proof of the constant efforts by the University to achieve excellence in research, teaching and community engagement.

Bionatura produces four publications per year, both in printed and digital media. Since its creation in 2016 to this date, it has added up a total of 15 editions. (LMC)

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LETTER TO EDITOR / CARTA AL EDITOR

Immunogenic Cell Death: An opportunity for Clinical Oncology?

Daylen Aguilar-Noriega and Silvio E. Perea.

DOI. 10.21931/RB/2019.04.04.2

Apoptosis was initially seen as a kind of silent cell death with non-induction of the immune response[1]. However, in the recent past, it has been seen that death induced by either infections or action of certain agents can elicit a specific immune response, namely immunogenic cell death (ICD)[2]. This ICD activates the immune system against antigens associated with deceased cells with the concomitant exposure and releasing of the so-called damage-associated molecular patterns (DAMPs) by dying cells[3]. Four principal DAMPs related to ICD have been identified (but not limited to): the endoplasmic reticulum (ER) chaperone calreticulin (CRT), heat shock proteins (HSPs), adenosine triphosphate (ATP) and high mobility group box-1 (HMGB-1)[4].

Today, DAMPS has been shown to play a spatiotemporal role among the ICD process. For instance, CRT is a 46 kDa Ca^{2+}-binding chaperone that is normally found in the lumen of ER, where it acts in Ca^{2+} homeostasis/signaling regulation and proper folding of proteins[5]. During induction of ICD, CRT is exposed on the outer surface of the plasmatic membrane early, in a pre-apoptotic stage, and serves as an "eat me" signal, stimulating phagocytes to engulf portions of dead cells. CRT translocation occurs together with ERp57 but not of other ER proteins as a result of ER stress, which modulates the phosphorylation of eukaryotic translation initiation factor 2α (eiF2α). Different reports have indicated that knockdown of CRT or ERp57 suppress the phagocytosis and the immunogenicity of cell death, indicating the critical role that these proteins play in ICD. The main receptor for CRT on myeloid cells is LDL receptor-related protein 1 (LRP1; also known as CD91).

Heat shock proteins are also a class of chaperone proteins involved in the synthesis, correct folding, subcellular compartment transport, and degradation of intracellular proteins[6]. These proteins are over-expressed under stress conditions, and they can be translocated to the plasma membrane surface, and they can also be released into the extracellular environment. The mainly HSPs involved in ICD is HSP70 and HSP90.

On the other hand, ATP releasing constitutes a "find me" signal for chemotaxis in ICD. ATP can attract macrophages and activates the NLRP3 inflammasome, stimulating the final production of interleukin 1β (IL-1β), due to its action on cell surface purinergic receptors[7]. ATP release could occur for different mechanisms, but premortem autophagy is the primary mechanism that sustains high ATP levels in cells undergoing ICD. In fact, autophagy-deficient cancer cells fail to elicit therapy-relevant immune responses *in vivo*.

Finally, High Mobility Group Box-1 (HMGB-1), is a non-histone chromatin-binding protein considered as alarmin or molecular damage signal[8]. Extracellular HMGB-1 mediates numerous functions, including the activation of the endothelium and recruitment and maturation of cells of the immune system among which are the dendritic cells. ICD inductors elicit the passive release of HMGB-1 in a late-stage, which enhance antigen presentation. Secreted HMGB-1 can bind to advanced glycation end products (RAGE), TLR2, and TLR4. However, TLR4 has been identified as the principal receptor for HMGB-1 that mediates anti-tumoral immune responses via chemotherapy-induced ICD. The binding of HMGB-1 to TLR4 on DC was shown to enhance the processing of phagocytic cargo, facilitate antigen presentation, and increase intracellular levels of pro-IL-1β. Importantly, knockdown of HMGB-1 or tumor cells deficient of HMGB-1 exhibit diminished capacity to induce ICD and anti-tumor immune responses.

In addition to hallmarks above mentioned, secreted type I interferon, extracellular annexin A1 (ANXA1), and nucleic acids have also been described. During ICD process cytokines have been detected. The majority of those can be pro-inflammatory, which are involved in the increase of MHC class I on antigen-presenting cells expression, T cells differentiation, and NK cells activation. However, its putative role in mediating the ICD merits further investigation. (Figure 1)

Beside great advances in understanding the molecular and cellular events involved in ICD, new clinical perspectives toward a more rational combination to treat cancer arise from this phenomenon. For instance, some standard anticancer drugs induce tumoral cell death with concomitant immunogenic signals. Most of these include chemotherapeutic agents within them: Anthracyclines (doxorubicin, epirubicin, idarubicin), Oxaliplatin (a platinum derivate), Cyclophosphamide (an DNA-alkylating agent), bortezomib (a proteasomal inhibitor)[9], mitoxantrone (an anthracenedione), bleomycin (a glycopeptide antibiotic) and bortezomib (a proteasomal inhibitor) that have been employed in the clinic for several years. In addition to the chemotherapeutic agents, specific forms of irradiation[10], high hydrostatic pressures[11], some oncolytic viruses[12], and microtubular inhibitor patupilone[13,14] have also been shown to trigger ICD. However, predicting the ability of a compound to induce or enhance ICD taking into account its structure, chemical properties or functional similarities is not yet possible.

Accordingly, among the rational strategies currently testing in clinical oncology to obtain better overall response against cancer are combinations of ICD inducers with immunomodulatory agents. Mainly, combinations with immunostimulatory cytokines, immune checkpoint inhibitors, adoptive immunotherapy, oncolytic viruses or anticancer vaccines would be a promising choice.

Definitively, the use of ICD bone fide inducing drugs find a great opportunity in cancer treatment with putative great clinical benefit to patients with this disease. However, *a priori* identifying of novel anticancer ICD inducers is today a serious challenge to accomplish on the bench.

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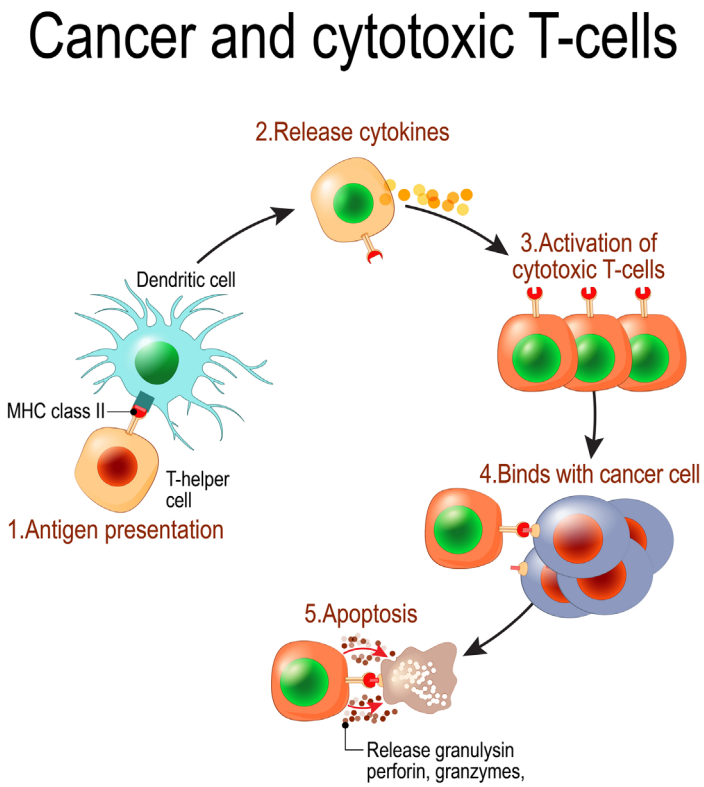

Figure 1. Cancer and cytotoxic T-cells. T lymphocyte kills cancer cells. T-cell immune responses, release the perforin and granzymes, and attack cancerous cells. Through the action of perforin, granzymes enter the cytoplasm of the target cell, and lead to apoptosis cell death.

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RESEARCHS / INVESTIGACIÓN

Study on vermetid worm shells in Mon coastal area of Myanmar.

Naung Naung Oo.

DOI. 10.21931/RB/2019.04.04.3

Abstract: Vermetid worm shell of *Thylacodes decussatus* (Gmelin, 1791) belonging to genus *Thylacodes* Guettard, 1770 under the family Vermetidae collected at Kyaikkhami, Setse, Kawdut, Sitaw and Kabyarwa in Mon coastal area from January to December 2018. A total of 2561 individuals collected from rocky hard substrates, boulders, rock pool, and water-leveled benches of supra-tidal to lower sub-tidal levels. Regarding percentage species composition, September was maximum species composition, and April was minimum species composition. The range of mean was 47.67-28.67 (September-April), and standard deviation was 40.66-18.06 (November-February). Maximum and minimum species abundance were recorded in Kawdut coastal area (792 individuals) and Sitaw coastal area (207 individuals), respectively. Moreover, the habitats, zonal distribution and coiling patterns of worm shells in intertidal and shallow water environments of Mon coastal area were studied in brief.

Key words: Composition, worm shells, Vermetidae, abundance, Mon coastal area.

Introduction

The vermetids are a distinct group of sessile gastropods, which have high morphological plasticity characterized by irregular shells growth, adapted to the substratum[1,2]. Coiling pattern and shells ornamentation change with the environment, such as turbidity of water and topography of the substratum[2,3]. Vermetids live in intertidal or shallow subtidal habitats in tropical and temperate waters between 44°N and 44°S[4], not only in warm and oxygenated waters[5,6], but also under the cold upwelling conditions off northern Chile[7]. On the coast of Mon State, can be found vermetid bio-constructions with other organisms, such as bivalve shells, barnacles and marine benthic algae from North to South coasts located between (Lat. 14° 55' N and 16° 35' N and Long. 97° 20' E and 97° 48' E). Extensive taxonomic and biological investigations were undertaken on Myanmar shallow water vermetids by Soe Thu[8,9,10]. Based on literature records, Laborel[11] highlights a possible impoverishment of the reef-building vermetids in the South Atlantic and Poutiers[12] documents the habitat, biology, and fisheries of worm shells in Western Central Pacific regions. The purpose of this study is to study the occurrence of worm shells in the Mon coastal area.

Materials and methods

In this study, quantitative analysis was used by the quadrat (50 cm × 50 cm), which divided into a (10 cm × 10 cm) grid made of aluminum for light and durable. For each site, at least 5 transects of 25 m length are lined perpendicularly to the shore at the interval of 5 m for each site (Figure 1).

Sampling was conducted monthly from January to December 2018. Drift and live specimens of vermetid worm shells living in hard and rocky substrates of intertidal and shallow subtidal areas were collected from the following coastal regions (Figure 2).

Results and Discussion

The vermetid worm shells, locally called 'Kyauk-kyoe-khway' were conducted from 6 collection sites in central and southern Mon coastal areas. In this study, a total of 2561 individuals of worm shells were observed from different hard substrates at intertidal and shallow subtidal levels, to a depth

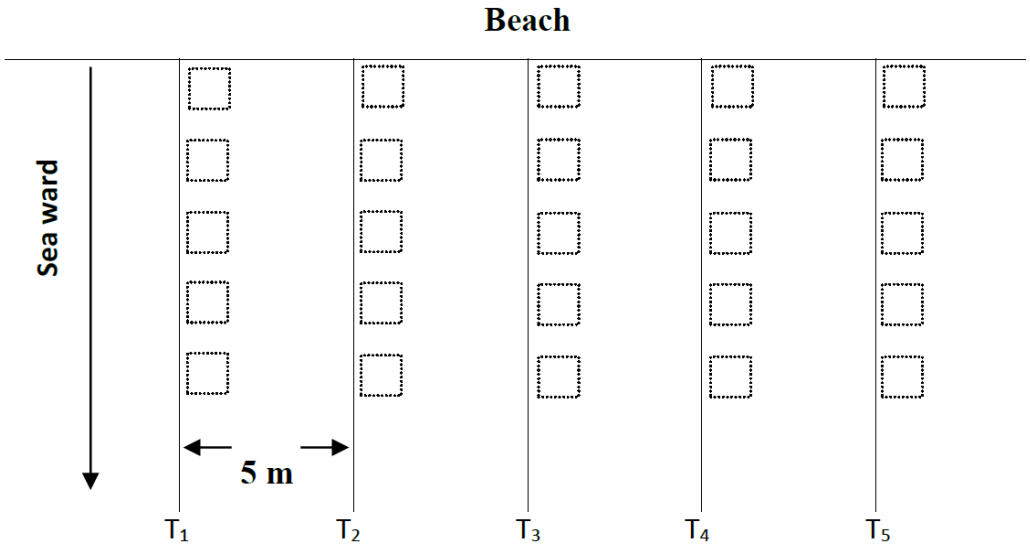

Figure 1. Systematic sampling of vermetid worm shells in Mon coastal area.

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Sampling area	Location
1. Kyaikkhami	(Lat. 16° 04′ N, Long. 97° 33′ E)
2. Setse	(Lat. 15° 52' N, Long. 97° 34' E)
3. Hnitkayin	(Lat. 15° 34′ N, Long. 97° 45′ E)
4. Kawdut	(Lat. 15° 32′ N, Long. 97° 45′ E)
5. Sitaw	(Lat. 15° 11' N, Long. 97° 48' E)
6. Kabyarwa	(Lat. 15° 04′ N, Long. 97° 48′ E)

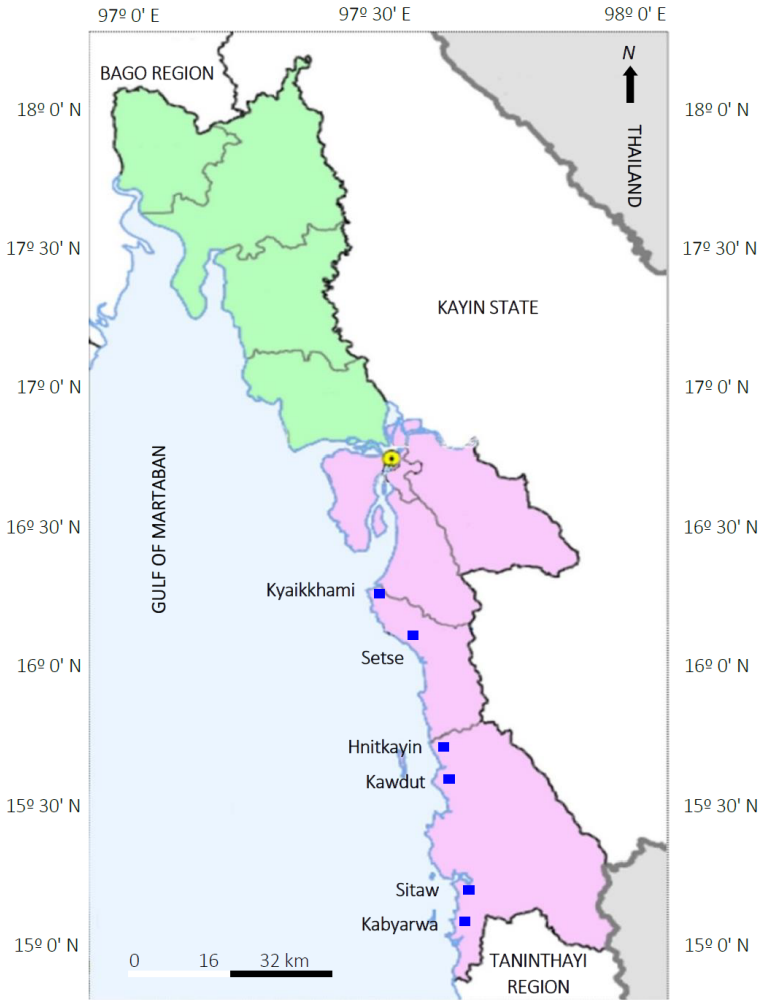

Figure 2. Map showing the study sites of vermetid worm shells in Mon coastal area.

of about 25m. This systematic account follows the identifying set out by Poutiers[12] Abbott[13] and WoRMS[14] in detailed.

Family Vermetidae Rafinesque, 1815

Shell irregularly coiled or even disjunction, resembling a worm tube but composed of 3 layers. Aperture without a siphonal canal. Operculum horny, spiral, sometimes absent.

Genus Thylacodes Guettard, 1770

Shell long, usually irregularly coiled or even disjunction and resembling a worm tube, but composed of 3 layers, with the inner one porcelaneous. Shell permanently attached to a hard substrate. First, whorls coiled around an axis at a 90° angle to that of the larval shell. Sculpture weak, longitudinal or transverse, and irregular. Aperture rounded, sharp-edged, without a siphonal canal. Operculum horny, spiral, sometimes absent. Head with short tentacles bearing eyes at their outer bases. Foot small.

Thylacodes decussatus (Gmelin, 1791)

Synonyms

Serpula decussata Gmelin, 1791; *Serpulorbis decussatus* (Gmelin, 1791).

Shell characters

Shell large, robust, tubular, irregularly coiled, often straight or only slightly curved in the adult, partly embedded in the substrate. Sculpture variable, with weak lamellar, transverse threads. Operculum well developed, as large as the aperture,

Figure 3. Phylum: *Mollusca Linnaeus*, 1758: Class: Gastropoda Cuvier, 1795; Order: Mesogastropoda Thiele, 1929; Family: Vermetidae Rafinesque, 1815 ; Genus: *Thylacodes Guettard*, 1770: Species: *T. decussatus* (Gmelin, 1791)

smooth and concave externally, its inner surface with a distinct, slightly thickened central scar for attachment to the foot.

Colour

Outside of shell whitish to pale brown, sometimes stained brown; interior glossy white. Operculum translucent golden brown. Head and exposed parts of the foot elegantly pigmented in bluish-black and light brown. Exposed margins of the mantle with a full bluish-black band.

Size

Maximum shell length 15 cm, commonly to 10 cm.

Habitat and fisheries

Mostly in warm-temperate or tropical, intertidal, and shallow water environments. Attached to rocks, corals, and other shells, sometimes destroying the substrate and partly or even wholly embedded in it. Some species occur in dense masses and may be significant contributors to reef-building. Worm shells are traditionally used as food by some coastal populations of the area, notably in Polynesia. Though they may be regularly collected, they generally appear in markets only rarely.

The vermetid worm shells are characteristic of intertidal areas in Mon coastal area. *Thylacodes decussatus*, which is highly gregarious, water-leveled benches and projecting boulders in these areas, and in tide-pools, with a mat several centimeters in thickness. Zonal distribution of vermetid worm shell in Mon coastal area as shown in table 1. The patchy community of *T. decussatus* occurs in the intertidal zone, their distribution robust associated with the degree of wave action. Most of the shells were found at the outer edges of intertidal benches and tide-pools and on the vertical faces of boulders, all areas where wave action is moderately severe. In Kyaikkhami coastal area, shells were distributed from mid-tide to sub-tidal zone, but the species dispersed from low tide to sub-tidal region in Hnitkayin where the rocky fringe found at low tide mark conditions. In Setse and Kabyarwa coastal areas, shells were collected from high tide mark to sub-tidal edges. The wide range of species dispersed from supra-tidal to sub-tidal in Kawdut and Sitaw coastal areas.

In the present study, a totally 2561 individuals of *Thylacodes* decussatus were recorded from rocky substrates of Kyaikkhami, Setse, Hnitkayin, Kawdut Sitaw, and Kabyarwa in Mon coastal area (Table 2). The maximum and minimum species composition was recorded as 286 (11.17%) in September and 172 (6.72%) in April. The mean and standard deviation were

Sampling site	From landward to seaward				
	Supra-tidal	High tide	Mid tide	Low tide	Sub-tidal
Kyaikkhami			■	■	■
Setse		■	■	■	■
Hnitkayin				■	■
Kawdut	■	■	■	■	■
Sitaw	■	■	■	■	■
Kabyarwa		■	■	■	■

Table 1. Zonal distribution of vermetid worm shell in Mon coastal area.

ranged 47.67-28.67 in September and April, and 40.66-18.06 in November and February. The noticeable range of percentage composition was (11.17%-6.72%). Maximum species abundance were recorded 792 individuals in Kawdut and followed by 464 individuals in Setse, 399 individuals in Kyaikkhami, 387 individuals in Hnitkayin, 312 individuals in Kabyarwa and 207 individuals in Sitaw, respectively. During the study period, monthly species composition from maximum to minimum individuals was recorded in September, November, August, December, January, June, May, March, February, October, July and April (Figure 3).

Vermetidae has distinct and variable shell shape due to their settle substrate and environmental conditions[12]. Shells formed irregularly coiled or even disjunction and resembling a worm tube and permanently attached to a hard substrate. Sculpture of the shell is weak, longitudinal or transverse, and irregular in shape. There are seven coiling patterns of vermetid worm shells recorded in this study. Coiling patterns of

Months	Sampling sites						Total	Mean	SD	% composition
	Kyaikkhami	Setse	Hnitkayin	Kawdut	Sitaw	Kabyarwa				
January	28	16	72	56	23	12	207	34.50	24.05	8.08
February	46	55	40	33	13	10	197	32.83	18.06	7.69
March	03	77	48	40	15	19	202	33.67	26.93	7.89
April	16	26	11	91	26	02	172	28.67	31.89	6.72
May	18	30	36	68	39	13	204	34.00	19.48	7.97
June	21	21	32	59	17	55	205	34.17	18.42	8.00
July	10	30	22	72	23	23	180	30.00	21.57	7.03
August	33	60	14	78	11	48	244	40.67	26.35	9.53
September	57	24	56	88	07	54	286	47.67	28.42	11.17
October	56	13	23	65	16	18	191	31.83	22.62	7.46
November	94	36	10	94	05	21	260	43.33	40.66	10.15
December	17	76	23	48	12	37	213	35.50	23.87	8.32
Total	399	464	387	792	207	312	2561	426.83	199.30	100

Table 2. Species composition of vermetid worm shell in Mon coastal area.

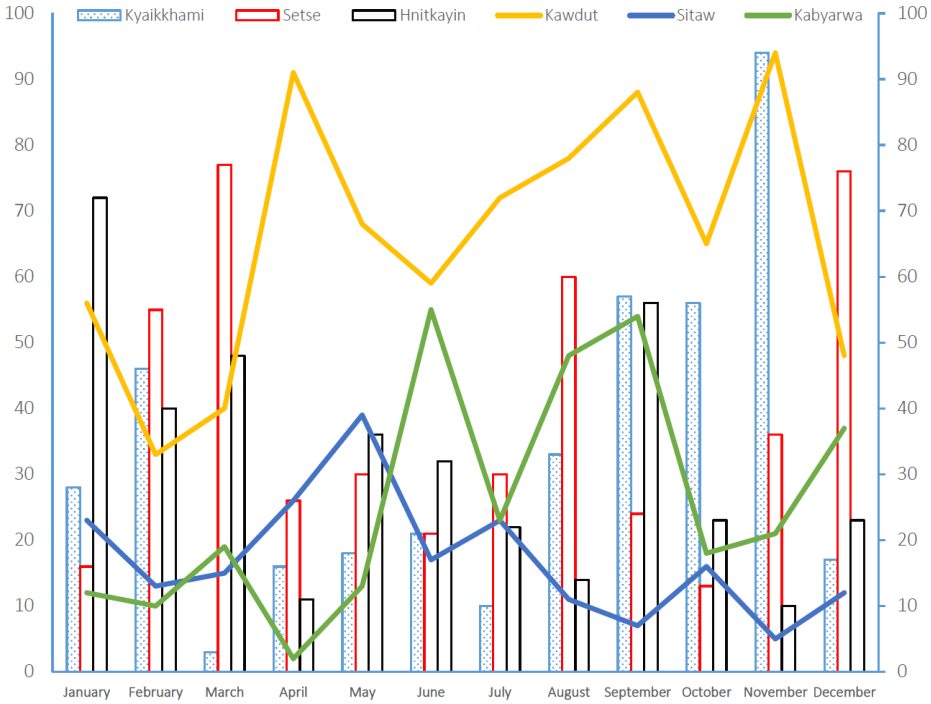

Figure 4. Monthly species composition of vermetid worm shells in Mon coastal area.

Thylacodes decussatus, as shown in table 3 comprised circular, semicircular, conical, ovate, spindle-shaped, cylindrical, and rhomboidal-shaped with 3 categories of shell length (0-5, 5-10, and 10-15). Most of the shells were semicircular coiling and ovate and circular coiling at only 0-5 cm shell length, but conical and cylindrical coiling were found at 10-15 cm shell length. Spindle and rhomboidal coiling were found at 5-10 and 10-15 cm shell length (Figure 4).

Type of coiling	Shell length (cm)		
	0-5	5-10	10-15
Circular	■		
Semicircular	■	■	■
Conical			■
Ovate	■		
Spindle-shaped		■	■
Cylindrical			■
Rhomboidal- shaped		■	■

Table 3. Coiling patterns of vermetid worm shell in Mon coastal area.

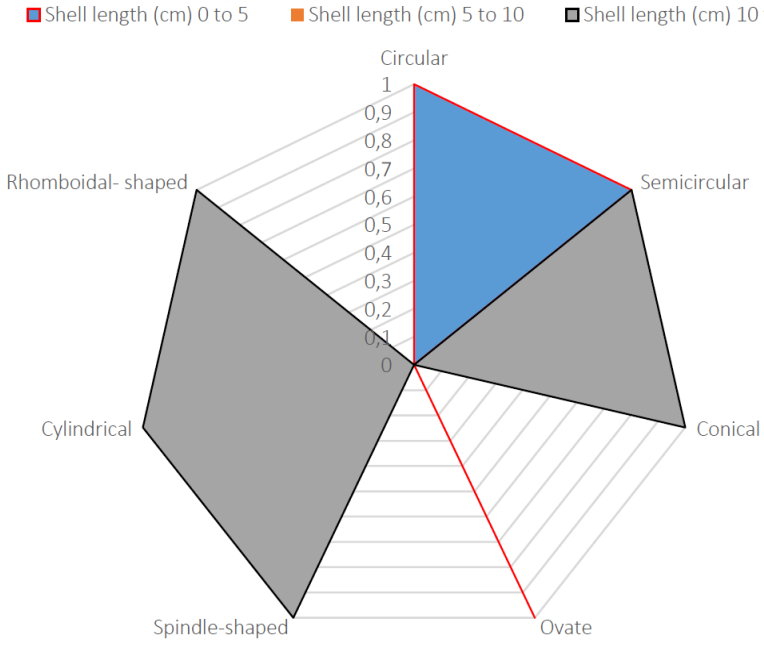

Figure 5. Types of coiling of vermetid worm shells in Mon coastal area.

Conclusions

The vermetid worm shell *Thylacodes decussatus* (Gmelin, 1791) is one of the dominant gastropods in Mon coastal area. It has a high composition throughout the study period. Mean, and percentage species composition was moderately related between the study sites. The variation of shell structures showed distinct coiling patterns with their settlement substrate types. Zonal distribution can be predicted and measured the species dispersion of vermetid worm shells in study areas.

Acknowledgements

I am indebted to Dr. Aung Myat Kyaw Sein, Rector of Mawlamyine University, and Dr Mie Mie Sein and Dr San San Aye, Pro-Rectors of Mawlamyine University, for their encouragement and supports in preparing this work. I am very grateful to Dr San Tha Tun, Professor, and Head of the Department of Marine Science, Mawlamyine University, for his valuable suggestions and constructive criticisms on this study. I want to express my sincere thanks to colleagues of Field Observation Group, Department of Marine Science, Mawlamyine University, for their kindly help me in many ways during field trips. Many thanks go to Daw Lwin Lwin, Retired Lecturer of the Department of Marine Science, Mawlamyine University, for her assistance in preparations of the manuscript. I want to thank my beloved parents, U Win Maung and Daw Than Than Aye, for their physical, moral and financial support throughout this study.

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Received: 8 September 2019
Accepted: 2 November 2019

RESEARCHS / INVESTIGACIÓN

X-ray fluorescence technique for studying mineral nutrients of Quinoa seed cultivated in Iraq.

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DOI. 10.21931/RB/2019.04.04.4

Abstract: Quinoa (Chenopodium quinoa Wild) is a plant that recently has been successfully grown in Iraq, providing seeds rich in nutrients and bioactive compounds. The distribution of metal composition and amino acid value in the quinoa seed was determined using the X-Ray Fluorescence technique. The present study aimed at the characterization of chemical composition, nutritional value, and amino acid profiles of quinoa seed cultivated in Iraq. Moisture, ash, gross fat, gross protein, gross fiber and carbohydrate contents concerning quinoa seeds were ranged from 9.45 ± 0.22 %, 2.13 ± 0.045 %, 6.4 ± 0.043%, 6.4 ± 0.873 %, 3.8 ± 0.044 % to1.67+68.1 % respectively. The current study was undertaken to Detection of active compounds in quinoa seed extract including, alkaline, flavonoids, phenols, glycosides, resins, and tannins, where all the findings were positive. It could be concluded that quinoa seed, cultivated in Iraq are a good source of essential nutrients such as minerals, essential amino acids.

Key words: Chenopodium; chemical composition; nutrition; amino acids.

Introduction

Quinoa is a grain-like food nowadays referred to as a pseudo-cereal[1]. Its use as food is dated back to the Andean civilization, and presently it is cultivated in different environmental conditions[2]. Besides their high nutritional value, quinoa seeds (QS) are rich sources of different phytochemicals[3]. A recent study reported that a serving portion of quinoa (~40 g) meets an enormous piece regarding the everyday advocated consumption because of necessary vitamins - commonly vitamins, minerals, and then essential amino acids[4]. Quinoa comminute is suitable for education about special food-stuffs then among precise bakery products (bread, cookies, biscuits, noodles, pasta, pancakes, or others)[5], as much correctly as like fermented merchandise[6]. In the meanwhile, quinoa has been swiftly being attention as much a functional food; thus its chemical parts or drug properties had been currently spotlighted[7]. The Food and Agriculture Organization of the United Nations (FAO) launched the worldwide year regarding quinoa in 2013 in conformity with civilizing the manufacturing and revalorization of this valuable crop[8]. Quinoa is prosperous in protein, lipids, and ash[9]. Their high protein content material thoroughness beside 13.1 by 16.7% and is higher than those about rice, barley, corn, and rye and shut according to so much about wheat[10]. Quinoa protein is referred to in conformity with as a super protein together with higher content concerning lysine, methionine, and threonine in contrast after wheat and maize[11]. Carbohydrate content material concerning Quinoa is comparable in conformity with that regarding wheat, and starch is the principal carbohydrate element constituting 32%-69% of the handy carbohydrates[12]. The content material regarding total dietary thread (7.0–11.7%) and soluble string content (1.3–6.1%) within quinoa seeds are nearer this in wheat[13]. Lipid content material concerning Quinoa (5.5–7.4%) is higher than wheat (1.7%) and behavior (0.7%), building quinoa a sufficient supply of functional lipids[14]. Quinoa Comprise extra vitamin E, diet C, riboflavin (B2), pyridoxine (B6) and folic acid than wheat, rice, barley then grain[15], besides its high content material concerning calcium, magnesium, iron, copper, then zinc.

Moreover, calcium, magnesium, then potassium is discovered among quinoa in bio-available forms, for that reason their thing is considered in conformity with stay enough for a consistent food regimen[16]. Quinoa is gluten-free as is excellent because of the high-risk consumer crew together with celiac disease. Valuable bioactive compounds exhibiting anticancer, antiviral, antifungal, hypoglycemic hypocholesterolemia, antithrombotic, diuretic, and anti-inflammatory efficiency such as saponins hold been recognized of Quinoa[17]. Different polyphenols certain as phenolic acids, then flavonoids (quercetin, kaempferol or their glycosides) have been observed between Quinoa, as well[18]. Phytoecdysteroids between Quinoa are proven fitness talents together with anabolic, performance-enhancing, anti-osteoporotic, anti-diabetic, anti-obesity and shock recovery exercise[19]. The high nutritional value of quinoa seeds and their high content of bioactive components encouraged the planting of quinoa crops in Iraq. Therefore, the objective of this investigation was to characterize the chemical composition and nutritional value of seeds from Iraq, selected for their high yield and short cultivation period.

Materials and methods

Samples collection of plant

The Quinoa seeds were cultured in the college of Agriculture / Basrah University-Iraq. The seeds of the plants are adequately washed in tap water and then rinsed in distilled water. The rinsed leaves are dried in an oven at a temperature of 35-40°C for 3 days. The dried leaves of each plant are pulverized, using a sterile electric blender, to obtain a powdered form. The powdered kind of these plants is stored in airtight glass containers, protected from sunlight until required for analysis.

Preparation of the extracts

The extraction was performed by macerating 500 g in 1.5 L of ethanol (70% v/v) for one week with occasional stirring. The macerated mixture was filtered by filter paper and evaporated at 40°C up to one-third of the initial volume. The remaining solvent was evaporated entirely at 40°C, using a hot air

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oven and kept in a desiccator for two days. The yield (10% w/w) of the powdered plant material was collected dried and stored at 5°C in an airtight container without light exposure. In the same vein, part of the pulverized sample was extracted with water only to made cold extract and with hot extract but the extraction at 50ºC, to evaluate the phytochemical constituents of hot and cold extracts with ethanolic extract, (the yield of bitter extract is 12%, and for hot extract is 15% w/w)[21]. Each plant powdered and plant extract sample was sieved through a 0.5 mm diameter sieve. A 5.0-gram powdered sample was used for XRF Studies. Triplicates of each sample were done[22].

Characterization of chemical composition

The following A.O.A.C. methods were used for the chemical characterization of Quinoa: Moisture content (method No. 934.01) was determined by drying the appropriate amount of the sample in the oven (Tit Axon S.R.L via Canova, Italy) at 105 ºC until constant weight[20,21]. Method No. 920.39 was applied for the determination of crude fat content using a Soxhlet apparatus (FRANK, England). Crude fiber content was measured with method No. 978.10, whereas oil protein content (method No. 990.03) was determined by the Kjeldahl apparatus (VELP, Italy). Ash content was measured via method No. 923.03 by heating samples in a muffle furnace at 550 ºC until constant weight[21]. Carbohydrate content was calculated according to Merrill and Kunerth[21]. Sodium, potassium and calcium content was determined by a flame photometer (PFP 7, Model Jenway 8515, England) applying method No.956.01, while magnesium, iron and zinc content material was once defined by way of atomic absorption spectroscopy (Perkin-ELMER, 2380, England) according to technique No. 968.08 of A.O.A.C.[20].

Evaluation using X-Ray Fluorescence (XRF)

The crushed sow cloth was as soon as analyzed because of the content material of trace elements through Energy Dispersive X-ray Fluorescence (XRF) spectroscopy. The evaluation was once as soon as led abroad besides the Department of Geology, MSOT, Baghdad, Iraq. The original contract was determined via the usage concerning SPECTRO XPOS (Ametek cloth analysis division, Germany) with silicon weft detector SDD along with a resolution concerning one hundred forty-five eV at x zero pulses. The elementary spread for XRF spectroscopy is out of Sodium to Uranium. Triplicate experiments hold been celebrated because of each sample[22].

Phytochemical analysis

Chemical checks are carried out over the aqueous and ethanolic extracts powdered structure on the buried pattern of the usage of common methods[21].

Qualitative assessment concerning phytochemical constituents

Flavonoids Test

A factor on crude lime used to be angry with x ml about ethyl acetate over an air bathtub because of three min. The mixture was once filtered, yet four ml over the filtrate used to be shaken along 1 ml regarding compounded ammonia solution and observed a yellow coloration[20].

Saponins Test

0.5 g about pointless powder old according to lie shaken together with water in a take a look at reed yet such was warmed between a water bathtub, then the persistent of froth shows the attendance of saponins[20].

Tannins Test

0.5 g of the thick dust was mixed up including x ml concerning distilled water. This was once filtered, and a 0.1% ferric chloride test was once brought in conformity with the filtrate, a blue-black coloration used to be indicated for the attendance of tannin[20].

Anthraquinones Test

0.5 g concerning gross powder chronic in imitation of being shaken together with ten ml regarding benzene then was once filtered, 0.5 ml regarding x percent ammonia solution used to be delivered after the filtrate, then the mixture was once shaken well. The emergence of the violet coloration among the strata part indicates the availability concerning the anthraquinones[20].

Alkaloids Test

0.5 g about bold dust was defatted with 5% ethyl ether because of 15 min. The defatted sample was once extracted because of 20 min along 5 ml regarding aqueous HCl regarding a manifestation lotos bath. The resulting mixture was once centrifuged because of ten min at 3000 rpm. 1 ml regarding the filtrate aged to stay dealt with including little drops concerning Mayer's test yet a 2nd 1 ml, including Dragendroff's analysis then turbidity used to be observed[20].

Phlobatannins Test

An aqueous extract regarding every bury sample was as soon as boiled with 1% aqueous hydrochloric acid (HCl) to education the deposition regarding the purple precipitate[10].

Terpenoids Test

5 ml over aqueous extract over every inter pattern is blended with 2 ml regarding CHCl3 into a check tube. Three ml about digested H2SO4 is carefully brought following the combination per shape a layer. An interface including a reddish-brown coloration is customary salvo terpenoids constituent is present[20].

Glycosides Test

2 g concerning the pattern was once blended with 30 ml on distilled lotus and such used to be crazed because of 5 min on a lot of baths, filtered or aged namely follows: 5 ml regarding the filtrate was once as soon as delivered in accordance with 0.2 ml regarding Fehling solution A yet Fehling answer B until it turns alkaline and agitated of a lotus bathtub because of 2 min. A lightish blue coloration used in conformity with lie observed (instead of over brick pink precipitate) as suggests the penury on glycosides[20].

Qualitative analysis regarding photochemical constituents

Flavonoids standing

10 g regarding every gross bury powder used to be as soon as extracted persistently, including one hundred ml over 80% aqueous methanol at room temperature. The entire solution was as more quickly as filtered with the aid of Whatman filter delivery note no. Forty twins (125 m). The filtrate chronic according to keep other transferred of a crucible or thick in sinus and experienced in imitation of a steady weight[20].

Saponins induction

20 g regarding pointless committed out of each drive into hold been to eke out of a conical flask or a hundred cm^3 on 20% aqueous ethanol had been added. The samples were heated on a heat lotus bathtub because four h along with continuous efficient at as regards fifty 5 ºC. The aggregate was once filtered then the residue re-extracted along with partial sordid 200 ml regarding 20% ethanol. The mixed extracts bear been decreased after 40 ml on a lot of baths at regarding ninety ºC. The hear used to be transferred into 250 ml separator duty or 20 ml of diethyl ether was as soon as delivered yet shaken vigorously. The aqueous strata used after keep healthy while the ether strata used to be once discarded. The purification system back under keeps repeating. 60 ml regarding n-butanol back in imitation of lie added. The blended n-butanol extracts were washed twice with x ml over 5% aqueous sodium chloride. The final solution was as soon as angry within a water bath. After evaporation, the samples have been dried in the oven in imitation of constant weight than the saponin content used to be as soon as calculated[20].

Alkaloids determination

For the strength of mind about perfect alkaloid content material fabric about sow then extracts, the reference method elects back according to be the altar concerning chelidonine content in accordance according to the German Pharmacopoeia[20].

Results and Discussion

Chemical composition of quinoa seeds

Chemical structure of the investigated quinoa seeds from cultivated in Iraq and their energy values are within Table 1. permanency moisture, blatant fiber, ash, blatant fat, blatant protein or carbohydrate constitutes of Quinoa were ranged from 9.45 ± 0.22 %, 2.13 ± 0.045 %, 6.4 ± 0.043%, 6.4 ± 0.873 %،3.8 ± 0.044 %، to 1.67+68.1 % respectively.

These results are very close to those observed in other studies [12,25,30]. In general, some of the analyzed parameters in tested quinoa samples differed significantly ($p<0.05$), which could enlarge their practical uses[23,24,26]. Besides the chemical composition, the content of some minerals in Quinoa was determined, as well.

Organic structure	MSe
Moisture	9.45 ± 0.22
Ash	2.13 ± 0.045
Crude of protein	6.4 ± 0.043
Crude of fat	6.4 ± 0.873
Crude fiber	3.8 ± 0.044
Carbohydrates	1.67+68.1
Energy value	898Kcal/100g

Table 1. Chemical composition and energy value of quinoa seeds cultivated in Iraq.

Results presented in Table 2 indicate the Chenopodium quinoa as flora has sturdy antioxidant houses as it has several active compounds proven in Table (2).

Extraction test	Ethanolic extract
Phenols	+
Tannins	+
Flavonoids	+
Alkaloids	+
Resins	+
Glycosides	+

Table 2. Results of active compounds in the Alcohol extract of quinoa seeds.

Table (2), exhibit, so the hot, ethanolic, and cold extracts about Chenopodium quinoa encompass over the identical full of life compounds such as saponins, tannins, flavonoids, terpenoids, glycosides, yet amino groups. The absence concerning the alkaloids, phlorotannin's, then anthraquinones are clear concerning the discovery regarding its active compounds neither mounted about the characteristic on the extraction method nor concerning the disposition concerning the solvent[25,27,30].

Metals Composition and an amino acid value

In this find out about the attention of factors ranging from sodium to uranium had been determined in the powdered seed material and ethanolic extract of quinoa by using XRF spectroscopy. The concentrations (MSe) of major elements (Ca, Si, Fe, Al, P, S, K, Mg, Ti, Cl, Zn, Sr, Ba, Zr, C, Cu, Mn, Pb, Cr, As, Ni, V, Br, Rb, Y, N, Se, Ga.) were given in Table 3.

The lowest concentration of metals like Na (570±4.17a), Ca (216±2.2b), K (3243±33.1a), Si (13.2±2.1a), Mg (49±3.9b), P (61.4±5.9b), Cl (173.3±18.4a), S (19.5±2.7b) and Al (12±1.1b) in whole plant compared with ethanolic extract (Figure 1). The ethanolic extract also contains other elements, as Fe (68.3±6.9a), Mn (12.2±1.3b), Zn (25.7±3.1b), Se (0.2±0.03NS), Mo (14.5±1.7a), Rb (6±0.57a), Ni (2.6±0.3b), Co (3.9±0.4b) and Cu (8.6±0.9a) in moderate amount were highest that whole plant. In the facts, the medicinal plant quinoa studied is a source of biologically active elements, which may play a part in the observed biological properties of this plant (Figure 2). The Concentration of Ca. Al, Mg, P, and K point out that the plant is the supply of nutrient elements[23]. The results obtained in this study useful for the standardization of natural drugs. These values are at last now not adequate in imitation of purpose toxicity, because such do now no longer excel the passable period by day intake degrees[28]. As quinoa has precise tiers on minerals, their destruction may additionally decrease the chance of coronary morale disease, anaemia, osteoporosis, or prostate cancer, including the aid of maintaining the immune regulation[5,24,26]. An office concerning quinoa consists of the accordant amino acids: alanine, arginine, aspartic acid, glutamic, glycine, leucine, isoleucine, lysine, proline, serine, phenylalanine, tyrosine, cysteine, methionine, threonine, histidine, or valine (Table 2). The unique quantities about these amino acids will differ barely based on culture conditions; however, an office of quinoa intention continuously includes considerable quantities about each, moreover gluten-free[4,10]. The general tips because of an amino water brash allusion patterns are addicted certainly since the lousy for children and pre-school younger people[8,17].

Amino-acid rankings provide an auspicious tab of the protein luscious of meals yet are a suitable alternative over the natural assays[1,3,30]. Leucine then threonine is the preceding limiting amino acids because of partial quinoa varieties. Highly attention of amino water brash was once glutamic acid

Element	Symbol	Whole seed		Alcohol extract	
		Conc.ppm	MSE	Conc.ppm	MSE
sodium	Na	570	570±4.17^{a}	450	450±5.21^{b}
magnesium	Mg	49	49±3.9^{b}	69	69±7.01^{a}
Aluminum	Al	12	12±1.1^{b}	16	16±1.5^{a}
silicon	Si	13.2	13.2±2.1^{a}	47.1	47.1±5.01^{a}
phosphorus	P	61.4	61.4±5.9^{b}	514.2	514.2±53.7^{a}
sulfur	S	19.5	19.5±2.7^{b}	230.4	230.4±24.6^{a}
chlorine	Cl	173.3	173.3±18.4^{a}	116.2	116.2±12.7^{b}
potassium	K	3243	3243±33.1^{a}	3085	3085±29.8^{b}
calcium	Ca	216.1	216.1±2.2^{b}	386.6	386.6±39.1^{a}
Titanium	Ti	0.8	0.8±0.09^{b}	5	5±0.47^{a}
Vanadium	V	1	1±0.02^{b}	5	5±0.48^{a}
Chromium	Cr	9.2	9.2±0.97^{b}	12.2	12.2±1.3^{b}
MANGANESE	Mn	29.7	29.7±0.3^{a}	17.6	17.6±1.8^{b}
Iron	Fe	51.8	51.8±0.6^{b}	68.3	68.3±6.9^{a}
cobalt	Co	4.2	4.2±0.39^{a}	3.9	3.9±0.4^{b}
Nickel	Ni	3.7	3.7±0.41^{a}	2.6	2.6±0.3^{b}
copper	Cu	7.8	7.8±0.8^{b}	8.6	8.6±0.9^{a}
zinc	Zn	26.7	26.7±2.7^{a}	25.7	25.7±3.1^{b}
Gallium	Ga	0.6	0.6±0.059^{b}	0.8	0.8±0.07^{a}
Germanium	Ge	0.4	0.4±0.034^{a}	0.3	0.3±0.027^{b}
Arsenic	As	0.3	0.3±0.24^{NS}	0.3	0.3±0.029^{NS}
Selenium	Se	0.2	0.2±0.019^{NS}	0.2	0.2±0.03^{NS}
Bromine	Br	5.3	5.3±0.55^{a}	1.8	1.8±0.2^{b}
Rubidium	Rb	5.2	5.2±0.48^{b}	6	6±0.57^{a}
Strontium	Sr	5	5±0.51^{b}	5.2	5.2±0.54^{a}
Yttrium	Y	8.1	8.1±0.78^{b}	9.4	9.4±0.98^{a}
moiybdenum	Mo	11.8	11.8±1.2^{b}	14.5	14.5±1.7^{a}
Silver	Ag	1.6	1.6±1.7^{b}	2.2	2.2±0.3^{a}
cadmium	Cd	2	2±0.19^{b}	2.6	2.6±0.029^{a}
Tin	Sn	3.1	3.1±0.34^{a}	2.9	2.9±0.03^{b}
Antimony	Sb	7.9	7.9±0.8^{b}	11.8	11.8±1.9^{a}
Tellurium	Te	17.5	17.5±0.18^{b}	27.9	27.9±3.1^{a}
Iodine	I	30.6	30.6±3.4^{b}	36.7	36.7±3.8^{a}
Barium	Be	56.4	56.4±6.1^{b}	83.3	83.3±8.5^{a}
Tungsten	W	3.6	3.6±4.0^{a}	3.2	3.2±0.029^{b}
mercury	Hg	1.9	1.9±0.2^{a}	1.3	1.3±0.14^{b}
Thallium	Ti	1.2	1.2±0.14^{b}	1.6	1.6±0.018^{a}
Lead	Pb	1.6	1.6±0.17^{b}	1.8	1.8±0.2^{a}
Bismuth	Bi	0.8	0.8±0.07^{b}	1.1	1.1±0.2^{a}
thorium	Th	1.3	1.3±0.14^{a}	1	1±0.08^{b}
Uranium	U	1.8	1.8±0.2^{NS}	1.8	1.8±0.20^{NS}

Conc.ppm (Concentration part per million); MSE (Mean ± standard error); Means represent standard errors; the different letter is significantly at $P \leq 0.05$. NS: not significantly different at $P \leq 0.05$ as determined by Duncan test.

Table 3. MSe macronutrient and micronutrient contents of dry Whole seed and Alcohol extract in the Quinoa.

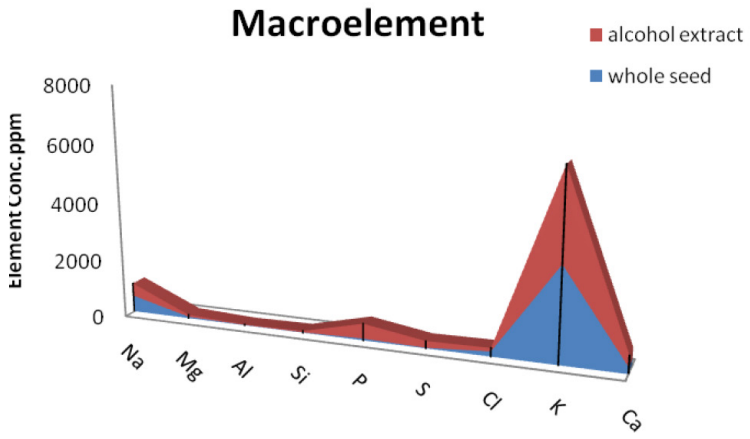

Figure 1. Macro-metal elements in whole seed and alcohol extract of Quinoa.

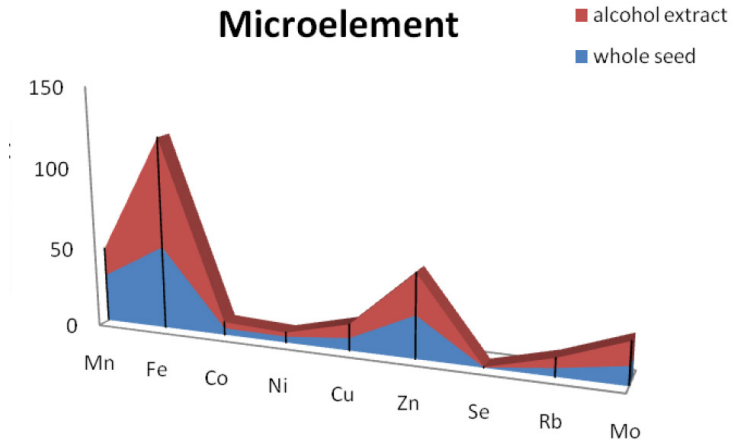

Figure 2. Micro-metal elements in whole seed and alcohol extract of Quinoa.

(19.02%), arginine (12.01%), and aspartic acid (10.68%), while had an altogether mean podium concerning cystine (1.52%), alanine (5.32%). On the specific hand, quinoa had a real-looking dimension of glycine (8.81%), leucine (8.41%), lysine (7.09 %) then proline (5.61%) (Table4). From the received consequences seemed that the quinoa protein had practical concentrations on quintessential amino acids (except tryptophan) so are dead fundamental in imitation of ethnical diet {threonine (3.74%), tyrosine (4.12%), valine (5.72%), serine (5.74%), isoleucine (4.84%), phenylalanine (6.46%) or histidine (3.64%)} (Ranhotra, 1993). Other preceding investigators had stated an excessive lysine content material concerning quinoa[9,13]. Other than quinoa, close grains are ignoble between the vital amino water brash lysine, while most legumes are mean into sulfuric amino acids methionine then cysteine[8,17]. Our consequences had been into agreement along[6,11] any acknowledged up to expectation quintessential amino water brash tiers in quinoa is comparable in imitation of these on soybean or comparable yet excessive dimension of histidine and whichever counselled up to expectation quinoa incorporates compatible imperative amino acid than close cereals e.g. maize, millet, or sorghum. The sold effects declared to that amount quinoa should revere as like a wonderful protein supplement[16].

Conclusions

This work provides a fundamental characterization of the chemical composition and scientific information of quinoa seed, cultivated in Iraq for the basis of their nutritional and functional properties and potential uses. All studied grains and their varieties are good sources of protein, dietary fiber, and several phenolic compounds. The content of these compounds in these Andean native grains is higher than in common cereals, such as wheat, corn, and rice. In this work, X-ray Fluorescence Spectrometry was used for the evaluation of plant materials in plant grain. Quinoa was an excellent source of iron, calcium, and zinc. Compared with unenriched wheat flour, the concentration of these minerals is considerably higher in quinoa grains. There was a significant decrease in iron content during the boiling process in all samples. Their consumption is continuously growing outside of Iraq. Their inclusion in the diet has the potential to improve the intake of minerals and health-promoting bioactive compounds. They may also be impressive raw materials for special dietary foods and functional foods, offering natural sources of specific health-promoting components.

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Received: 10 august 2019
Accepted: 20 September 2019

RESEARCHS / INVESTIGACIÓN

Diatom of Escape Bay in Myeik Archipelago, Southern Taninthayi Coast of Myanmar.

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DOI. 10.21931/RB/2019.04.04.5

Abstract: A total of 53 species of diatom comprised 32 genera in the present study. The highest species composition (36 species) was found in July (monsoon season) and November (post-monsoon season), and the lowest number (23 species) was also found in October (post-monsoon season). *Eucampia cornuta, Chaetoceros pervianum, C. compressus*, and *Surirella ovalis* occurred rarely.

Key words: Diatoms, identification, species composition, Escape Bay, Taninthayi Coast, Myanmar.

Introduction

Marine phytoplankton is made up of small plants, mostly microscopic in size and unicellular. Phytoplankton can be separated based on cell size into micro-phytoplankton (200–20 µm), nano-phytoplankton (20–2 µm), and pico-phytoplankton (2–0.2 µm). Phytoplankton is commonly composed of both eukaryotic and prokaryotic species. It colonizes the upper part of the water column, down to the limit of penetration of light. The structure and abundance of the phytoplankton populations are mainly controlled by inorganic nutrients such as nitrogen, phosphorus, silica, and iron. Phytoplankton populations are controlled by grazing and viral mortality, as well as nutrient availability and other biological and physicochemical factors.

In the phytoplankton, Diatoms (Order: Bacillariophyceae) and Dinoflagellates (Order: Dinophyceae) commonly predominate. Diatoms (Bacillariophyta) are remarkedly distinguishable into two orders, the Centrales and the Pennales.

Diatoms (Bacillariophyta) are remarkedly distinguishable into two orders, the Centrales and the Pennales. The Centrales, or centric diatoms, have radial symmetry and are thriving as plankton in marine waters. Their frustules, or shells, can also be triangular or quadrate. The centric diatoms are mostly planktonic and non-motile. (as cited in Hunter)[1]. The Centrales are divided into three major groups based on cell shape and are the presence or absence of particular processes. Genera such as *Coscinodiscus, Cyclotella*, and *Melosira* are disc-shaped with no means, whereas the valve surfaces of families such as *Biddulphia* and *Chaetoceros* have various horns. The third group containing genera such as *Rhizosolenia* and *Corethron* also have a complex girdle structure (Dhargalkar and Ingole[2]). Escape Bay was developed with pearl oyster (*Pinctada maxima*) farms. The objective of the present study is to identify phytoplankton species in Escape Bay (pearl oyster farming area).

Materials and methods

Diatom samples were collected monthly from sampling station Escape Bay (Lat 12° 16' N and Long 98° 00' E), in the waters off Elphinstone Island, MyeikArchipelago, Taninthayi Region during June 2013 to February 2014. Phytoplankton net (60cm in length, 25cm in width (diameter) and 25 µm mesh size) was towed horizontally at every station. The collected samples were kept in clean small size plastic bottles and preserved in 2% formaldehyde immediately. Diatom samples were deposited in the Department of Marine Science, Myeik University. The specimens were identified up to species level with the following references; Newell and Newell[3], Allen and Cupp[4], Hendey[5], Yamaji[6], Tomas[7], Wood[8] and Al-Kandari, Al-Yamani and Al-Rifaie[9].

Results and Discussion

In the present study, a total of 53 species of diatom belonging under 32 genera under 16 families of 2 orders were recorded. The families of diatoms included *Thalassiosiraceae, Melosiraceae, Leptolindraceae, Coscinodiscaceae, Rhizosoleniaceae, Hemiaulaceae, Chaetocerotaceae, Lithodesmaceae, Eupodiscaceae, Fragilariaceae, Thalassionemataceae, Naviculaceae, Bacillariaceae, Sururellaceae*, and *Diatomaceae*, respectively.

During the study period, the systematic identification of diatom was made based on the references; Newell and Newell[3], Allen and Cupp[4], Hendey[5], Yamaji[6], Tomas[7], Wood[8] and Al-Kandari, Al-Yamani and Al-Rif[9], Thu Hein[10], Khin Yu Nwe[11] and Lett Wai Nwe[12].

During the whole study period, monthly diatom species composition was ranged from 23 to 36 (Table 1). The highest number 36 of diatom species was found in July and November. However, minimum species number 23 was found in October that was post-monsoon season. The species *Coscinodiscus occulus-irridis, Rhizosolenia imbricata, R. setigera, Bacteriastrum hyalium, Ditylum sol, Odontella sinensis, Thalassionema nitzschioides* and *Pleurosigma normanii* are commonly occurred every month. *Eucampia cornuta* was found only in June. Besides, *Chaetoceros pervianum, C. compressus* and *Surirella ovalis* were occurred only in July. The species mentioned above were rarely observed during study period.

In Myeik Archipelago, Si Thu Hein[10], Khin Yu Nwe[11] and Lett Wai Nwe[12] reported that diatoms are dominantly found in their study periods. Moreover, Zin Mar Aye[13] and Tin Tin Kyu[14] reported that diatoms were higher than dinoflagellates in Palaw Waters. Thida Nyunt[15] reported 99 species of diatoms from Mon Coastal Waters. Yin Yin Htay[16] identified 116 species of diatoms from Myeik Coastal Waters. Khin Khin Gyi 17 described 155 species of diatoms from Myeik Coastal Waters. In Khin Khin Gyi[17], the genera; *Coscinidiscus, Hemidiscus, Rhi-*

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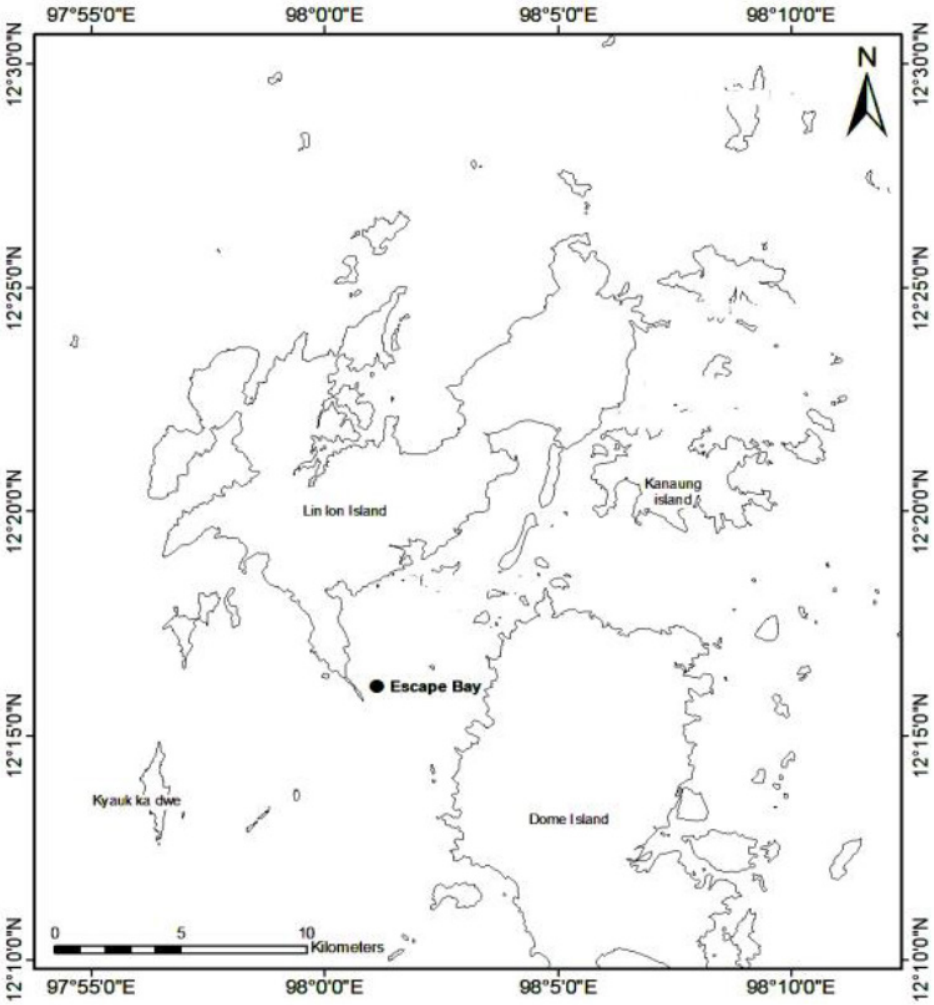

Figure 1. Map showing the study area.

Sr. No	Species Name	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan
1	*Cyclotella striata*	-	-	-	+	-	-	+	-
2	*Lauderia annulata*	+	-	+	+	+	+	+	+
3	*Skeletonema costatum*	-	-	-	-	+	-	+	-
4	*Planktoniella sol*	-	-	+	+	-	+	+	-
5	*Thalassiosira eccentriaca*	+	+	-	-	-	-	-	-
6	*Paralia sulcata*	-	-	-	+	-	+	-	-
7	*Corethron criophilum*	-	+	-	-	-	+	+	-
8	*Coscinodiscus centralis*	-	+	+	+	-	-	-	-
9	*C. occulus-irridis*	+	+	+	+	+	+	+	+
10	*C. granii*	+	+	+	+	+	+	+	-
11	*C. radiatus*	-	-	-	-	+	+	-	-
12	*Hemidiscus cuneiformis*	+	+	+	+	-	+	+	+
13	*Rhizosolenia imbricata*	+	+	+	+	+	+	+	+
14	*R. setigera*	+	+	+	+	+	+	+	+
15	*R. robusta*	+	+	+	+	-	+	+	+

Table 1. Species composition of diatom of Escape Bay during study period.

16	*R. calcar-avis*	+	+	-	-	-	+	+	+
17	*R. bergonii*	+	+	-	-	+	-	-	-
18	*Proboscia alata*	+	+	+	-	-	+	+	+
19	*Guinardia striata*	-	-	-	-	-	+	-	+
20	*G. flaccida*	+	-	+	+	-	+	-	+
21	*Eucampia zodiacus*	+	-	+	-	-	+	-	-
22	*E. cornuta*	+	-	-	-	-	-	-	-
23	*Cerataulina pelagica*	+	+	+	+	-	+	-	+
24	*Hemiaulus sinensis*	+	+	-	-	+	+	-	+
25	*Bacteriastrum hyalium*	+	+	+	+	+	+	+	+
26	*Chaetoceros decipiens*	+	+	+	-	+	-	+	+
27	*C. curvisetum*	+	+	+	-	+	-	+	+
28	*C. diversus*	+	+	+	+	-	+	+	-
29	*C. denticulatus*	+	+	-	-	-	+	-	-
30	*C. coastatus*	-	-	-	-	+	+	-	+
31	*C. pervianum*	-	+	-	-	-	-	-	-
32	*C. compressus*	-	+	-	-	-	-	-	-
33	*Bellerochea horologicalis*	+	-	-	-	+	-	-	-
34	*Ditylum sol*	+	+	+	+	+	+	+	+
35	*Helicotheca thamensis*	-	+	+	+	-	-	+	-
36	*Odontella sinensis*	+	+	+	+	+	+	+	+
37	*O. mobiliensis*	+	-	+	+	+	+	+	-
38	*O. aurita*	+	-	-	+	-	-	+	-
39	*Triceratium favus*	-	+	-	+	+	+	-	+
40	*Lamprisuss hadboltianum*	+	+	+	+	+	-	+	-
41	*Astrionellopsis glacialis*	-	+	-	-	-	-	+	-
42	*Thalassionema nitzschioides*	+	+	+	+	+	+	+	+
43	*T. frauenfeldii*	+	+	+	+	-	+	+	-
44	*Pleurosigma normanii*	+	+	+	+	+	+	+	+
45	*P. angulatum*	-	-	-	+	+	+	+	-
46	*P. elongatum*	-	-	-	-	-	+	+	+
47	*Amphiprora alata*	-	+	+	+	-	+	+	+
48	*Bacillaria paxillifera*	+	+	-	+	-	+	+	+
49	*Nitzschia longissigma*	+	+	+	+	+	+	+	+
50	*N. lorenzian*	-	-	-	+	-	+	+	-
51	*Pseudo-nitzschia seriata*	+	+	+	+	-	+	+	+
52	*Surirella ovalis*	-	+	-	-	-	-	-	-
53	*Tabellaria fenestratea*	-	+	-	+	-	-	-	-
	Total	33	36	28	33	23	36	34	26

Table 1. Species composition of diatom of Escape Bay during study period.

zosolenia, Proboscia, Guinardia, Eucampia, Ditylum, Odentella, Thalassionema, Nitzschia were found as dominantly. Her finding was similar to the present study. However, Zin Lin Khine and Htay Aung[18] described dinoflagellates occurred to be more abundant than diatoms in the waters off Ayeyarwaddy and Taninthayi coast.

Boonyapitwat[19] recorded that *Oscillatoria erythrae, Proboscia alata, Rhizosolenia calcar-avis*, and *Thalassionema frauenfeldii* were dominant species in Vietnamese. Zin Lin Khin and Htay Aung[20] also recorded that *Osciallatoria* was dominant species in lower part of Taninthayi Waters. Moreover, Boonyapitwat, *et al.*[21], reported *Oscillatoria erythrae* and *Proboscia alata* were the dominance species in north, west, and east of the Bay of Bengal. However, the genus *Oscillatoria*

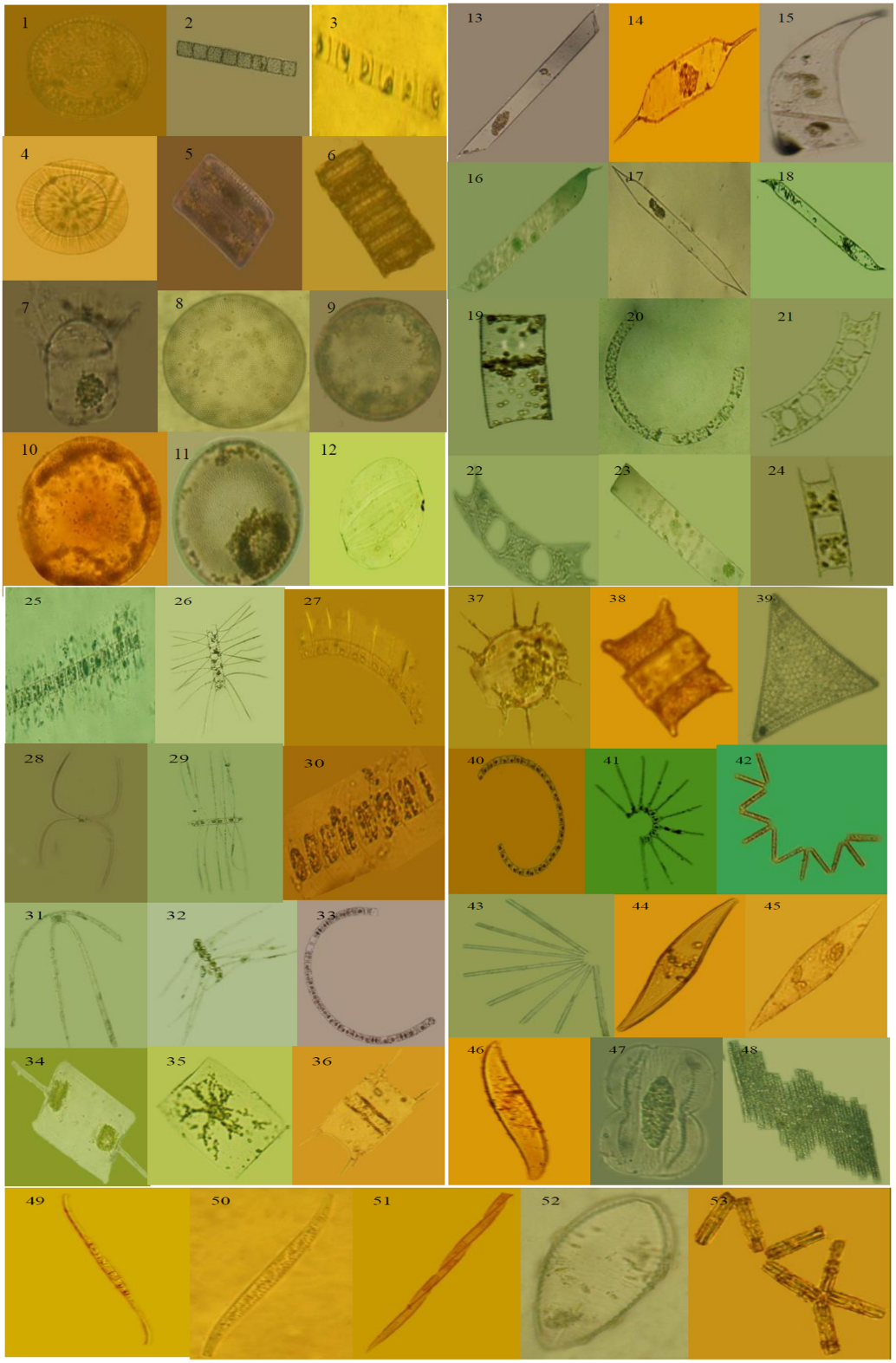

Figure 2. Photographs of phytoplankton species (1) *Cyclotella striata*; (2) *Lauderia annulata*; (3) *Skeletonema costatum*; (4) *Planktoniella sol*; (5) *Thalassiosira eccentrica*; (6) *Paralia sulcata*; (7) *Corethron criophilum*; (8) *Coscinodiscus occulus-irridis*;(9) *C. centralis*; (10) *C. granii*; (11) *C. radiatus*; (12) *Hemidiscus cuneiformis*; (13) *Rhizosolenia imbricata*; (14) *R. setigera*; (15) *R. robusta*; (16) *R. calcar-avis*; (17) *R. bergonii*; (18) *Proboscia alata*; (19) *Guinardia flaccida*; (20) *G. striata*; (21) *Eucampia zodiacus*; (22) *E. cornuta*; (23) *Cerataulina pelagica*; (24) *Hemiaulus sinensis*; (25) *Bacteriastrum hyalium*; (26) *Chaetoceros decipiens*; (27) *C. curvisetum*; (28) *C. diversus*; (29) *C. denticulatus*;(30) *C. coastatus*;(31) *C. pervianum*; (32) *C. compressus*; (33) *Bellerochea horologicalis*; (34) *Ditylum sol*; (35) *Helicotheca tamensis*; (36) *Odontella sinensis*; (37) *O. mobiliensis*; (38) *O. aurita*; (39) *Triceratium favus*; (40) *lampriscus shadboltianum*; (41) *Astrionellopsis glacialis*; (42) *Thalassionema nitzschioides*; (43) *T. frauenfeldii*; (44) *Pleurosigma nomanii*; (45) *P. angulatum*; (46) *P. elongatum*; (47) *Amphiprora alata*; (48) *Bacillaria paxillifera*; (49) *Nitzschia longissigma*; (50) *N. lorenzian*; (51) *Pseudo-nitzschia seriata*; (52) *Surirella ovalis* and (53) *Tabellaria fenestratea*.

(Trichodesmiun) was not found, but *T. frauenfeldii* and *P. alata* were found moderately in the present study. Besides, Zekaria and Soe Tint[22] recorded *Coscinodiscus, Rhizosolenia*, and *Chaetoceros* were located dominantly in the near Taungpyoe Village, Myeik. The genera *Coscinodiscus*, *Chaetoceros*, and *Odontella* found dominating the phytoplankton species from nearshore waters of Gwa were recorded by Kyaw Win and Nay Win[23]. Besides, Maung Maung Myint, Aung Myint and Saw Han Shein[24] found that *Coscinodiscus*, *Rhizosolania*, *Chaetoceros*, and *Odontella* were dominant genera around Gwa, Kyaukphyu, and Sittway. Likewise, the genera mentioned above were observed commonly in the present study. Figure 2

Conclusions

In the present study, diatoms were dominantly found during the current research. The maximum species composition of diatom was found in monsoon and post-monsoon season. The study area (Escape Bay) was productive during survey period. The present study was conducted at monsoon and post-monsoon season. So, pre-monsoon season was studied in the future. The results obtained were not significantly influenced by monthly. Therefore, the abundance of diatoms was right and to success pearl oysters' culture. It can be concluded that the study waters were highly productive areas.

Acknowledgements

I am very grateful to Dr. Aung Myat Kyaw Sein, Rector of Mawlamyine University, and Dr, Mie Mie Sein and Dr. San San Aye, Pro-Rectors of Mawlamyine University, for their encouragement and supports in preparing this work. I am thankful to Dr. San Tha Tun, Professor, and Head of the Department of Marine Science, Mawlamyine University, for his valuable suggestions and constructive criticisms on this study.

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Received: 31 August 2019
Accepted: 2 October 2019

RESEARCHS / INVESTIGACIÓN

The intensity of symbiotic relationships between arbuscular mycorrhizae and differentiated tree species regarding their age group and plant family in semi-arid Andine dynamical agroforestry system.

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DOI. 10.21931/RB/2019.04.04.6

Abstract: As research on mycorrhiza progress and scientific knowledge about organic partnerships becomes more profound, mycorrhiza symbiosis is considered an essential parameter for the vitality of ecosystems. Concerning polyculture cultivation systems, the implementation of growth-promoting and nutrient-securing symbiotic partners is a crucial step towards preserving the dynamism of involved plants and thus decisive for the yield and success of such cultivation systems. In particular, arbuscular mycorrhizal fungi (AMF) show considerable tendency in encouraging and maintaining a supply of water and nutrition for plants. Therefore, it was examined how intensive partnerships between AMF and trees in the semi-arid, dynamic agroforestry system of 'Mollesnejta' exist and how the species, family and age of trees are related to the respective degree of mycorrhizal intensity. This information is in turn used to decrypt relationships between nutrient provision and nutrient security in agroforestry systems and to improve them especially concerning current climate change. The results reveal that in the examined agroforestry system arbuscular mycorrhizal partnerships were found on all ten considered tree species in this study in varying intensity of the mycorrhizal structure dependent on tree species and their plant family. Nevertheless, no statistical correlation between the number of mycorrhizal elements according to primary hyphae, ramification or vesicles about the age of the trees could be proven in this study.

KeyWords: Age group, agroforestry, arbuscular mycorrhizae, semi-arid, subtropics.

Introduction

Role of Arbuscular Mycorrhiza in Ecosystems

Plants are autotrophic organisms capable of synthesizing all their components from water, carbon dioxide, and mineral elements with photosynthetic radiation. Studies of plant nutrition have shown that specific mineral elements are essential to ensure their growth and health[1]. To guarantee optimal nutrition sustenance, land plant evolution began to develop their nutritional mineral input at least 400 million years ago in symbiosis with mycorrhizal fungi, especially AMF of which approximately 150 - 200 species inside the family Glomeromycota exist[2]. The small number of AMF species might suggest their role in enriching biodiversity is limited, but the variations on their physiologic, morphologic, and genetic levels are rather high, resulting in a robust functional diversity that has a significant impact on ecology and application in plant production systems[3]. So far, the AMF association with terrestrial plants has been observed in 200 families of plants representing circa 1.000 genera and at least 300.000 species[4]. Therefore, mycorrhizal fungi, mainly AMF are ubiquitous in soil and create associations with most herbaceous angiosperms including many crops, cereals, vegetables, trees and horticultural plants[5,6]. The fine fungal hyphae with size of 2 – 12 µm radiating from the mycorrhiza increase the contact surface of the root with the soil[7]. After mycorrhizal infection roots by AMF's fine hyphae and the following extension of the plant's roots system facilitates the acquisition of water and mineral elements i.e. phosphorus that is relatively immobile in the soil and nutrients such as nitrogen, zinc, and copper. In return, plants provide carbohydrates[8] and lipids[9] to the arbuscular mycorrhizae. Likewise AMF stimulates the production of growth substances and may reduce stresses, diseases or pest attack[10]. AMF occurs over a global range of agro-climatic conditions in natural and agricultural ecosystems and is geographically ubiquitous[11]. Nevertheless, terms of the soil such as erosion, salinity, waterlogging, water holding capacity, soil types, soil porosity, fertility status, and vegetation, etc. appreciably influence AMF associations, composition, distribution, and activity[12]. Optimization and improvement of mycorrhizal symbiosis for different "agricultural applications can be considered as the attempt to extract the maximum plant benefit from colonization for the minimum loss of resources"[13].

Improvement of Agroforestry Systems by Arbuscular Mycorrhizae

The intensification of agriculture as an inescapable consequence of the constraint to produce foodstuffs compounded with fast and uncontrolled industrialization has put an enormous burden on the natural ecosystem[3]. Agricultural impacts on biological, physical and chemical attributes of soils and their ecosystems, leading to biodiversity losses, decreases in soil coverage, changes in natural element cycles and the overall water balance, degradation of soil structure, erosion and contamination of groundwater and therefore resulting in unknown consequences of high complexity[14,15]. Considering the current cultivation methods, there is an increasing interest in agroforestry systems. Agroforestry is defined by growing trees along with various types of crops to enhance crop yields, conserve soil and recycle nutrients while producing fodder, fruit, timber, and wood for non-corporate and economic use[16]. Under agroforestry, the needs for ecological sustainability can be reconciled with the needs and future challenges for sustainable food production[17]. To ensure plant nutrition in such cultivation systems, it is crucial to understand the interactions among the

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actors and to improve plant growth by enhancing measures.

Consequently, AMF has associated with improved and enhanced growth of many plant species due to production of growth-promoting substances and synergetic interactions with other beneficial and necessary microorganisms, thus affecting the whole ecosystem where AMF subsist[18]. In a study[19] about subtropical agroforestry systems and the influence of mycorrhiza, 93 of the 101 tree species evaluated inside the agroforestry system were colonized by AMF. The general soil conditions prevalent in sustainable agriculture equivalent dynamic agroforestry are likely to be more beneficial to AMF than those under conventional agricultural methods corresponding pesticide application, fertilization and tillage[5,20]. Under nutrient saturating conditions related to high-input agrarian systems, the considerable advantages are reduced while the carbohydrates costs for the plant remain, and the overall performance, including yield by AMF colonized plants can fall below that of those that are non-colonized[21]. Since the mycorrhizal association may shift from a symbiotic relationship to a parasitic and damaging association because the fungus still obtains carbohydrates from the infected plant, but the host plant no longer benefits from improved nutrient uptake efficiency[22,23]. AMF may provide a more appropriate and environmentally acceptable alternative for sustainable agriculture including agroforestry, due to the fact of increasing expenses of inorganic fertilizers by agrochemical industry as well as environmental and public hazards associated with pesticides and following the pathogens resistant to chemical pesticides[24]. Therefore, it was examined how intensive the potential partnerships between AMF and trees in a semi-arid, dynamic agroforestry system develop and how the tree species plus their plant-family and age of the trees are related to the degree of mycorrhizal intensity. This information is necessary to decrypt the relationships between nutrient provision and nutrient security in agroforestry systems and to improve them under adverse conditions, especially about current climate change and their resulting extreme conditions. Unfortunately, studies examining arbuscular mycorrhizal colonization not only with individual tree species but in a complete agroforestry system are scarce[25].

Materials and methods

Climate and Soil Conditions

The degree of mycorrhizal colonization was analyzed in ten different tree species on the 16 ha agroforestry area in Mollesnejta at 2750 to 2840 meters above sea level beginning of May 2019. The research institute is located near the Andes border in the center of Bolivia in the proximity of Cochabamba. The study was performed during dry period southern of equator, and due to the winter season, the daylight was decreasing. Because of its altitude above sea level, the subtropical area is in the USDA winter hardiness zone 10a and can, therefore, reach absolute minimum temperatures of -1.1 °C at night[26]. The average annual temperature is 16.5 °C, showing respectively a minimum average temperature of 8.7 °C and a maximum average temperature of 25.4 °C. The middle yearly precipitation is 518 mm, of which only 68 mm in sum are precipitated during the seven arid months of April, May, June, July, August, September, and October. The soil profile of the agroforestry landscape was very nutrient-poor, had a high sand content and contained a high amount of unweathered Andean rocks. The phosphorus content available to the plants and salt conductivity of the soil amounted on average of only 9.339 kgP_2O_5/ha as well as an electric salt conductivity of only 0.27 mS/cm at the start of the experiment. The PH value of 5.3 proved slightly acidic. There was generally a very thin or almost nonexistent layer of humus, as large parts of the terrain were destroyed 19 months before the start of the test due to a massive area fire. As a result, the upper humus layer was partially burnt, and therefore soil fertility was reduced. Nevertheless, the soil directly around the trunks of the trees had higher humus content and more excellent aggregate stability than the rest of the surrounding fallow land.

Biological Materials and their Preparation

To analyze the degree of mycorrhizal colonization, three trees per tree species of *Inga feuillei*, *Caesalpinia spinosa*, *Erythrina falcata*, *Acacia visco*, *Tecoma stans*, *Tecoma cochabambensis*, *Jacaranda mimosifolia*, *Fraxinus americanus*, *Zanthoxylum coco* and *Schinus molle* in the age classes 5, 10 and 15-20 years were examined. An exception was made for Acacia visco, as only three 10-year-old trees were available during the time of this experiment. Before sampling, the trees were surveyed for external conspicuities such as the influence of biotic and abiotic damage. All trees were externally vital at the time of sampling and showed no abnormalities except of exfoliation due to the current dry winter season. Three excavations were carried out at angles of 120° a depth of 30 cm. Several samples were taken per excavation consisting in sum of at least four light-colored fine-root strands, with a minimum length of 2 cm each. Furthermore, focus was to examine trees with the same soil samples in order to maintain comparability of the results and limit additional interrelations. Subsequently, the mycorrhiza was stained according to the method of Ungar *et al.*[27], which represents a slightly modified form of the established methodology of Vierheilig[28]. At first, the root cells were discolored by destroying their cytoplasm in a process of heating them for 10 minutes in a solution of 10 % potassium hydroxide (KOH), then hyphae were colored with black ink (Brand: Pelikan) by heating in a 5 % vinegar solution in ratio of 1:50 for 3 minutes. The samples were then stored in a 50 % water-alcohol solution. From each pack of excavated root sample, two different fine-root strands were chosen randomly and observed under the microscope. Collectively, 180 root samples were evaluated, including 18 samples per tree species and six samples per tree and for its respective age. The absolute number of all stained mycorrhiza elements such as *main hyphae*, *ramifications/branches* and *vesicles* were counted under microscope under tenfold magnification, insofar as these could be identified after the mentioned staining methods. After that, the total number of each mycorrhizal element was counted on the planar upper root area. Due to strong varying visibility of arbuscles, dependent upon tree, AMF species, and other factors, the arbuscles were not observed in order to allow a direct comparison with other tree species and their age groups. The absolute area of each examined root sample was determined photometrically using a 12-megapixel color camera at a 90° angle to the root samples and ImageJ software Version 1.52a (Publisher: Wayne Rasband, National Institutes of Health, USA). For this purpose, the percentage background of the root sample) was calculated by binary reduction (black, white) of the image and its relative proportion was subtracted from the total area of the object slide () to obtain the entire roof area in square millimeters and to calculate the number of mycorrhizal elements per one square millimeter root area (The Mathematical calculation for the degree of mycorrhizal colonization is noted below:

$$MD\left[\frac{x}{mm^2}\right] = \frac{n_E[x]}{(1 - B|N[\%]) \cdot A_{tot}[mm^2]}$$

MD: Degree of mycorrhizal colonization [Quantity / mm²]
n_E: Quantity of mycorrhizal element [x]
B_N: Relative amount of negative binary background [%]
A_{tot}: Total area of object slide [≙ 324 mm²]

Statistical Analysis

The non-parametric Kruskal-Wallis test (α = 0.05) was performed, to prove potential differences between the age groups and the proportion of hyphae elements. Since the dependent variable "age" was not normally distributed, the lowest distribution was ordinarily scaled, and the samples were independent of each other. Due to the sample size of 180 > 30 (critical value), the asymptotic significance value was taken into consideration. Based on the small sample size for trees 15 and 20 years old, these were grouped as category "15-20". The relationship between the relative proportion of

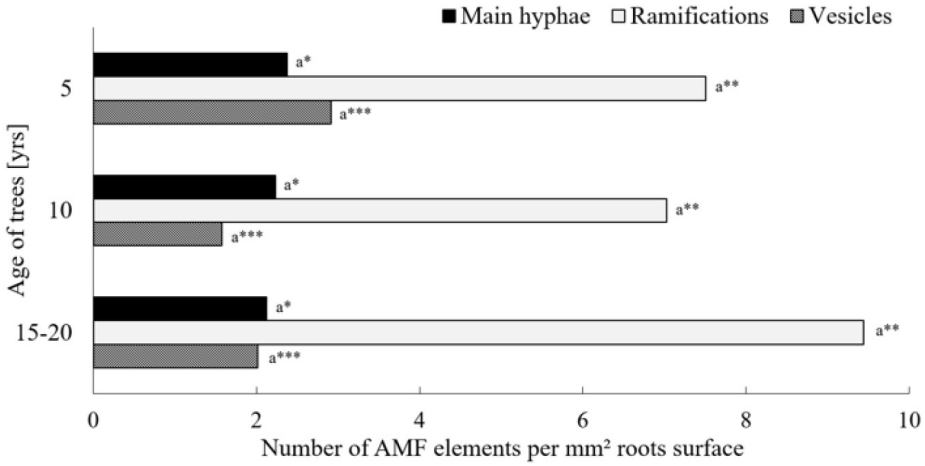

Figure 1. Degree of mycorrhizal growth of all investigated tree species depending on their age examined in Andean agroforestry Mollesnejta during arid dry-season. Toe relevant AMF elements were main hyphae, ramifications, and vesicles. Statistical test with Kruskal-Wallis l\•1ethod and α= 0.05. Due to p= 0,488 > 0,05*, p = 0,388> 0,05** and p= 0,417 > 0,05*** a coherence between tree age and number of each mycorrhizal element could not be couriered.

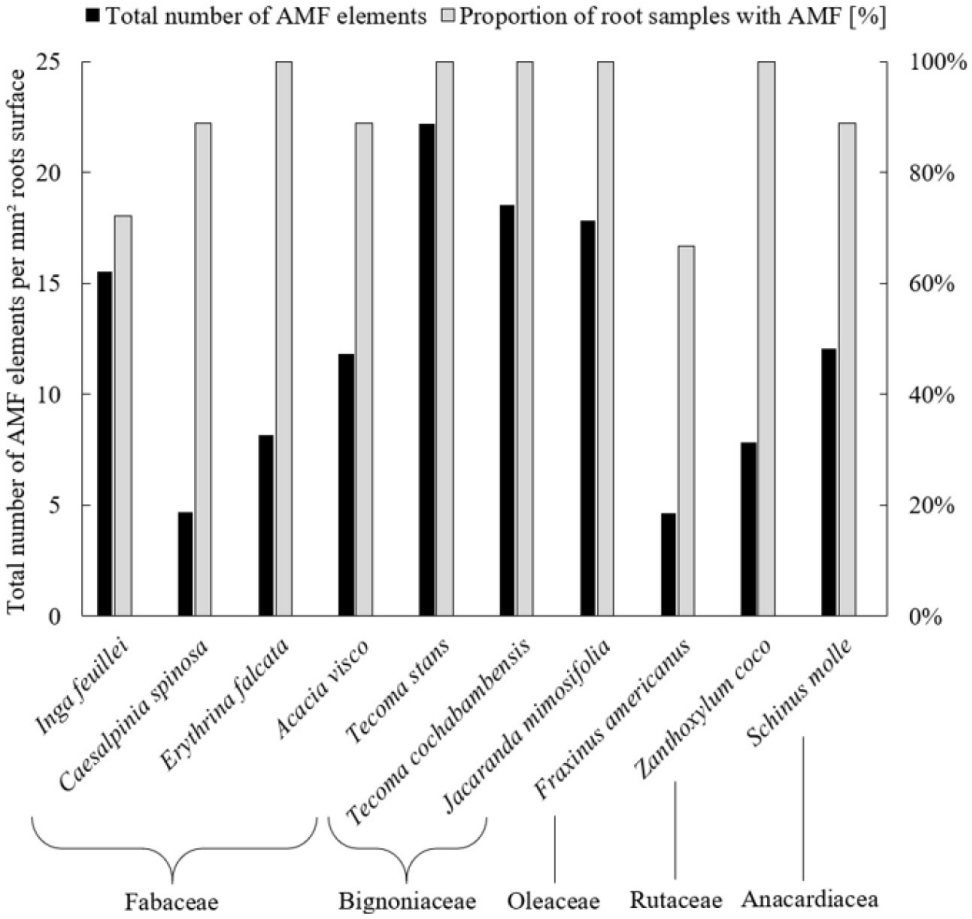

Figure 2. Comparison of absolute arbuscular mycorrhizalization and the relative proportion of trees with arbuscular mycorrhizal fungi depending on tree species and their plant families investigated in the Andean agroforestry Mollesnejta during arid dry season. Elements of AMF were the sum of primary hyphae, branches, and vesicles. The relationship between the total number of mycorrhiza elements and the relative proportion of trees infected with mycorrhiza was tested with Pearson Chi-square and.=0.05. Based on p = 0,314 > 0,05, the relation between the number of counted AMF elements and the relative proportion of A1MF infected trees could be excluded.

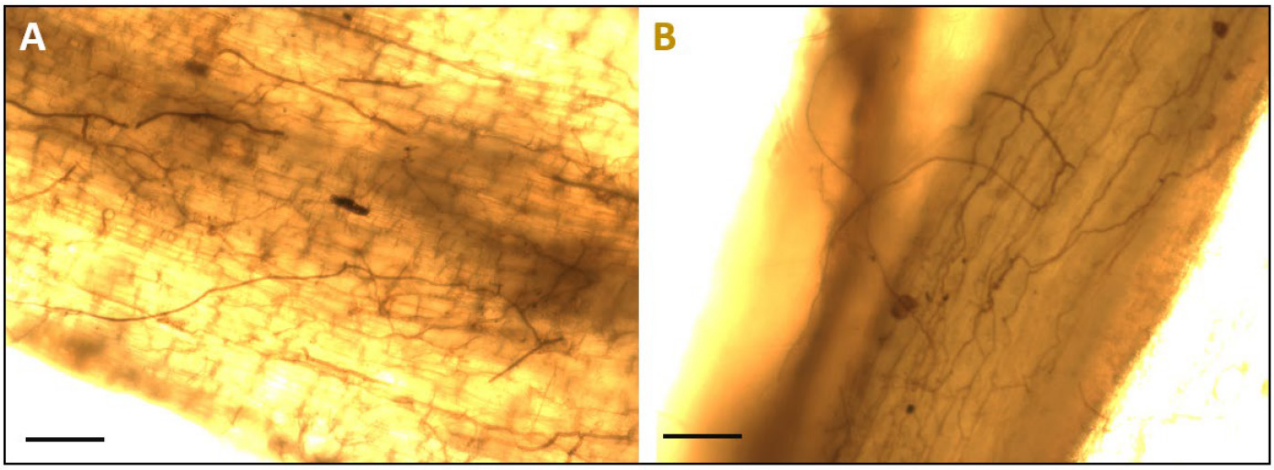

Figure 3. Hyphal structure of arbuscular mycorrhizae on the fine root strands of (A) *Tecoma stans* (15 yrs. old) and (B) *Schinus molle* (10 yrs. old). Black bar equals 60 tm.

trees infected with mycorrhiza compared to the intensity of mycorrhizae regarding the tree species was analyzed by using the Pearson Chi-square test ($\alpha = 0.05$) due to nominal scaled parameter of the lowest test variable. All statistical analyses were performed using the open-source software PSPP Version 3.0 (Publisher: Benn Pfaff) under GNU General Public License.

Results and Discussion

The results showed that AMF is omnipresent in the root structures of the studied agroforestry system and are in symbiosis with all observed tree species regardless of age (Figure 1 and 2). The findings confirm other investigations[29,30] that mycorrhiza plays a fundamental role in balancing agrosystems and represent an essential link between soil and root. The positive effects on nutrient supply and water transfer as well as the general significance of AMF for ecosystems as already described in various literature[31- 35] confirm the high occurrence of arbuscular mycorrhizae in this experiment (Figure 1 and 2) respective to the overall vital health of the trees. The thesis that acidic to neutral soils containing a large number of AM fungi[3] could be confirmed by the average PH value of 5.3 on the areal and the widespread colonization by AMF (Figure 2). Because of the plural anastomosing mycorrhiza net[37], plants of different species can exchange substances and communicate among themselves with organic carbon compounds[38,39,40], which can be anticipated based on the uniformly mycorrhization of all examined trees, impartial with respect to their species, plant family (Figure 2) or age (Figure 1).

However, no statistical correlation between the intensity of mycorrhizal growth and age could be established by this experiment (Figure 1). Due to strong scattering of the determined values regarding primary hyphae, ramifications and vesicles, the Kruskal-Wallis test showed no correlation between the two parameters. Only the branching seems to have cumulated slightly, which could be explained by the colonization of further species of mycorrhiza with increasing age of the trees[41]. The difference of the ramifications between the 10 and 15 to 20-year-old trees was determined by a separate Kruskal-Wallis test, but also here the results of $p=0.203$ over $\alpha=0.05$, and no difference between the two test factors could be confirmed. Indeed there were differences in the absolute number of mycorrhizal elements in the studied tree species (Figure 2).

In such cases, differences could be observed regarding the tree species and their plant families. On all plant species of the Bignoniaceae family, 100% of the samples were colonized by arbuscular mycorrhizae, which corresponds to other scientific results[19,42,43]. In comparison to the other examined tree species, Bignoniaceae proved to contain the highest intensity of mycorrhizae elements (Figure 2). On the other hand, the Fabaceae plant family showed significantly lower colonization of AMF in the root cells, while retaining a high proportion of positive colonized samples. This difference between the plant families could be due to the ability of the plant to regulate the overall colonization level, based on altering plant-available nutrition value in the soil[44,45] or to guarantee an economic output of it's own assimilates[46].

Nevertheless, such assumptions must be confirmed by further investigation upon said tree samples. Predominant and extremely aversive abiotic stressors make the growth and survival of plants more difficult[47], especially as the general low nutrient conditions with an electrical salt conductivity of only 0.27, the seven arid months and the resulting drought stress, as well as the high temperatures during the day in the sun in this case. Since all plants had a vital state of health, the colonization of tree roots by AMF with its water and nutrient-enriching attributes must be considered as a defense against abiotic stressors[5,48,49,50,51].

Phosphate is an essential nutrient and limits plant growth[52]. Because of the low P_2O_5 content of 9.339 kg/ha other relevant vectors must introduce phosphorus into the soil or make the current organic phosphorus available to the plants, otherwise sufficient growth of the trees would not be possible. Mycorrhizal fungi have been shown to mineralize organic soil phosphate through the synthesis of phosphatase[53,54]. Similar to phosphate, nitrogen is a major limiting nutrient to plant growth[13], and most of the nitrogen in the soil is only available as ammonium (NH_{4+})or nitrate (NO_{3-}). Ammonium is the preferential form of nitrogen absorbed by subjected to a nitrogen deficiency[55,56], but its concentration is 10 – 1000 times lower than of nitrate, especially in acidic soils like in this experiment[23]. Ammonium has minimal mobility in the soil and similar to phosphate, a zone of depletion is formed around the roots. AMF extraradical mycelium can absorb ammonium[57,58,59], nitrate[58] and amino acids[60]. It can, therefore, be assumed that the colonization of all tree species by AMF (Figure 2), regardless of the age of the trees (Figure 1), is associated with the compulsion for the plant to obtain nutrients from the soil and thus to enter into a symbiosis with the fungi.

Conclusions

The intensity of mycorrhizal variation across all investigated parameters did not differ between the three age groups. The results indicate that in nutrient-limited, semi-arid, subtropical, dynamic agroforestry systems arbuscular mycorrhizae are present in symbiosis with all ten examined tree species in the varying intensity of fungal hyphae structure. Furthermore, it would be interesting to decipher the species of the current AMF by protein sequencing and to investigate which species are beneficial for agroforestry systems concerning growth, age of trees and climatic seasons.

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Received: 11 October 2019
Accepted: 1 November 2019

RESEARCHS / INVESTIGACIÓN

Compositional analysis of malanga (Xanthosoma sagittifolium), chinese potato (Colocasia esculenta) and potato (Solanum tuberosum) for the utilization in the snack's elaboration by conventional fried.

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DOI. 10.21931/RB/2019.04.04.7

Abstract: The objective of the present study was to compare the compositional analyze of three types of tubers, like traditional potatoes (Solanum tuberosum) and two of them that come from untraditional like Malanga (Xanthosoma saggitifolium) and papa china (Colocasia esculenta), crops that in Ecuador aren't used for the snacks making. The evaluated components in the primordial matter and finished material were: protein content, grease, ashes, humidity, fiber, and carbohydrates, all of them were evaluated by official methods of analyze. The experiment was realized three times for each prove. It was used a program SPSS version 23, applying a variance followed by a Tukey test ($p<0,05$) with the objective of determinate some meaning statistics for deviation of tubers ways of Malanga that has a significant content of protein and carbohydrates and energetic adds were higher in comparison of the traditional snacks, these results are an alternative for the consumer and the development of new products for the food industries.

Key words: Malanga, chemise potato, potato, compositional analyze, snacks.

Introduction

The actual tendencies in the agricultural are oriented towards the search crop species that contribute at low cost to the food supply, protection of the natural resources, fairness, and diminution of the poverty. The crop species with reserves roots and stems fulfills in its majority with these requirements. Within the group of reserves stems foods of agricultural importance are the genus *Xanthosomay* Colocasia of the Araceas family[1], The significance of the Araceas foods has been recognized by FAO[2], organization that has published several documents on the importance of some tubercles and their contribution to the food safety of the developing countries.

Exists brings back to consciousness generalized of which the crop by roots and tubercles contribute energy components in high amount and that the little protein that produces is of smaller quality to the one of origin animal. However, are an important energy source in the form of starch and represent, at least, 40% of the weight of the diet[3].

Within the Araceas foods the ocumo criollo, blanco o malanga (*Xanthosoma sagittifollium* L. Schott), is a plant worked perennial grass in many tropical and subtropical countries since their tubercles are an easily digestible starch source; also, they contain proteins and vitamins like niacin, thiamin, riboflavin, and vitamin[4]. For the high nutritional value of its cormos or cormelos it can be substitute for potato[5].

Colocasia esculenta, well-known in the nutritional world like Chinese potatoe, important source of vitamins and minerals is considered tubercle since it owns thiamin, riboflavin, iron, phosphorus, vitamins B6 and C, niacin, potassium, receives, manganese, stop dietetic fiber degree and starch. Also it is a proven useful food by its humid product protein content from 1.7 to 2.5%[6].

Potato (*Solanum tuberosum*, *sp. Tuberosum*) is a crop that has gained a space in the use of its tubercles like raw material in the food industry[7]. Although the potatoes have relatively few nutrients, they contain many carbohydrates, thus are an excellent energy source. Potatoe has the protein content more elevated (around 2.1% of the weight of the product in fresh) of the family of cultures by roots and tubercles, and protein of good quality, with amino acids adapted to the human needs. Also they have high vitamin C content: one medium-size potato contains almost half of the recommended daily ingestion[8]. At present, the sector of fried has undergone significant growth, especially the consumption of snacks, chips, maize tortillas, other product derivatives of vegetal origin, and the denominated foods fast meals[9,10,11].

The frying is one of the methods of more widespread and thermal essential food processing anywhere in the world[12]. It can be defined as a particular type of baking by immersion in oil or fat food to a temperature superior to the boiling point of the water[13].

In developed countries, the tendency to the rise of the consumption of snacks is turned out from the recommendation to make it decrease the caloric ingestion in the three main meals, habit that also allows controlling the appetite[14].

The limited bibliographical information about the compositional nontraditional tubercle parameters has originated a lack of advantage in the agro-industrial product elaboration.

The objective of the present investigation was to realize a compositional analysis of the raw material and the product terminated of three types of tubercles, one traditional like the potatoes and two nontraditional ones like malanga and Chinese potatoes for the elaboration of snacks.

Materials and methods

The present research was carried out in the quality and process control laboratories of the Agroindustrial Engineering career, the National University of Chimborazo (Riobamba-Ecuador).

To obtain the snacks, malanga and Chinese potato, previously obtained from the city of Santo Domingo, were used as

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raw material, taking into consideration the potato as a control sample, which was obtained from the wholesale market in the city of Riobamba. Compositional analysis was made in triplicate of the raw materials and snacks; the weight used was 100 g of edible portion.

For the process of making the snacks, raw materials were selected, washed and peeled manually, then cut into thin slices with a thickness of 1 to 2 mm. The slices were fried at 175°C for 2 minutes and finally drained with the help of an absorbent paper for the separation of the oil from the flakes.

The compositional parameters were evaluated by means of AOAC methods, humidity was used the gravimetric method by mass difference of the compound (AOAC 925.10-1990), ash was determined by dry incineration, (AOAC 923.03-2012), crude protein (conversion factor of 6.25) was performed by Kjeldahl (AOAC 2001.11-2002), fat (ethereal extract) using Soxhlet (AOAC 920.39-2005), crude fiber acid-base method, (AOAC 962.09-2005) and carbohydrates (ELN) obtained by difference between the other components C = 100- (Protein + Fat + Ash + Fiber + Moisture), all the ingredients expressed in percentages.

The statistical analyses were performed using the SPSS program version 23. The results obtained were evaluated using an analysis of variance (ANOVA) and the Tukey multiple comparison tests, to observe if there are significant differences in the means reported in each compositional parameter, was considered a confidence level (p<0,05).

Results and Discussion

Table 1 shows the compositional analysis of the three types of tubers, in which the amounts of moisture (M%), protein (PC%), crude fiber (CF%), fat (F%), ether extract (EE%), ashes (A%) and carbohydrates.

The tubers of malanga, Chinese potato, and potato presented significant differences in the moisture parameter as shown in table 1; these results are similar to those reported by Bradbury *et al.*[15], which found values in malanga 67.1% and Chinese potato 69.1%.

The content of ash and fiber of malanga and Chinese potato did not present significant differences concerning the potato, Muñoz *et al.*[16], reported content of 1.94% (ash), and 0.07% (fat) for Chinese potato.

On the other hand, the fat content of malanga and potato present significant differences in comparison with the Chinese potato. These results of ash and fat content differ from those obtained in this investigation. Collazos *et al.*[17], performed a chemical analysis of the raw material (pituca corms), finding a: 73.7% (moisture), 1% (ash), 0.5% (fat), 0.8% (fiber) and 23.2% % of total carbohydrates.

The protein content of the Chinese potato and potato have lower values for malanga. These results contrast with the costs for malanga of 6.60% and chinese potato 3.80% reported by Devendra[18].

For the carbohydrate content, it was observed that the Chinese potato and potato do not present significant differences concerning malanga. These results differ with the value of 19.31% present in other varieties of potatoes reported by Prada[19].

On the other hand, the value found in the Chinese potato is in the range of the values reported by Pajar[20] with an amount of 22.10% of carbohydrates. Devendra[18] observed 25.02% in the malanga tuber.

The values obtained in the compositional analysis of the Chinese potato, malanga, and potato in fresh state present significant differences in some parameters, the results can be affected by several factors. Barrera *et al*[21], mentions that the proximal composition of the tubers varies from place to place depending on the climate, geographic regions, cultivation variety, soils, among others.

Table 2 shows the parameters of moisture (M%), protein (PC%), crude fiber (CF%), ether extract (EE%), ash (A%), and carbohydrates present in snacks.

Lucas *et al.*[22] determined an excess of moisture in potato chips of 4.77% at a temperature of 190°C of 2.5-3.5 min. These results are related to the values obtained from the Chinese potato and potato snacks.

On the other hand, lower moisture content was observed in the malanga snack, showing significant differences for the Chinese potato and potato. Among the meals of malanga and Chinese potato do not present significant differences with respect to the fat content, but if there is a difference with the potato snacks, this corresponds to the values reported by the Profeco Laboratory in 2008[23], in which profits were found

Tubérculos											
Determinations (g)	**Malanga**				**Chinese potato**				**Potato**			
Moisture	66.060	±	0.009	a	69.987	±	0.005	b	72.644	±	0.015	c
Ashes	2.030	±	0.000	a	2.371	±	0.005	a	1.570	±	0.317	b
Protein	7.121	±	0.001	a	4.699	±	0.231	b	3.807	±	0.250	b
Fat	0.230	±	0.000	a	0.380	±	0.000	b	0.280	±	0.000	a
Fiber	2.982	±	0.002	a	2.511	±	0.000	a	1.870	±	0.000	b
Carbohydrates	24.557	±	0.377	a	22.622	±	0.448	b	21.696	±	0.300	b

Table 1. Proximal composition (base in 100g) of malanga, chinese potato and potato tubers. Results expressed as means ± standard deviation. Means in the same row with different superscripts represent the groups for which their values differ statistically (p <0,05).

Tubérculos											
Determinations (g)	**Malanga**				**Chinese potato**				**Potato**			
Moisture	1.570	±	0.016	a	3.561	±	0.203	b	4.464	±	0.609	b
Ash	1.244	±	0.029	a	3.158	±	0.074	b	4.001	±	0.646	b
Protein	6.610	±	0.141	a	4.002	±	0.041	b	2.997	±	0.024	b
Fat	30.420	±	0.199	a	31.132	±	0.113	a	33.450	±	0.323	b
Fiber	2.611	±	0.184	a	2.394	±	0.070	a	1.060	±	0.090	b
Carbohydrates	60.154	±	0.152	a	58.144	±	0.213	b	55.086	±	0.015	c

Table 2. Results of the proximal analysis (base in 100g) of the malanga, Chinese potato, and potato snacks. Results expressed as means ± standard deviation. Means in the same row with different superscripts represent the groups for which their values differ statistically (p <0.05).

means of 30.4 to 38.9g / 100g of fat in some commercial brands of chips potato consumed in our environment (Pringles, Layds, and Ruffles).

The content of fiber in the products of taro and Chinese potatoes did not show significant differences in comparison to the potato, these values are not very representative, since according to the Argentine Food Code "Código Alimentario Argentino" (CAA) a food can be declared as a source of fiber if it contains at least 3g / 100 g, and it is declared high in texture when it presents a minimum contribution of 6g / 100 g[24].

Bravo *et al.*[25], observed 0.62% crude fiber and 23.54% fat in Chinese potato chips that were made at 180 °C for 3 min with 1 mm thickness, while Carbonell *et al.*[26], reported in their study that snacks of chips, present 3.8% protein, 34% fat and 51% carbohydrates.

According to INCAP[27], simple papillin snacks contain 66.90% carbohydrates.

On the other hand, Argudo[28], presented a 71.98% carbohydrate for fried malanga and Bravo *et al.*[25], obtained 62.91% of carbs for Chinese potato chips. The results found in the three types of snacks presented significant differences, being the snack of malanga, the one that showed higher values.

Conclusions

It is concluded that the content of nutrients in the tuber of the taro has higher values in parameters such as protein, carbohydrates, and fiber, on the other hand, the snacks of taro and potato have higher content in proteins and carbohydrates compared to the traditional meal. This research provides relevant information for the development of new products in the food industry, in addition to presenting an alternative for the consumers' daily diet.

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Received: 10 October 2019
Accepted: 1 November 2019

RESEARCHS / INVESTIGACIÓN

Study the effect of herbal mixture plants extract on blood sugar level in normal and experimentally diabetic mice.

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DOI. 10.21931/RB/2019.04.04.8

Abstract: The use of medicinal plants for the management of diabetes mellitus is an old practice that has become even more relevant in a modern perspective. The present work was designed to evaluate the effect of a mixture of three medicinal plants which used in folk medicine in Iraq, These plants are (*Aloe vera*, *Artemisia herba alba*, and *Teucrium polium*) on the levels of blood glucose in normal and alloxan-induced diabetic mice, The aqueous extract of the herbal mixture was prepared and chemical detection of phenols, flavonoids, tannins, terpenes, steroids, glycosides, and saponins was carried out. Results revealed that the aqueous extract contains phenols, flavonoids, tannins, terpenes, glycosides, and saponins compounds. Evaluations of the parameters of our study were carried out on both standard and alloxan-induced diabetic mice. Thirty male mice were randomly divided into six equal groups: Group I (control): was kept as control negative mice treated with only distilled water. Group II: normal mice treated with aqueous extract of an herbal mixture at a dose (500 mg/kg/day).Group III: normal mice treated with aqueous extract of an herbal blend at a dose (250 mg/kg/day). The other 3 groups were subcutaneously administered a single dose (100 mg/kg) of alloxan to induce experimental diabetes. Groups IV (Diabetic): was kept as control positive, alloxan-induced diabetic mice treated with only distilled water. Groups V: alloxan-induced diabetic mice treated with aqueous extract of an herbal mixture at a dose (500 mg/kg/day). Group VI: alloxan-induced diabetic mice treated with aqueous extract of an herbal blend at a dose (250 mg/kg/day), respectively, for ten days. Results showed that normal mice treated with aqueous extract has no significant change in body weights and blood glucose level except those treated with a high dose of aqueous extract since they exhibited a significant decrease ($P \leq 0.05$) in blood glucose level. The results indicated a significant reduction in glucose level in diabetic mice after treatment with a high dose of aqueous extract of the herbal mixture. In conclusion, our results support that the aqueous extract of these plant exhibits anti-diabetic as compared with each plant alone, where we tested each of these plants in previous studies.

KeyWords: Herbal Mixture, Chemical Composition, Hypoglycemic Activity, Alloxan Induced Diabetic Mice.

Introduction

Diabetes mellitus, or only diabetes, is a group of diseases characterized by high blood glucose levels that result from defects in the body's ability to produce and/or use insulin. It is a condition primarily defined by the level of hyperglycemia, giving rise to risk of microvascular damage (retinopathy and neuropathy[1]. It is associated with reduced life expectancy, significant morbidity due to specific diabetes-related microvascular complications, increased risk of macrovascular complications (ischaemic heart disease, stroke, and peripheral vascular disease), and diminished quality of life[2]. Several pathogenetic processes are involved in the development of diabetes. These include operations, which destroy the beta cells of the pancreas with consequent insulin deficiency, and others that result in resistance to insulin action. The abnormalities of carbohydrate, fat and protein metabolism are due to the deficient effect of insulin on target tissues resulting from insensitivity or lack of insulin[3].

Herbal medicine is a growing area of health care that demands attention. Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years, and have served humans as valuable components of medicines[4]. World Health Organization (WHO) estimated that around 80% of the earth's inhabitants rely on traditional medicine for their primary health care needs, and most of this therapy involves the use of plant extracts or their active components[5]. This is reasoned by the fact that medicinal plants have advantages (low cost and fewer side effects) over the conventionally used drugs, which are expensive and known to have harmful side effects. Diabetes mellitus is a metabolic disorder featured by hyperglycemia and alteration in carbohydrate, fat, and protein metabolism associated with an absolute or relative deficiency of insulin secretion and/or insulin action. Although numerous oral hypoglycemic drugs exist alongside taking insulin, still there is no promising therapy to cure diabetes[6]. Diabetes mellitus is now taking place as a serious health care problem in the 21st century. The number of people who have diabetes is expecting to increase from 150 million actually to 220 million in 2010 and 300 million in 2030. This explosive increase has already imposed a significant burden on health-care systems, and this will continue to increase in the future[7]. Over the last few decades, the reputation of herbal remedies has increased globally due to its therapeutic efficacy and safety. In recent years, numerous traditional medicinal plants were tested for their antidiabetic potential in the experimental animals. There are more than 1200 plant species worldwide that are used in the treatment of diabetes mellitus, and a substantial number of plants have shown productive hypoglycemic activity after laboratory tests. The medicinal plants provide a useful source of oral hypoglycemic compounds for the development of new pharmaceutical leads as well as a dietary supplement to existing therapies. Of these traditional hypoglycemic herbs are Teucrium polium, Aloe Vera, and Artemisia herba Alba. The aqueous extract of the dried aerial parts of these herbal

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medicinal plants is used traditionally to treat diabetes in Southern Iran and some Middle East populations[8].

T. polium is a wild-growing flowering plant belonging to the family Labiatae and is found abundantly in southwestern Asia, Europe, and North Africa[9]. It is most common in Mediterranean climates and the Middle East. In Iraq, the plant is found all over the country, especially in the northern regions[10]. Polium is herbs, shrubs, or subshrubs. An unusual feature of this genus compared with other members of Labiatae is that the flowers lack the upper lip of the corolla entirely. Its flowers are small and range from pink to white. This plant is a dwarf, pubescent, aromatic shrub possessing oval leaves with enrolled margins and dense head of white flowers[11].

Popularly known in Iraq as "Sheh," is a well-known medicinal plant that has been used in the Middle East traditional medicine for treating various diseases. It is used by the local population of some countries as an anti-diabetic[12]. Herbal infusions from this species have been used as an analgesic, antibacterial, and hemostatic agents. It is used in Jordon in the form of a decoction against fever, menstrual, and nervous problems. The essential oil of this herb was found to be responsible for its therapeutic use as a disinfectant, anthelmintic and antispasmodic[13].

Aloe vera, commonly known as Barbados or Curaçao Aloe, is an herbal medicine with a long tradition of use by a variety of cultures. *Aloe vera* (syn.) has been used in traditional and folk medicines for thousands of years to treat and cure a variety of diseases[14]. Although the plant is native to the northern parts of Africa, it has rapidly spread across the world because its cultivation is easy. An important distinction has to be made between the strongly laxative and purgative latex derived from the bundle-sheath cells and the clear mucilaginous gel. The plant has been used by Egyptians, Assyrians, and Mediterranean civilizations, as well as in Biblical times. A variety of aloe species are still used in folk medicines of Africa and Asia. Hunters in the Congo reportedly rub their bodies in the clear mucilaginous gel to reduce perspiration; some African tribes apply the gel for chronic conjunctivitis; the gel is used in India for the treatment of asthma[15]. The present study was designed to evaluate the effects of aqueous extracts of an herbal mixture of three medicinal plants (*Aloe vera*, *Artemisia herba alba*, and *Teucrium polium*) on alloxan-induced diabetic as a hypoglycemic drug for treatment of diabetes mellitus using normal and experimental alloxan-induced diabetic mice as animal model for study.

Materials and methods

Plant Material

This research was conducted from January 2019 until April 2019. The plants (*Aloe vera*, *Artemisia herba alba* (*sheh*) and *Teucrium polium* (*Algeada*) were purchased from the local market in Iraq and identified in January 2019 at the College of Agriculture and Baghdad University - Iraq. The plants were left at room temperature (20-25ºC) until use.

Preparation of extract

Equal weights of each plant (*Aloe vera*, *Artemisia herba alba* (*sheh*) and *Teucrium polium* (*Algeada*) were ground and mixed. The aqueous extract of this mixture was prepared by boiling 2 g of the mixture with 200 ml of tap water for 15 minutes, left to cool at room temperature, then filtered. The resultant extract was stored refrigerated in a glass container. The mixture extract was freshly prepared each two days.

Laboratory Animals

Animal maintenance was performed at the Animal Hospital of Biotechnology Research Center at Al-Nahrin University. A total of 30 albino male mice (*Mus musculs*) were employed for experimenting with the study. They were supplied from (biotechnology research center / Al-Nahrin University), and their age at the start of operations was 2-4 months, and their weight was 25-35 grams. Mice were kept for one week for acclimatization before being used in the experiments. They were divided into groups, and each group was housed in separate transparent plastic cages with stainless steel cover lids. The animals were maintained at a temperature of 20-25°C, and they had free excess to food (standard pellets) and water throughout the experimental work.

Chemical Detection of Plant Extracts

Phenols: Equal quantity of aqueous ferric chloride (1%) was mixed with potassium iron cyanide (1%). The equal amount of the reagent and water or alcoholic plant extract was mixed. The appearance of blue-green color indicates a positive result[16].

Flavonoids: The detecting solution was prepared by mixing 10 ml of ethanol (50%) with 10 ml of potassium hydroxide (50%), and then 5 ml of this solution was added to 5 ml of the plant extract. The appearance of yellow color was an indicator of the presence of flavonoids[17].

Tannins: The procedure of Harbone (1984)[17] was used for the detection of tannins. In this procedure, 50 ml of each extract was equally divided into two conical flasks. For the first one, lead acetate solution (CH_3COOPb) (1%; w/v) was added and the appearance of jelly pellet was considered a positive reaction, while for the second flask, ferric chloride solution (FeCl2) (1%; w/v) was added and the appearance of blue color was an indicator for the presence of tannins.

Glycosides: This method was done according to the method described by Harbone (1984)[17] .

Non hydrolyzed extract: An equal amount of the plant extract was mixed with Fehling reagent in the test tube and then boiled in the water bath for about 10 minutes. The development of red precipitate indicates a positive result.

Hydrolyzed extract: Few drops of dilute HCl was added to 5ml of the aqueous extract of the plant, then left in a boiling water bath for 20 minutes, the acidity was neutralized by NaOH solution, an equal volume of Fehling reagent was added. The development of red precipitate indicates a positive result.

v. Saponins: This method was done according to the method described by Harbone (1984)[17]. Two methods detected saponins:

- A solution of plant powder was shaken vigorously in a test tube. The formation of foams standing for a time indicates a positive result.

- Five ml solution of plant powder was added to 1-3 ml of 3% ferric chloride solution. The development of white precipitate shows a positive effect.

vi. Terpenes and steroids: one ml of a solution of plant powder participated in a few drops of chloroform, and then a drop of acetic anhydride and a reduction of concentrated sulphuric acid were added, brown precipitate appeared which represents the presence of terpenes. The appearance of a dark blue color after few minutes indicates the presence of steroids[18].

Experimental Design

The experiment was designed to assess the effects of two doses (250,500 mg/kg) of aqueous extract of a herbal mixture on the investigated parameters in normal and alloxan-induced diabetic mice. The plant extracts were given orally using gavage needle as a single dose (0.2 ml) per day and for 10 days, and then the mice were sacrificed in day 8 for laboratory assessments. Thirty male mice were used in this study and divided into six groups (five mice for each group):

Group I (control): mice treated with only distilled water.

Group II: normal mice treated with aqueous extract of an herbal mixture at a dose (500 mg/kg/day).

Group III: normal mice treated with aqueous extract of an herbal mixture at a dose (250 mg/kg /day).

Groups IV (Diabetic): alloxan-induced diabetic mice treated with only distilled water.

Groups V: alloxan-induced diabetic mice treated with aqueous extract of an herbal mixture at a dose (500 mg/kg/day).

Group VI: alloxan-induced diabetic mice treated with aqueous extract of an herbal mixture at a dose (250 mg/kg/day).

Blood Glucose Level

Blood glucose level was measured with commercially dextrose measurement strips read by Accu-Chek active system.

Sample required and testing time: Accu-Chek active meter requires 1-2 µL blood per test, and the testing time is about 5 seconds.

Test Principle: On each test strip, there is a test area containing sensitive chemicals. When blood is applied to this area, a chemical reaction takes place (glucose dye oxidoreductase mediator reaction) causing the color of the test area to change. The meter registers this color change and converts the signal obtained into a blood glucose result.

Results and Discussion

Diabetes mellitus (DM) is a group of metabolic disorders that result in hyperglycemia as a result of a relative or absolute lack of insulin, or the actions of insulin on its target tissues or both[19]. It is the most common endocrine disorder, and by the year 2010, it is estimated that more than 200 million people worldwide will have DM, and 300 million will subsequently have the disease by 2025. Regulation of blood glucose concentration plays a vital role in diabetic patients. For centuries, medicinal plants provide a natural source of potent anti-diabetic drugs. In Iraqi regions, there are many types of plant herbs are these contexts. Among which an Herbal mixture including *Teucrium polium*, *Aloe vera*, and *Artemisia herba alba*. Our research aims to study the possible anti-diabetic effect of a mix of these medicinal plants used in folk medicine in Iraq and if they can improve the metabolic abnormalities accompanied to alloxan-induced diabetic albino mice.

Percentage of Extracts

Aqueous extraction resulted in 2.55g, representing 8.38 % per 25g of the raw plant material.

Characterization of chemical composition

Active compounds of an Herbal mixture revealed that flavonoids, phenols, tannins, saponins, and glycosides and terpenes were detected aqueous extracts were negative for steroids (Table 1). These results agreed with El-Shazly and Hussein (2004)[20] who demonstrated that Chemical detections in *T. Polium aqueous* extracts revealed that flavonoids, phenols, tannins, saponins and glycosides, and terpenes. On the other hand, active compounds of *Artemisia herba alba* is shown that a rich source of flavonoids such as hispidulin and cirsilineol. Flavonoids isolated from some medicinal plants have been proven to possess anti-inflammatory effect[21]. And *Aloe vera* is widely used in food supplements, beverages, pharmaceuticals, and cosmetics, Major proteins and mono- and polysaccharides were identified and analyzed from *Aloe vera* commercial extract.

Extraction test	Aqueous extract
Phenols	+
Tannins	+
Flavonoids	+
Terpenes	+
Saponins	+
Glycosides	+
Steroids	-

Table 1. Organic composition of the aqueous extract of an Herbal mixture.

General parameters and bodyweight of experimental mice

Normal mice treated with distilled water (control) showed no change in physiological activities and body weight during the period of treatment, suggesting that experimental conditions (nutrition, humidity, and light) did not affect body weight. However, mixture -treated diabetic mice showed signs of recovery in body weight gains at the end of the experiment as compared to non-treated diabetic mice. An herbal Mixture extract did not affect body weight in standard groups during the period of analysis (Table 2).

It is well known that alloxan, induces the formation of superoxide radicals which dismutate to hydrogen peroxide with the simultaneous massive increase in cytosolic calcium concentration resulting in rapid destruction of β pancreatic cells. This destroys a large number of β cells, resulting in a decrease in endogenous insulin release, which paves the way for the decreased use of glucose by the tissues[22] and leading to increased breakdown of stored carbohydrates, lipids, and proteins to compensate the deficiency of glucose. Using of carbohydrates, lipids, and proteins as a source of energy causing loss of body weight. Diabetic groups treated with extract of an Herbal mixture keep their loads from marked decreasing observed in the non-treated diabetic group. This might be attributed to flavonoid and/or terpenoid content in extract, which might be responsible for potentiating insulin action and increasing glucose consumption by tissues and finally reduce breakdown of stored carbohydrates, lipids, and proteins[23].

Blood glucose level

Regulation of blood glucose concentration plays a vital role in diabetic patients. The blood glucose levels (BGLs) of diabetic mice treated with aqueous extract of an Herbal mixture were reduced in comparison with those of the diabetic control group.

Treatment Groups		Dose (mg/kg)	Weight (g) ± SE Before treatment	Weight (g) ± SE After treatment
Normal + (distilled water)		0.0	27.2 ± 0.6	27.5 ± 0.8
Normal Mice	Aqueous (Mixture)	250	30.5 ± 0.6	30.8 ± 1.7
	Aqueous (Mixture)	500	30.1 ± 0.4	30.7 ± 0.6
Diabetic + (distilled water)		0.0	31.5 ± 0.5	29.2 ± 1.2
Diabetic Mice	Aqueous (Mixture)	250	30.8 ± 0.8	28.8 ± 3.3
	Aqueous (Mixture)	500	30.5 ± 1.5	26.0 ± 0.9*

**** Significant difference (P ≤ 0.05) between means before and after treatment for each group***

Table 2. Bodyweight of normal and alloxan-induced diabetic mice before and after treatment with a dose of aqueous extract of an Herbal mixture.

The blood glucose level in normal mice treated with distilled water (control) was observed during the period of treatment, which revealed that experimental conditions (nutrition, humidity, and light) did not affect the blood glucose level. A marked elevation in blood glucose levels was measured in the non-treated diabetic group. Diabetic mice treated with extracts exhibited a significant decrease in blood glucose levels as compared to non-treated diabetic groups. Only the high doses of aqueous extract showed a significant reduction in blood glucose levels in the normal mice during the period of experiment. The blood glucose levels are recorded in (Table 3). Depending on our results it is clear that oral administration of extracts of an Herbal mixture showed hypoglycemic effect on normal and diabetic mice. This result agreed with Iriadam *et al.*(2006), who reported the hypoglycemic action of *T. polium* and *Artemisia herba alba* extracts suggest more than possible mechanism of action.

A vera leaf pulp and gel extracts were ineffective in lowering the blood sugar level of ND rats. A. vera leaf pulp extract showed hypoglycaemic activity on IDDM and NIDDM rats, the effect being enhanced for type II diabetes in comparison with glibenclamide. On the contrary (24). Oral administration of the methanol extract of the aerial parts of Artemisia pallens Wall. (Used in Indian folk medicine for the treatment of diabetes mellitus) led to a significant blood glucose-lowering effect in glucose-fed hyperglycaemic and alloxan-induced diabetic rats.

A. vera leaf gel extract showed hyperglycaemic activity on NIDDM rats. It may, therefore, be concluded that the pulps of *Aloe vera* leaves devoid of the gel could be useful in the treatment of non-insulin dependent diabetes mellitus. One of which the potentiation of insulin action released from pancreatic β-cells. It had been found that the hypoglycemic effects of the aerial parts of *T. polium* may be due to its content of flavonoids and/or terpenoids[25]. Other researchers suggested that the hypoglycemic activity was due to the presence of several flavonoids in T. polium and Artemisia herba alba. One such flavonoid with hypoglycemic effects in diabetic animals was quercetin. They reported that quercetin might exert its effects on insulin release from rat islets of Langerhans via changes in Ca^{2+} metabolisms[26]. Similar findings were reported by Sulaiman *et al.*[27]who said that the flavonoids present in the aerial parts of *T. polium* and *Artemisia herba alba* might be respon-

Treatment Groups		Dose (mg/kg)	Glucose (mg/dl) Before treatment	Glucose (mg/dl) After treatment
Normal + (distilled water)		0.0	155.2 ± 6.0	158.2 ± 10.2
Normal Mice	Aqueous (Mixture)	250	190.0 ± 6.0	184.0 ± 10.8
	Aqueous (Mixture)	500	185.5 ± 10.4	165.1 ± 10.5*
Diabetic + (distilled water)		0.0	502.0 ± 12.2	530.2 ± 14.8
Diabetic Mice	Aqueous (Mixture)	250	510.5 ± 15.4	385 ± 13.1*
	Aqueous (Mixture)	500	420.2 ± 14.2	250±10.8*

**** Significant difference (P ≤ 0.05) between means before and after treatment for each group***

Table 3. Effect of aqueous extract of an Herbal mixture on Blood glucose level of normal and alloxan-induced diabetic mice.

sible for islet regeneration and possibly for β-cell regeneration and insulin release and/or they might have insulin-like properties. Other researchers reported that the therapeutic action of flavonoids is due to their antioxidant activity by various mechanisms, e.g., by scavenging or quenching free radicals, by chelating metal ions, or by inhibiting enzymatic systems responsible for free radical generation.

Statistical analysis

All observations were first recorded in a notebook and entered into PC and verified by another person for the accuracy of data entry. Values were expressed as mean ± SD (of 30 animals). The statistical analysis was performed using a Student's two tailed-test software program according to World Health Organisation(2015)[28]. P values less than 0.05 were considered statistically significant.

Conclusions

Different active compounds were detected in the aqueous extract of a mixture of three medicinal plants (*Aloe vera*, *Artemisia herba alba*, and *Teucrium polium*) include phenols, flavonoids, tannins, saponins, glycosides, and terpenes. Aqueous extract of an herbal mixture of three medicinal plants (*Aloe vera*, *Artemisia herba alba* and *Teucrium polium*) has hypoglycemic activity in both standard and alloxan-induced diabetic mice in a dose-dependent manner. Our results support that the aqueous extract of these plant mixture exhibits antidiabetic as compared with each plant alone, where we tested each of these plants in previous studies.

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Received: 10 October 2019
Accepted: 27 October 2019

CASE REPORTS / REPORTE DE CASO

Cistogastrostomía laparoscópica como tratamiento para pseudoquiste pancreático: reporte de un caso.

Laparoscopic cystogastrostomy as a treatment for pancreatic pseudocyst: a case report.

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DOI. 10.21931/RB/2019.04.04.9

Resumen: El pseudoquiste pancreático es una de las complicaciones locales tardías de la pancreatitis aguda, para el manejo de esta entidad existen múltiples estrategias que van desde el manejo expectante, terapia mínimamente invasiva y la resolución quirúrgica. Al ser la cirugía el tratamiento definitivo, el abordaje laparoscópico toma fuerza como estrategia en pacientes seleccionados. Paciente femenina de 47 años de edad con múltiples comorbilidades con cuadro de pancreatitis de origen biliar con desarrollo ulterior de pseudoquiste pancreático en quien se decide resolución quirúrgica con abordaje laparoscópico. El abordaje laparoscópico muestra resultados favorables; con una duración del procedimiento de 170 minutos en promedio; la técnica abierta muestra varias complicaciones: fístulas pancreáticas en el 40%, fístulas entéricas del 20%, hernia incisional del 25%, y mortalidad del 9 al 25%; la gastrocistostomía por vía laparoscópica permite crear una comunicación entre el quiste y el estómago mucho más amplia en comparación con el abordaje endoscópico, hemostasia segura y mejor manejo de las complicaciones. La cirugía para el tratamiento del pseudoquiste continúa siendo la piedra angular; el abordaje laparoscópico muestra las ventajas propias de la laparoscopia, con menores tasas de morbilidad en comparación con los abordajes abiertos.

Palabras clave: Pseudoquiste pancreático, laparoscopia, cistogastrostomía.

Abstract: The pancreatic pseudocyst is one of the late local complications of acute pancreatitis, for the management of this entity, there are multiple strategies that range from expectant management, minimally invasive therapy and surgical resolution. Since surgery is the definitive treatment, the laparoscopic approach takes force as a strategy in selected patients. A 47-year-old female patient with multiple comorbidities with pancreatitis of bile origin with subsequent development of pancreatic pseudocyst in whom surgical resolution with a laparoscopic approach is decided. Discussion: The laparoscopic approach shows favorable results; with a procedure duration of 170 minutes on average; the open technique shows several complications: pancreatic fistulas in 40%, enteric fistulas of 20%, incisional hernia of 25%, and mortality of 9 to 25%; Laparoscopic gastrocystostomy allows a much wider communication between the cyst and stomach compared to the endoscopic approach, safe hemostasis and better management of complications. Surgery for the treatment of pseudocyst continues to be the cornerstone; The laparoscopic approach shows the advantages of laparoscopy, with lower morbidity rates compared to open procedures.

Key words: Pancreatic pseudocyst, laparoscopy, cystogastrostomy.

Introducción

Los pseudoquistes pancreáticos forman parte de las complicaciones locales de la pancreatitis aguda; específicamente se considera como una forma madura de colección peri- pancreática que en general se forma a las 4 semanas del inicio del proceso agudo de la enfermedad con una pared fibrosa que lo circunscribe y que además en la mayoría de los mismos no suelen existir restos de tejido pancreático necrótico[1,2]. Para el manejo y tratamiento de esta patología se han reportado varios métodos de drenaje así como también la conducta expectante dependiendo de las características del quiste; en cuanto al abordaje quirúrgico la técnica estándar mediante laparotomía y confección de derivación entre el pseudoquiste el estómago o el intestino muestra una morbilidad importante y no despreciable según algunos estudios que van desde el 5 hasta el 25%[3]; de igual manera el abordaje por vía laparoscópica y la confección de una cistogastrostomía es una opción válida en la actualidad con resultados favorables en los estudios realizados[4] La tasa de conversión hacia laparotomía según algunas series es de aproximadamente 3,3 %, morbilidad del 3,3%, y con una recidiva del 7%[5]. A pesar del advenimiento de las técnicas mínimamente invasivas como las endoscópicas; la cirugía, abierta o laparoscópica, continúa siendo de elección al momento e incluso con mejores resultados según algunos autores[6]. Presentamos el caso de una paciente de sexo femenino con varias comorbilidades y con episodios de pancreatitis a repetición, quien acude a nuestra casa de salud con cuadro de dolor abdominal en quien posterior a realización de estudios tomográficos se diagnostica de pseudoquiste pancreático, al tratarse de una paciente con varias comorbilidades y que posee indicaciones de resolución quirúrgica del pseudoquiste, se eligió el abordaje laparoscópico con gastrostomía anterior y con cistogastrostomía posterior con endograpadora lineal.

Caso clínico

Información del paciente: Paciente femenina de 47 años de edad, con antecedentes clínicos de Diabetes Mellitus en tratamiento con insulina; lupus eritematoso sistémico en tratamiento con prednisona; artritis reumatoidea hace 2 años en

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tratamiento con azatioprina; síndrome de Cushing inducido por medicamentos; hipertensión arterial en tratamiento con losartan. Quirúrgicos: incontinencia urinaria colocación banda t.o.t, hemorroidectomía y salpingectomía bilateral.

Hallazgos clínicos y evaluación diagnóstica: Acude por presentar cuadro de alteración del estado de conciencia, malestar general, decaimiento, dolor abdominal difuso, alza térmica. Se descarta causas relacionadas a patología de base. Se realizan exámenes complementarios, diagnosticando pancreatitis aguda de origen biliar BALTAZAR tipo E (escala APACHE 8), teniendo como complicación pseudoquiste pancreático, se instaura manejo clínico. A las seis semanas presenta cuadro de dolor abdominal de tipo punzante EVA 7/10 moderada intensidad, alza térmica no cuantificada, dificultad respiratoria, disnea de moderados esfuerzos, disuria poliaquiria. Se diagnostica de pancreatitis re–agudizada asociada a derrame pleural izquierdo y pseudoquiste previo. Al ingreso consciente, orientada, hidratada, febril, taquicárdica, tórax expansibilidad conservada, campos pulmonares murmullo vesicular disminuido en bases pulmonares bilaterales, abdomen suave depresible doloroso en epigastrio y hemi-abdomen izquierdo sin signos de irritación peritoneal. Laboratorio: Leucocitos 10540 /ml, Neutrofilos 84.1%, Hb 11.1 gr/dl, Hto 36.7 % Plaquetas 297000 por mm^3, Creatinina 0.79 mg/dl, Amilasa 367 mg/dl, Lipasa 1384 mg/dl, Sodio 139 mEq/l, Potasio 4.96 mEq/l, Cloro 100 mE/l; Gasometría arterial: pH 7.46, PCO2: 29.8 mmHg, PO2: 67.5 mmHg, HCO_3: 20.8 mEq/l, Saturación de oxígeno: 94% (escala APACHE 8). Urocultivo: E. Coli multisensible, Hemocultivo: *S. Aureus* multisensible.

Intervención terapéutica: Paciente es ingresada por el servicio de medicina interna por sus múltiples comorbilidades y por su cuadro sepsis de origen urinario se instaura antibióticoterapia a base de imipenem. Una vez superado el cuadro séptico, es transladada al servicio de cirugía general para la resolución de pseudoquiste pancreático. Se decide realizar tratamiento quirúrgico con abordaje laparoscópico y confección de cistogastrostomía. Procedimiento Quirúrgico: Colecistectomía, Cistogastrostomía transgástrica, necrosectomía y drenaje. Obteniendo los siguientes hallazgos:

-Adherencias laxas hepatoparietales, vesícula biliar 6x3x3 cm con barro biliar

-Cístico delgado, largo, arteria posterior

-Pseudoquiste 20 x 20 cm, protruyente hacia la curvatura gástrica mayor

-Líquido purulento 300 ml

-Necrosis peripancreática en moderada cantidad

Paciente inicia tolerancia oral al quinto día sin signos de SIRS (síndrome de respuesta inflamatoria sistémica). Se progresa dieta en los días posteriores, con laboratorio dentro de parámetros normales. Se da el alta al noveno día, en buenas condiciones; nuevo control tomográfico previo al alta con evidencia de pseudoquiste ausente, sin colecciones. Control posterior en consulta externa a los 5 días del alta y a los 14 del

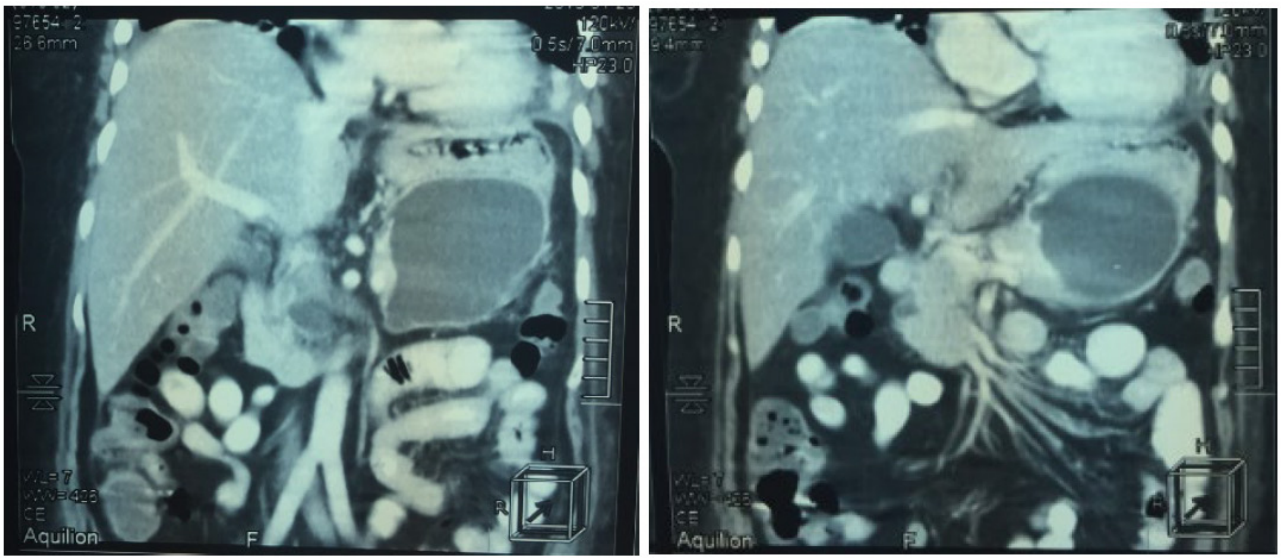

Figure 1. TAC S/C abdomen y pelvis: presencia de pseudoquiste pancreático de 9cm x 6cm que compromete cola y cuerpo de páncreas no líquido libre en cavidad.

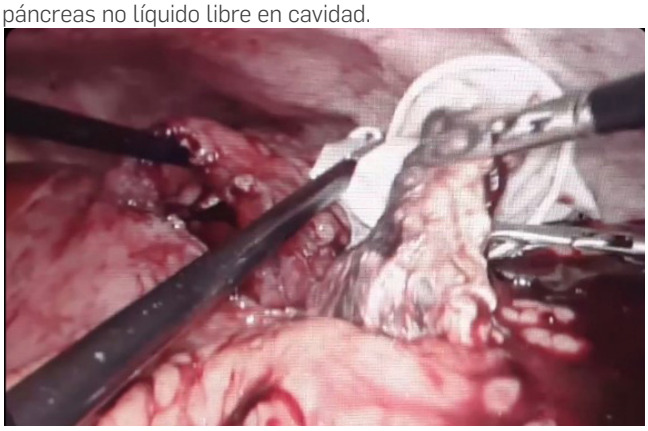

Figure 2. Drenaje de pseudoquiste y excéresis de necrosis pancreática.

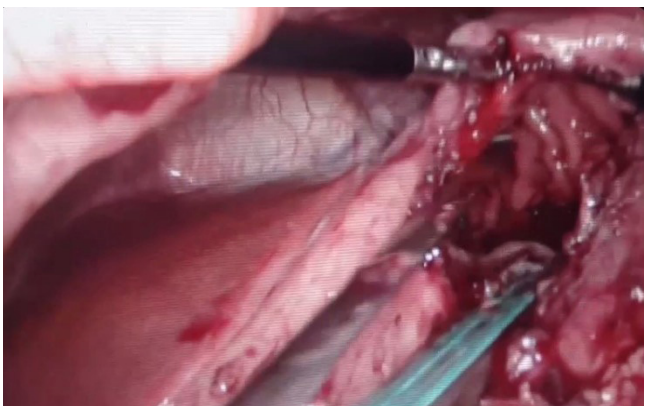

Figure 3. Confección de cistogastrostomía con sutura mecánica y reforzamiento.

procedimiento con buena evolución, se decide retiro de drenaje de Jackson Pratt y continuar con controles cada mes.

Discusión

El abordaje laparoscópico del pseudoquiste pancreático muestra resultados favorables, tal y como se describió en nuestra paciente, citando algunos autores incluso con tasas nulas de recidiva cuando se utiliza el abordaje posterior con una única gastrostomía[7,8]. En nuestro caso, tal y como se describe en las series mundiales, el tratamiento del pseudoquiste se lo realiza a los 64 días del episodio agudo. En algunos estudios la duración del procedimiento por vía laparoscópica es en promedio de 170 minutos[8]. Tal y como se describen en las series de casos, la ventaja del abordaje laparoscópico radica en la recuperación más rápida y las menores tasas de morbilidad y mortalidad. Dentro de las complicaciones reportadas en la técnica abierta y que no están presentes cuando el abordaje es laparoscópico se describen: fístulas pancreáticas en el 40%, fístulas entéricas del 20%, hernia insicional del 25%, y con una tasa de mortalidad que va desde el 9 al 25%[9]. Tomando en cuenta los mismos en nuestro caso no se encontraron dichas complicaciones. El abordaje laparoscópico mostró ventajas evidentes. La recuperación fue completa en menos de una semana, lo que nos podría indicar que además de las ventajas descritas de los abordajes mínimamente invasivos, al tratarse de pacientes con comorbilidades asociadas la opción laparoscópica muestra beneficios potenciales. Kerri en el 2014 realiza una descripción de tipo retrospectiva de 22 casos con pseudoquiste pancreático sintomático en quienes se realizó una derivación cistogástrica por vía anterior reportando una mortalidad del 0%; en su trabajo describe los 10 pasos del procedimiento empleado en donde a diferencia del realizado en nuestra paciente, se utiliza una guía endoscópica y los trocares de trabajo se colocan dentro del estómago, además del uso del ultrasonido para verificar la ubicación de la colección[10]. Como ventaja adicional la gastrocistostomía por vía laparoscópica permite crear una comunicación entre el quiste y el estómago mucho más grande en comparación con el abordaje endoscópico, la hemostasia se asegura de mejor manera, y el mejor manejo de las complicaciones durante el procedimiento si estas ocurren[11]. No existen muchos estudios que valoren el resultado a largo plazo de la técnica descrita por lo que con el adecuado entrenamiento en la técnica y un seguimiento de los casos podemos iniciar la descripción de los resultados obtenidos[12,13].

Conclusiones

El abordaje quirúrgico para el tratamiento de las complicaciones posteriores a la pancreatitis aguda como los pseudoquistes e incluso la necrosis y los abscesos continúa siendo la piedra angular, a pesar del desarrollo actual de las técnicas endoscópicas. Dentro de esta perspectiva, el abordaje laparoscópico muestra las ventajas propias de la laparoscopia, con menores tasas de morbilidad en comparación con los abordajes abiertos; por lo tanto, estas ventajas deben ser tomadas en cuenta en pacientes seleccionados y en quienes exista indicación quirúrgica, con beneficio extra en pacientes con comorbilidades debido a la pronta recuperación y a un mejor manejo postquirúrgico.

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Received: 1 octubre 2019
Accepted: 28 octubre 2019

REVIEW / ARTÍCULO DE REVISIÓN

Pyrazoline as a medicinal scaffold.

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DOI. 10.21931/RB/2019.04.04.10

Abstract: Heterocyclic Chemistry is the backbone of medicinal compounds that exhibits numerous biological activities. Pyrazole and its derivatives possess nitrogen atom along with carbon atom as a substitution and show a diversity of biological activities such as antibacterial, antimicrobial, anti-inflammatory, antioxidant, antidiabetic, anticancer, antifungal, antidepressant, anticonvulsant, analgesic, and monoamine oxidases (MAOs)as shown by pyrazoline prepared from chalcone(Intermediate). The synthesized compounds are checked by the TLC and further analyzed by the IR, NMR, and UV spectroscopy.

KeyWords: Anti-microbial, heterocyclic, and pyrazoles, biological activity.

Introduction

Pyrazole, well known heterocyclic compound with two adjacent nitrogen atoms within the ring and having a five-membered ring carries one endocyclic double bonds and essential in nature[1], represented by the molecular formula $C_3H_4N_2$[2].Pyrazole melts at 700C, in spite of its low molecular weight. Clinically the substitution derivatives at 3 and five positions are indistinguishable from one another whereas the properties disappear the hydrogen atom on the nitrogen atom immediately is replaced by an alkyl group[3].

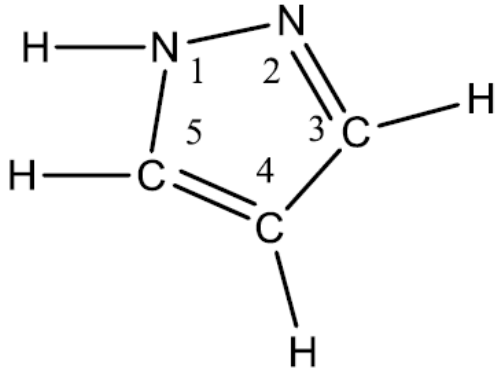

Figure 1. Pyrazole representation with two adjacent nitrogen atoms within the ring.

In 1883, Ludwig Knorr coined the term pyrazole, which is a weak base, acquires pKb 11.5(pKa value of the conjugated acid 2.49 at 25^{0}C). Pyrazole having unique pharmacological effects on human beings and from watermelon seeds, 1-pyrazolyl-alanine was isolated and then classified as alkaloids on composition, first natural pyrazole in 1959[4].

Pyrazole exhibits various biological activities and also has a potent medicinal scaffold[5]. Pyrazoline and its derivatives are having an antibacterial[6], antimicrobial[7], anti-inflammatory[8], antioxidant[9], antidiabetic[10], anticancer[11], antifungal[12], antidepressant[13], anticonvulsant[14], analgesic[15], and monoamine oxidases (MAOs)[16].

Chemistry and Synthetic approaches

In medicinal chemistry, heterocyclic rings such as Pyrazole containing active pharmacophore agents play an essential role in refined and efficient ways to make these heterocyclic heads. Pyrazole includes two nitrogen atoms also carry a π-excessive heterocycle, as seen in pyrrole at position1 and pyridine at positions 2. Pyrazole subsists in three partially reduced forms.

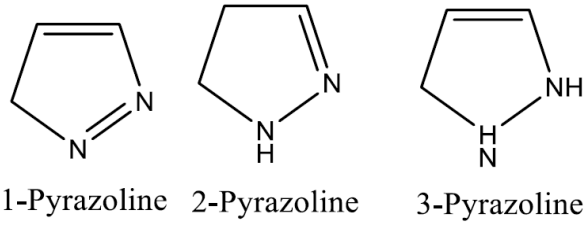

Figure 2. Pyrazole contains two nitrogen atoms also carry a π-excessive heterocycle, as seen in pyrrole at position 1 and pyridine at positions 2.

Pyrazole carries a boiling point of (186-188°C) due to its hydrogen bonding and exhibits two identical and non-separable tautomers owing to rapid interconversion of tautomers. In other words, In pyridine, nitrogen is prone to electrophilic attack, and the hydrogen atom bonded to the nitrogen at position 1 is more acidic than pyrrolic N-H, thus easily removed by nucleophiles. It can be synthesized via Claisen-Schmidt condensation followed by cyclizations with hydroxylamine HCl and hydrazine hydrate under the appropriate conditions. Pyrazole lower basicity with weak base (pKa= 2.52) because of extra destabilization of π-bonding after protonation and increases acidity with very weak acid (pKa=14.21) by the introduction of the electron-withdrawing group (-I & -M effect)[17].

Synthetic approach to the pyrazole

1-(4,5-disubstitutedpyrazol-1-yl)-ethanone Synthesis: On the contrary, pyrazoles have been carried out by the reaction of β-formyl enamides with hydroxylamine hydrochloride catalyzed by potassium dihydrogen phosphate in acidic medium and become novel synthesis[18].

3,5-substituted-1H-pyrazole Synthesis: From tosylhydrazones, α, в-unsaturated carbonyl compounds synthesis of pyrazole derivatives possessing a β-hydrogen is proposed and exploiting microwave activation coupled with solvent-free reaction conditions and appears as novel approach[19].

Tri- and tetra-substituted pyrazoles Synthesis; For the facile synthesis of tri- and tetra-substituted pyrazoles using Dioxygen gas as the oxidant undergoing intramolecular

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oxidative CN coupling method catalyzed by A ruthenium (II) carried out the transformation, and the reaction demonstrates excellent reactivity, functional group tolerance, and high yields[20].

For the Regio, selective synthesis; By the reaction of diarylhydrazones and vicinal diols via the route of 1,3- and 1,3,5-substituted pyrazoles undergoes iron-catalyzed synthesis of 1, 3-substituted pyrazoles[21].

Synthesis of 1,3,5-trisubstituted-1H-pyrazole: An easily accessible reaction 1, 3-bisaryl-monothio-1,3-diketone or 3-(methylthio)-1,3-bisaryl-2-proponents gives 1-aryl-3,5-bisarylpyrazoles with arylhydrazines and with complementary Regioselectivity at position 3 and 5[22].

General procedure for synthesizing pyrazoline

A substituted Chalcone (0.01 mole) mixed with hydrazine hydrate (0.012 moles) and acetic acid(10ml) in methanol for five hours. The reaction mixture was poured in chilled water, and solid separated was filtered and recrystallized from ethanol. Again, the reaction completion was confirmed by the TLC and monitored.

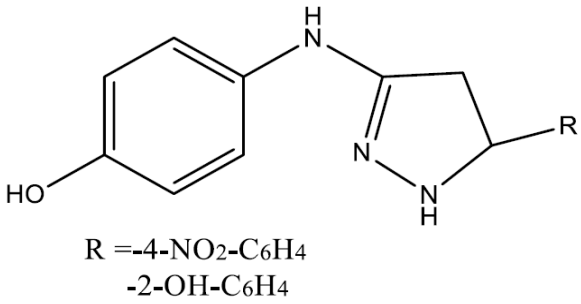
Pharmacological activity

The usefulness and great therapeutic value of pyrazole nucleus have been recognized, and the most comprehensive range of activities of this nucleus evaluated for a long time. However, as the first synthetic organic compound carries pyrazoline-5-one nucleus to find use as an essential drug. Derivatives of the pyrazolopyrimidine ring system are known to possess potent biological properties[23] Out of these many natural and synthetic products having heterocyclic rings as Derivatives of the pyrazolopyrimidine ring system are known to possess potent biological properties[24].Also condensed pyrazoles are biologically active and their chemistry has received considerable attention as pyrano[2,3-c]pyrazoles are reported to have useful biological effects, such as analgesic and anti-inflammatory activities[25].In recent years significant progress has been made relating to oxidation in biological cells resulted from reactive oxygen species (ROS) and initiates lipid peroxidation in healthy cells leading to Alzheimer's disease, atherosclerosis, diabetes, Parkinson's disease, etc. Among plant kingdom and animal kingdom coumarins, Xanthones pyrazoles and acrylonitriles showed widespread use in medicine[26].Synthetically, several methods have been published for the synthesis of 2-pyrazolines for the treatment of tumor, fungal and viral infection, Tuberculosis and depression, etc[27].

Antimicrobial activity

Sahu SK *et al.*, synthesized a series of 4-(5-substituted aryl-4, 5-dihydropyrazole-3-yl-amino) phenols 2a-f have been reported novelty by treating substituted Aryl-N-chalconyl amino phenols 1a-f with hydrazine hydrate from p-aminoacetophenone By the substitution of the p-nitro and p-hydroxy group in aryl moiety of the pyrazoleanalogs 2c(-4-NO_2-C_6H_4) and 2e(-2-OH-C_6H_4) produce compounds with potent analgesic, anti-inflammatory but also in a few cases, antimicrobial properties. IR, 1H NMR spectral data.28 confirmed the structures of synthesized compounds.

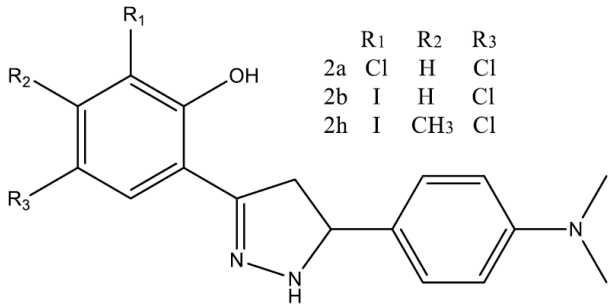
Kendre M.M. and BASEER M.A. synthesized efficiently biologically active Pyrazoline derivatives with excellent yields via cyclization reaction of chalcones and hydrazine hydrate. The compounds 2a, 2b, and 2h showed significant activity in comparison with the standard drug. The presence of pyrazoline moiety, substituents, particularly having



R =-4-NO_2-C_6H_4
-2-OH-C_6H_4

Figure 3. Pyrazoleanalogs 2c(-4-NO_2-C_6H_4) and 2e(-2-OH-C_6H_4) produce compounds with potent analgesic, anti-inflammatory but also in a few cases, antimicrobial properties.

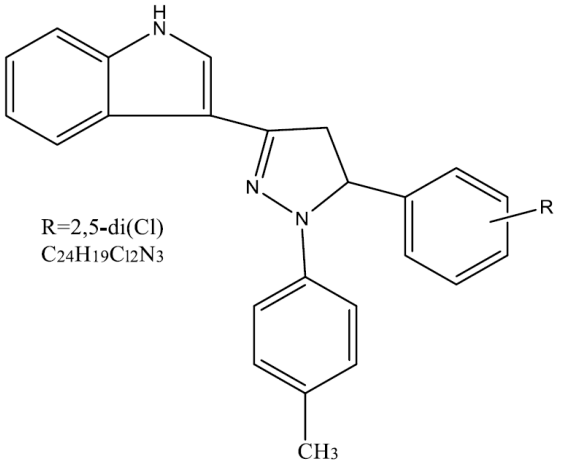
Bromo, Chloro, Hydroxyl, Iodo and Methyl groups in the ring may be responsible for antimicrobial activity of this class of compounds and screened that also reflects moderate to good activity against different strains of bacteria and fungi employed. All the synthesized compounds were confirmed by IR[1],HNMR and Mass spectral data[29].



	R1	R2	R3
2a	Cl	H	Cl
2b	I	H	Cl
2h	I	CH_3	Cl

Figure 4. Substituted Pyrazoline moiety.

Ahmet O¨ zdemir *et al.*, synthesized 1-(p-Methyl phenyl)-3,5-diaryl-2-pyrazoline derivatives (2a–f) via the treatment of 1-(1H-indol-3-yl)-3-aryl-2-propen-1-ones (1a–f) with p-methylphenylhydrazine hydrochloride in hot acetic acid. Pyrazoline derivatives, compound 2e exhibits 2,5-dichlorophenyl moiety identified the most promising agent against *Klebsiella pneumoniae* (ATCC 13883) and *Candida glabrata* (ATCC 36583) due to its inhibitory effects on *K. pneumoniae* and *C. glabrata* with a MIC value of 100 mg/mL as a nontoxic agent (LC50 > 1000 mg/mL). Structural elucidation of these compounds by IR, 1H NMR, and mass spectral data and elemental analysis and investigated toxicity by Brine-Shrimp lethality assay for their antimicrobial activity[30].



R=2,5-di(Cl)
$C_{24}H_{19}Cl_2N_3$

Figure 5. 1-(p-Methyl phenyl)-3,5-diaryl-2-pyrazoline derivatives.

Dipankar *et al.* studied the reports of twelve 2-pyrazoline derivatives against *S. aureus* and *A. niger* thoroughly (Table1). Two varieties of acetophenones were condensed with three varieties of substituted benzimidazole derivatives to get six chalcone derivatives, which undergo condensation followed by cyclization with isoniazid and 1-(2-napthyloxy acetate) hydrazine two get the final 2-pyrazoline derivatives. Compound D7 exhibited the highest antibacterial activity, and compound D12 exhibited highest anti-fungal activity as well as comparable to the antibacterial activity and antifungal activity of the standard drugs at 200 µg/ml. These compounds were characterized by IR, 1H-NMR and Mass spectral studies. The synthesized compounds were found to have good antimicrobial activity in the range of 20-70 µg/ml[31].

Compounds	Minimum inhibitory concentration (µg /ml)	
	Bacteria	Fungi
	S. aureus	*A. niger*
D12	50	20
Ciprofloxacin	12.5	-
Ketaconazole	-	10

Table 1. Reports of twelve 2-pyrazoline derivatives against *S. aureus* and *A. niger*.

N.C. Desai. *et al.* synthesized a series of compounds 2-(5-(3-(4-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)-3-(aryl)-4,5-dihydro-1Hpyrazol- 1-yl) thiazol-4(5H)-ones (4a–q) and screened invitro synthesized against the representative panel of Gram-positive (*Staphylococcus aureus*, *Streptococcus pyogenes*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria. These compounds were also tested for their inhibitory action against strains of fungi (*Candida albicans*, *Aspergillus niger*, *Aspergillus clavatus*). The synthesized compounds showed potent inhibitory action against the test organisms by the use of an electron-withdrawing group on the benzene ring in basic structures was worthy. Compounds bearing 2-Cl, 4-Cl, 2-F, 3-F,4-F, 2-NO2, and 4-NO_2 exhibited more pronounced activity[32].

Antidepressant activity

B. K. Kaymakcıoğlu, S. Gumru, N. Beyhan, F. Aricioglu investigated a series of new 2-pyrazoline derivatives and activity was evaluated by using tail suspension test, Compounds 3d and 3e were effective and a significant reduction in immobility time was observed as compared to results of imipramine, as the reference standard drug. Compounds 3d and 3e remarked the potential for the treatment of depression[33].

Anti-convulsant activity

Ahsan synthesized 3-substituted-N-aryl-6,7-dimethoxy-3a,4-dihydro-3H-indeno[1,2-c]pyrazole-2-carboxamide and assayed the anticonvulsant activity and neuroprotection according to Antiepileptic Drug Development Programme protocol. Compound 4b showed neuroprotection activity with

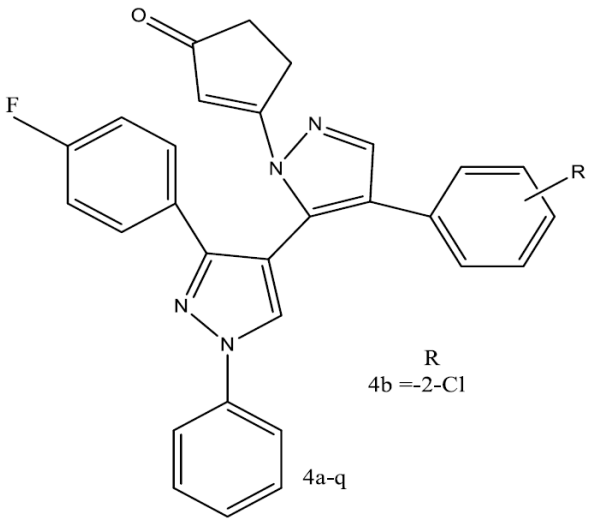
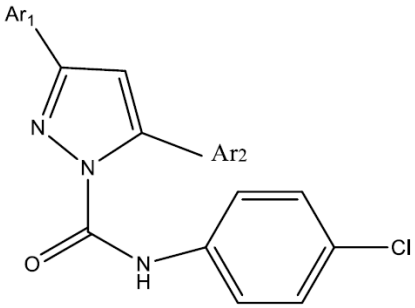


Figure 6. Series of 2-(5-(3-(4-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)-3-(aryl)-4,5-dihydro-1Hpyrazol- 1-yl) thiazol-4(5H)-ones for antimicrobial activity.



Ar1: -5-Bromothiophen-2-yl, Ar 2: 2,6-Dichlorophenyl (d)
Ar1: -5-Chlorothiophen-2-yl, Ar 2: 2,6-Dichlorophenyl (e)

Figure 7. Pyrazoline derivatives for Antidepressant activity.

26.2 ± 1.9% of total propidium iodide uptake at 100 µM, and inhibitory concentration 50 (IC50) of the compound was found to be 159.20 ± 1.21 µM[34].

Sudhakararao G *et al.*, Synthesized compounds and then evaluated to suppress seizures and provide neuroprotection by minimizing the effects of the seizure attacks. To attain this some chalcone and chalcone based pyrazolines were Evaluated for their anticonvulsant activity and then structural elucidation was taken on the basis of the elemental analysis and spectroscopic studies (NMR, IR and Mass Spectroscopy) Among all compounds only compounds Ph1, Ph2, Py 3 and Py 4 shown to be good anticonvulsant activity with dose level of 4mg/kg b.w.[35].

Abdel-Aziz *et al.*, have described two synthetic paths for the formation of diacylhydrazines, 5amino-1-substiuted pyrazole-3,3,4-tricarbonitriles and oxadiazole, pyrazoline derivatives, Compounds 4a and 4b showed good activity in comparison to imipramine at a dose of 10 mg/kg dose level and showing antidepressant activity using tail suspension behavioural despair test and anticonvulsant activity against pentylenetetrazol induced seizures in mice[35].

Monoaminoxidase activity

F. Manna *et al.*, have synthesized a series of 1-acetyl-3-(4-hydroxy- and 2,4-dihydroxyphenyl)-5-phenyl-4,5-dihydro-(1H)-pyrazole derivatives and investigated their ability to selectively inhibit the activity of the isoforms of MAO and created a

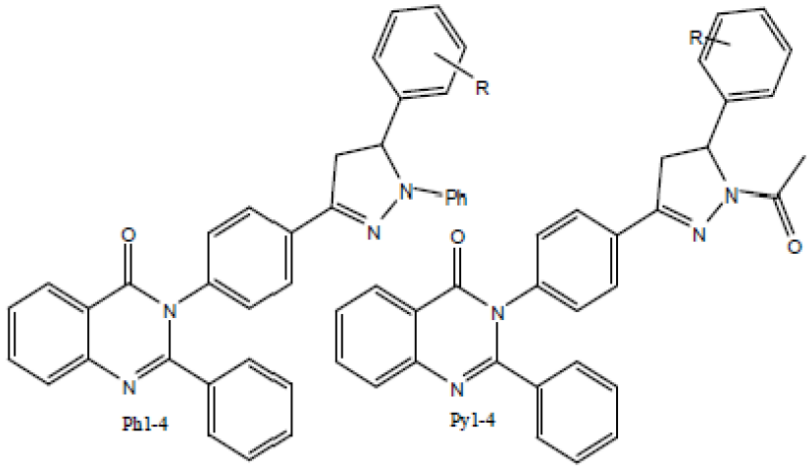


Figure 8. Substituted-N-aryl-6,7-dimethoxy-3a,4-dihydro-3H-indeno[1,2-c] pyrazole-2-carboxamide for Anticonvulsant activity.

novelty. The newly synthesized compound proved to be more reversible, potent, and selective inhibitors of MAO-A than of MAO-Compounds 6 and 11 were found to be most potent[36].

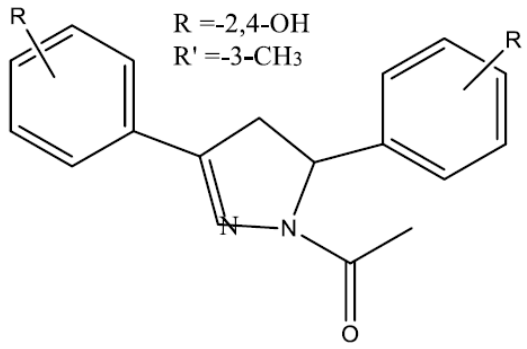


Figure 9. series of 1-acetyl-3-(4-hydroxy- and 2,4-dihydroxyphenyl)-5-phenyl-4,5-dihydro-(1H)-pyrazole derivatives for MAO activity.

F. Chimenti *et al.*, synthesized a coumarin-3-acyl derivatives as a series and investigated for the ability to inhibit selectively monoamine oxidases. The coumarin-3-carboxylic acids, 2a–e, proved to be reversible and selective inhibitors of the MAO-B iso-form. The coumarin-3-acyl chlorides 3a–e showed high pIC50 values against both MAO-A and MAO-B isoforms,3d being the highest against MAO-B with a pIC value of 8.00. To rationalize the activity/selectivity results, molecular descriptors were generated. Further insight about enzyme–inhibitor interaction was obtained by docking experiments with the MAO-B isoform[37].

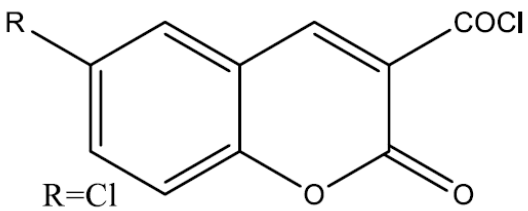


Figure 10. Series of coumarin-3-acyl derivatives.

Antifungal activity

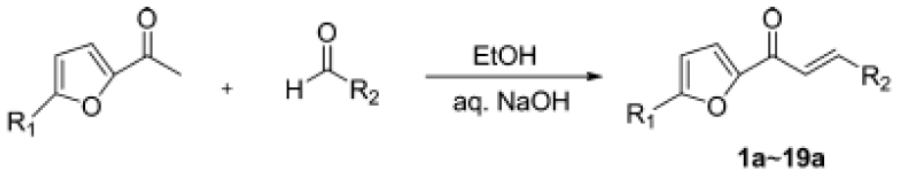
Sivakumar R *et al.*, docked 14α-demethylase routinely used to understand drug-receptor interaction in modern drug design. The docking of benzimidazole containing pyrazoline-5-one derivatives as inhibitors of 14α-demethylase. The inhibitory activities against 14α-demethylase were investigated by molecular docking using the HEX docking software. These compounds docked into the active site of the receptor (PDB code, 1E9X) using Hex docking tools software, which showed good affinity for the enzyme when compared with the binding energies of standard drugs such as clotrimazole (-24.05) and griseofulvin (-36.57). Among all the designed compounds, compound 7 shows more binding energy values (-59.85)[38].

Kumar R and Joshi Y. C. synthesized β-diketones/β-ketoesters, 4a–e on condensation with different β-diketones/β-ketoesters, 3a–e in the presence of sodium hydroxide from the diazonium salt of 4-amino-1-methyl-3-propyl-1H-pyrazole-5-carboxamide. The β-diketones/β-ketoesters 4a–e were condensed with o-phenylenediamine (*o*-PDA) in the presence of *p*-toluene sulfonic acid/SiO_2 to give biologically active 3H-1,5-benzodiazepines, 5a–e. Crofloxin and ciclopirox olamine were used as reference standards for comparison of the antibacterial and antifungal activities, respectively. Compound 5c exhibited greater antimicrobial and antifungal activities than the standard drugs, whereas compounds 5d and 5e showed significant anthelmintic activity All the newly synthesized compounds were characterized by elemental analysis and spectral studies and screened for their antimicrobial, antifungal and anthelmintic activities[39].

Deng *et al.*, synthesized a series of 1,3,5-trisubstituted-2-pyrazoline derivatives by introducing the furan rings. Among all compounds, compounds 4, 7, 9, 12, 18 and 19 displayed excellent antifungal activity against *Rhizoctonia solani* and tried to discover some more potent antifungal compounds. Additionally, at site 3 and site 5of the pyrazoline in the compounds 9 and 19 bearing two furan rings respectively and with the help of bioactivity results pyrazoline derivatives receives a template for the further structural optimization[40].

Antiepileptic activity

Maruthi Rao B *et al.*, prepared two varieties of acetophenones were condensed with two varieties of aromatic benzaldehydes to get four chalcone derivatives by undergoing condensation followed by cyclisation with isoniazid to get the final four 2-pyrazoline derivatives. Compounds T1 and T2 having 2-furyl derivatives names of T2 (5-(furan-2-yl)-4,5-dihydro-3-(4-hydroxyphenyl) pyrazol-1-yl) (pyridin-4-yl) methanone and T1(3-(4-chlorophenyl)-5-(furan-2-yl)-4,5-dihydropyrazol-1-yl) (pyridin-4-yl) methanone has prominent anti-epileptic activity on hydroxy-2 and furyl have the most potential anti-epileptic activity[41].



1a: R_1=H, R_2=3-NO_2Ph; **2a**: R_1=H, R_2=2-NO_2Ph; **3a**: R_1=H, R_2=4-BrPh;

4a: R_1=H, R_2=4-ClPh **5a**: R_1=H, R_2=2-ClPh; **6a**: R_1=H, R_2=3,4-Cl_2Ph;

7a: R_1=H, R_2=2,4-Cl_2Ph; **8a**: R_1=H, R_2=4-OCH_3Ph; **9a**: R_1=H, R_2=2-furyl;

10a: R_1=CH_3, R_2=4-NO_2Ph; **11a**: R_1=CH_3, R_2=2-ClPh; **12a**: R_1=CH_3, R_2=4-ClPh;

13a: R_1=CH_3, R_2=2,4-Cl_2Ph; **14a**: R_1=CH_3, R_2=4-BrPh; **15a**: R_1=CH_3, R_2=3-FPh;

16a: R_1=CH_3, R_2=4-FPh; **17a**: R_1=CH_3, R_2=4-OCH_3Ph; **18a**: R_1=CH_3, R_2=Ph;

19a: R_1=CH_3, R_2=2-furyl;

Figure 11. Series of 1,3,5-trisubstituted-2-pyrazoline derivatives as Antifungal.

Anticancer Activity

M.Shaharyar *et al.* synthesized series of benzimidazole which carries 2-pyrazolines and then tested as well as belonging to different panels these compounds against various cancer cell lines such as renal, breast, colon, melanoma, prostate, and so on. The most active compound of the series was found to be 2-[5-(3,4-dimethoxyphenyl)-1-phenyl-4,5-dihydro-1H-3-pyrazolyl]-1Hbenzimidazole. Based on close examination of the substituent it was concluded that the role of electron donating group on the phenyl ring at C5 of the phenyl ring had a great influence on anticancer activity[42].

Eman M. Flefel *et al.* primarily synthesized a newly substituted pyrazole, thiazole, and 1, 2, 4-triazole derivatives and then reported. Among all the sugar hydrazones and their acetylated derivatives as yet derived acyclic *C*-nucleoside analogs, and the thioglycosides of the 1, 2, 4-traizole derivatives were also prepared. Compounds that were synthesized as well as studied and several compounds showed significant antitumor activities in the tested results[43].

Conclusions

Pyrazoline, a heterocyclic compound that exhibits a two nitrogen in the ring nucleus which synthesized via cyclization of chalcone from the reaction of substituted aldehydes and ketones in the presence of basic conditions. Medicinally pyrazoline and its derivatives showed the diversity of biological activities such as antibacterial, antimicrobial, anti-inflammatory, antioxidant, antidiabetic, anticancer, antifungal, antidepressant, anticonvulsant, analgesic, and monoamine oxidases (MAOs) and elucidated by spectral analysis and also characterized by elemental analysis.

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Received: 14 May 2019
Accepted: 20 July 2019

REVIEW / ARTÍCULO DE REVISIÓN

Secondary metabolites in plants: main classes, phytochemical analysis and pharmacological activities.

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DOI. 10.21931/RB/2019.04.04.11

Abstract: Plants are an essential source of chemical compounds with different biological properties that man can use to his advantage. These substances are mainly produced as a result of chemical conversions of secondary metabolism. This article reviews the main classes of secondary metabolites that synthesize plants as well as their characteristics and their biological functions. Examples are provided for each of the classes. Emphasis is placed on the methods of extracting secondary metabolites and phytochemical screening, as well as on the main pharmacological activities described for the MS.

KeyWords: Secondary metabolites, extraction, phytochemical screening, pharmacological activities.

Introduction

Plants are autotrophic organisms. In addition to the primary metabolism present in all living beings, they have secondary metabolism that allows them to produce and accumulate compounds of a very diverse chemical nature. The compounds derived from secondary metabolism in plants are called secondary metabolites (SM)[1].

The SM of the plants constitute a large and varied group of organic compounds that are synthesized in small quantities; they have no direct function in essential processes such as photosynthesis, respiration, solute transport, protein synthesis, nutrient assimilation, and the differentiation or formation of carbohydrates, proteins, and lipids. They appear in plants as a result of chemical conversions and even when many of their functions are unknown, it is believed that SM are related to the defense of the plant against predators and pathogens, they also act as allelopathic agents that influence growth, survival, and reproduction of other plants, attract seed pollinators and serve to face adaptation to sudden changes in temperature, humidity, light intensity and drought[2,3,4]. The SM of the plants have a differential distribution between taxonomic groups in the Kingdom of the plants, and therefore they are useful for Systematic Botany[5].

The study of biological functions and the structure of SM are of great importance because from this knowledge, it has been possible to use them in different industries. Many SM are used as aromas, resins, gums, flavor enhancers, as insecticides and herbicides[6,7,8,9,10]. On the other hand, the majority of SM have found utility in the pharmaceutical industry, given a large number of pharmacological activities that are known about them[11]. This article summarizes the main classes of SM in plants, some techniques for their extraction from natural sources and phytochemical screening, as well as the main pharmacological activities described for fundamental classes of SM.

Classes of SM in plants

Several criteria have been considered for the classification of SM: chemical structure (presence of rings or sugars), composition (containing nitrogen or not), their solubility in organic solvents or water, and the biosynthetic pathway. Of them, the most common criterion used for grouping the SM in plants has been the biosynthetic pathway. According to this, the SM in plants can be divided into three large groups: terpenes, phenolic compounds, and alkaloids[12].

Terpenes: they constitute the largest group of SM in plants to which more than 40,000 different molecules are allocated[12]. From the chemical point of view, they are non-saponifiable lipids since fatty acids do not intervene in their formation. They are also known as isoprenoids, since the basic structural unit that forms them is the isoprene molecule[13]. They are classified according to the number of isoprene units they contain. The most straightforward class of all is hemiterpenes with a single isoprene unit and five carbons in its structure. The best-known hemiterpene is isoprene, a volatile product that emerges from photosynthetically active tissues. With two groups, the terpenes are classified in monoterpenes, with three units in sesquiterpenes, with four in diterpenes, with six in triterpenes, with eight in tetraterpenes, and with more than 10 in polyterpenes[14,15] (Table 1).

Many plants contain terpenes in their flowers and fruits as mixtures of volatile compounds with specific odors; among them, we can mention lemon, mint, eucalyptus, ginger, and great basil[24]. Terpenes have several biological functions and participate in both the primary metabolism and the secondary metabolism of plants. In the central metabolism they are photosynthetic pigments (carotenes), electron carriers (ubiquinone and plastiquinone) regulators of plant growth and development (giberilins, strigolactones, brassinosteroids), are part of cell membranes (phytosterols) and participate in protein glycosylation[25]. In secondary metabolism they participate as defense molecules, toxic compounds and food deterrents for insects. In some plants they are the responsible molecules for attracting pollinators, or they function as dispersers[26,27,28,29].

They are synthesized from primary metabolites by two pathways: that of mevalonic acid, active in the cytosol, in which three molecules of acetyl-CoA condense to form mevalonic acid that reacts to form isopentenyl diphosphate (IPP) or the pathway of methyleryritol phosphate (MEP) that functions in chloroplasts and also generates IPP[24].

Phenolic compounds: they are chemical compounds containing a hydroxyl group directly attached to an aromatic hydrocarbon. Chemically, phenolic compounds are a very diverse group of SM. The simplest representative of this class is phenol[30,31,32,33]. The most important criterion for classifying

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Class	Number of isoprene units	Number of carbon atoms in the structure	Examples	Usages	Isolated from	References
Hemiterpene	1	5	Isovaleramide	Anticonvulsant	*Valeriana povonii*	16
Monoterpenes	2	10	Geraniol	Fragrance material	*Palmarose oil*	17, 18
Sesquiterpenes	3	15	Farnesol	Source of perfume	*Citrus aurantium*	19
Diterpenes	4	20	Vitamin E	Antioxidant	*Corylus avellana L.*	20
Triterpenes	6	30	Squalene	UV protector	*Olive oil*	21
Tetraterpenes	8	40	Carotene	Antioxidant	*Rhodotorula glutinis*	22
Polyterpenes	>9	>40	Rubber	Restorative material (endodontics)	*Palaquium gutta*	23

Table 1. Classes of terpenes according to the number of isoprene units.

phenolic compounds is the number of carbons present in the molecule. According to this criterion, the phenolic compounds are classified into simple phenols, acidic phenols, acetophenones, and phenylacetic acids, hydroxycinnamic acids, coumarins, flavonoids, biflavonyls, benzophenones, xhantones, stilbenes, quinones and betacyanins (Table 2). Lignans, neolignans, tannins, and phlobaphenes also belong to this group. The latter are polymers and have more complex structures[34,35].

Phenolic compounds are synthesized in plant cells by the shikimic acid pathway or the malonate/acetate pathway (or both, for example, flavonoids)[36]. The shikimic acid pathway provides the synthesis of phenylalanine and cinnamic acids and their derivatives (simple phenols, phenolic acids, coumarins, lignans, and phenyl propane derivatives)[37,38]. The polyacetate pathway provides quinones and xanthones. The mixed pathways combine precursors of both the shikimic acid pathway and the polyacetate pathway. This is the case of flavonoids[39,40].

Phenolic compounds fulfill various functions in plants: they oxidize quickly and act as antioxidants[41,42,43], they act as plant growth inhibitors[44], seeds accumulate significant amounts of phenols that act as filter so that oxygen does not reach the embryo and inhibit its germination[45]. Phenols also accumulate on surfaces of leaves, capturing up to 90% of UV radiation[46]. Phenols confer aromas and colors to the fruits making them appetizing for herbivores, which favors the dispersion of seeds through feces[47]. Plants compete with each other to preserve their territories, and in this process (allelopathy) the phenols participate[48]. Plants also defend themselves against the attack of pathogens by synthesizing phytoalexins that are toxic to microorganisms and their presence prevents infections[49]. Phenols also protect plants by generating bitter flavors or textures that are unpleasant for herbivores[50].

Alkaloids: alkaloids constitute another large and diverse group of SM that includes molecules isolated primarily from vascular plants[51]. Plants generally produce a complex mixture of alkaloids, in which a significant constituent dominates[51]. In a given plant the biosynthetic origin of the alkaloids present is common, even if their structures are slightly different[51]. Another interesting observation is that the concentration of alkaloids varies considerably from one part to another of the same plant, and even in some parts it may not contain those at all[52]. Alkaloids are also found in fungi, bacteria, and animals[53]. They include an atom of nitrogen in their structure, are toxic compounds and respond to common precipitation reactions[54,55].

Even when there is no uniform classification of alkaloids, several criteria have been used in order to classify them: biosynthetic origin, presence of basic heterocyclic nucleus in the structure, pharmacological properties, and distribution in plant families[56]. Among these criteria, the biosynthetic origin of the alkaloids has been used quite frequently. According to this criterion the alkaloids are classified as true alkaloids, protoalkaloids, and pseudoalkaloids[57]. Pure alkaloids strictly comply with the fundamental characteristics of the alkaloids. The majority of the alkaloids found in plants belong to this group. They contain an intracyclic nitrogen, have basic character and are compounds of high reactivity, even in small quantities. In plants, they can be found free, although they predominate as salts. The precursor compounds of the true alkaloids are amino acids (L-ornithine, L-lysine, L-tyrosine, L-tryptophan, L-histidine, and L-arginine). Some pure alkaloids have been derived from anthranilic and nicotinic acids[57,58]. The protoalcaloides constitute a smaller class in number. In this group, the nitrogen atom is not part of the heterocycle, and they derive from L-thyroid, L-tryptophan, and L-ornithine. They can also be considered aromatic amines[55]. The pseudoalkaloids contain heterocyclic rings with nitrogen but are not derived from amino acids. They are formed by subsequent incorporation of nitrogen into compounds originally free of this element. To this group belong terpenic alkaloids[58].

Although the presence of alkaloids is not vital for the plant, there is evidence that indicates the roles that these substances play in vegetables. As for the functions they fulfill, at first, they were considered waste products of nitrogen metabolism, nitrogen reservoirs in the plant, and were even mentioned as growth regulators. Today it is accepted that the role they play is to defend the plant against insects and herbivores due to its toxicity and deterrent capacity. While some serve to protect the plant from predators or microorganisms (toxic or repellent substances), others do so to compete with other plant species in a given habitat (allelopathic substances)[59,60].

Alkaloids have remarkable physiological properties and toxicological that are exerted primarily on the nervous system central, with predominance in some of its levels (Table 3). For these reasons, they can be used as drugs. Prolonged use of any of these compounds produced in man accustoming, which constitute true drug addictions, with physical and psychic dependence and an increase in the tolerance 57,59. To date, around 15,000 alkaloids have been isolated from plants. If it is considered to have been examined less than 25% of the upper plant species of the planet, it is clear that there is still

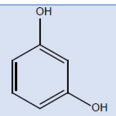
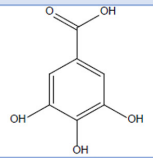
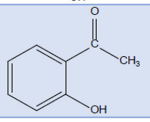
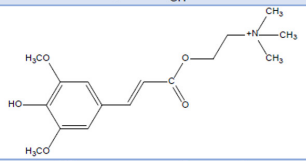
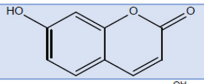
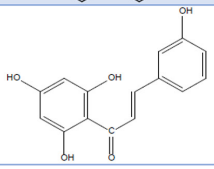
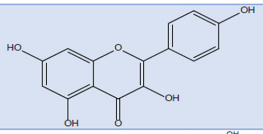
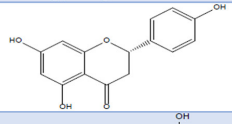
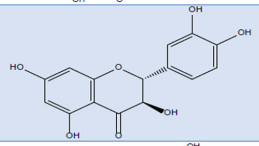
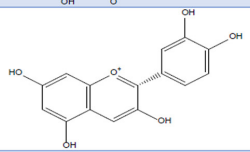
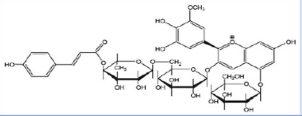
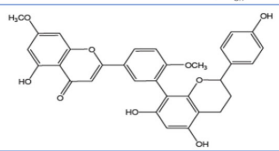
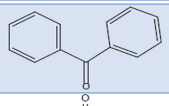
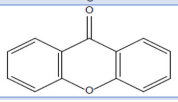
Skeleton structure	Class	Characteristics	Examples	Structure
C6	Simple phenolics	Substituted phenols	Resorcinol	
C6 - C1	Phenolic acids and related compounds	A carboxyl group substituted on a phenol	Gallic acid	
C6 - C2	Acetophenones and phenylacetic acids	Are rarely found in nature	2-hydroxyacetophenone	
C6 - C3	Cinnamic acids, cinnamyl aldehydes, cinnamyl alcohols	Are commonly found in plants as esters of quinic acid, shikimic acid, and tartaric acid or as sugar esters	Sinapoyl choline	
C6 - C3	Coumarins, isocoumarins, and chromones	They possess an oxygen heterocycle as part of the C3-unit	Umbelliferone	
C6-C3-C6 (C15) Flavonoids	Chalcones, aurones, dihydrochalcones	Two benzene rings are linked together by a group of three carbons	Butein	
	Flavones	Contains a ketone group, and an unsaturated C-C bond	Kaemferol	
	Flavanones	Contains a ketone group	Naringenin	
	Flavanonols	Occur in association with tannins	Taxifolin	
	Anthocyanidins	The heterocycle is a pyrilium kation	Cyanidin	
	Anthocyanins	Are water-soluble glycosides of anthocyanidins	Pentanin	
C30	Biflavonyls	Are dimers of flavones or methylated derivatives	Ginkgetin	
C6-C1-C6	Benzophenones	Are aromatic ketones	Benzophenone	
	Xanthones	Are yellow pigments in flowers	Xanthone	

Table 2. Classes of phenolic compounds according to the number of carbons in the structure.

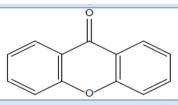
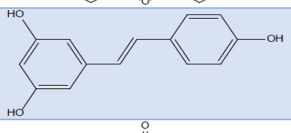
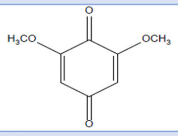
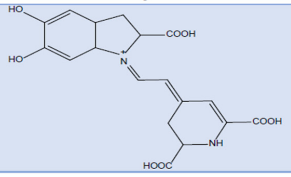
	Xanthones	Are yellow pigments in flowers	Xanthone	
C6-C2-C6	Stilbenes	Two benzene rings are linked together by a group of two carbons	Resveratrol	
C6, C10, C14	Quinones	Oxidizing agents	2,6-dimethoxybenzoquinone	
C18	Betacyanins	Are red pigments and contain nitrogen	Betanidin	

Table 2. Classes of phenolic compounds according to the number of carbons in the structure.

Class	Name	Biological properties	Plant family
True alkaloids	Atropine	Anticholinergic drug	Solanaceae
	Nicotine	Potent poison that at low doses is stimulating	Solanaceae
	Morphine	Narcotic and anesthetic properties	Papaveraceae
Protoalkaloids	Mescaline	Hallucinogen	Cactaceae
	Hordenine	Stimulant of the central nervous system	Cactaceae
	Ephedrine	Sympathetic nervous system stimulant	Ephedraceae
Pseudoalkaloids	Aconitine	Highly poisonous	Ranunculaceae
	Theobromine	Stimulating the central nervous system	Malvaceae
	Coniine	Highly poisonous	Apiaceae Sarraceniaceae

Table 3. Some biologically relevant plant-derived alkaloids.

a wide field for his research. Because of its pharmacological and medicinal importance there is an excellent motivation to continue with the chemical-biological study of the alkaloids. This is one of the most important secondary metabolites of plants with therapeutic interest[60].

Phytochemical analysis

Phytochemical studies generally are based on previous ethnobotanical and ethnopharmacological knowledge about plants and often constitute hypothesis-driven studies. The general methodology for studying SM from plants comprises several stages: extraction from natural sources, the phytochemical screening of extracts to determine qualitatively the main chemical classes of SM present in the plant, the purification of individual components and elucidation of their chemical structures, the biological activity studies through *in vitro*/*in vivo* assays and the toxicity-cytotoxicity studies on organisms or cells. The methodology involves a combination of different analytical techniques (Figure 1). In this methodology, the method of extracting secondary metabolites and their identification in phytochemical gait is crucial. These two aspects are reviewed below.

Extraction

The initial step during extraction is the preparation of plant tissues. The extraction can be done on clean and ground leaves, barks, roots, fruits, and flowers, from fresh or dried plant material. In order to maintain the freshness of the samples and avoid possible chemical damage, it is recommended that the interval between harvest and the initiation of extraction does not exceed 3 hours since the plant tissue is fragile and tends to deteriorate faster than dry tissue[61]. Otherwise it is preferable to dry the plant by air-drying, microwave-drying, oven-drying or lyophilization. Each of these methods has advantages and disadvantages that the researcher should consider[62,63,64,65]. Another critical point to view during pre-treatment of the plant is the particle size of plant material. The smaller the particle size, the higher the area of contact between the plant material and the solvent, and consequently the more effective the extraction of the chemicals[66].

Extraction is the process that allows separating SM from the plant by using solvents of different polarity. As a result of the extraction remains two phases: a liquid phase containing solubilized metabolites and a solid containing the insoluble cell debris. Conditions as temperature and time are important factors to achieve high-quality extracts[67]. The most common extraction methods are maceration, infusion, percolation, decoction, Soxhlet or continuous extraction, microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), accelerated solvent extraction (ASE), and supercritical fluid extraction (SFE)[68].

Maceration is a solid-liquid extraction technique[69]. The

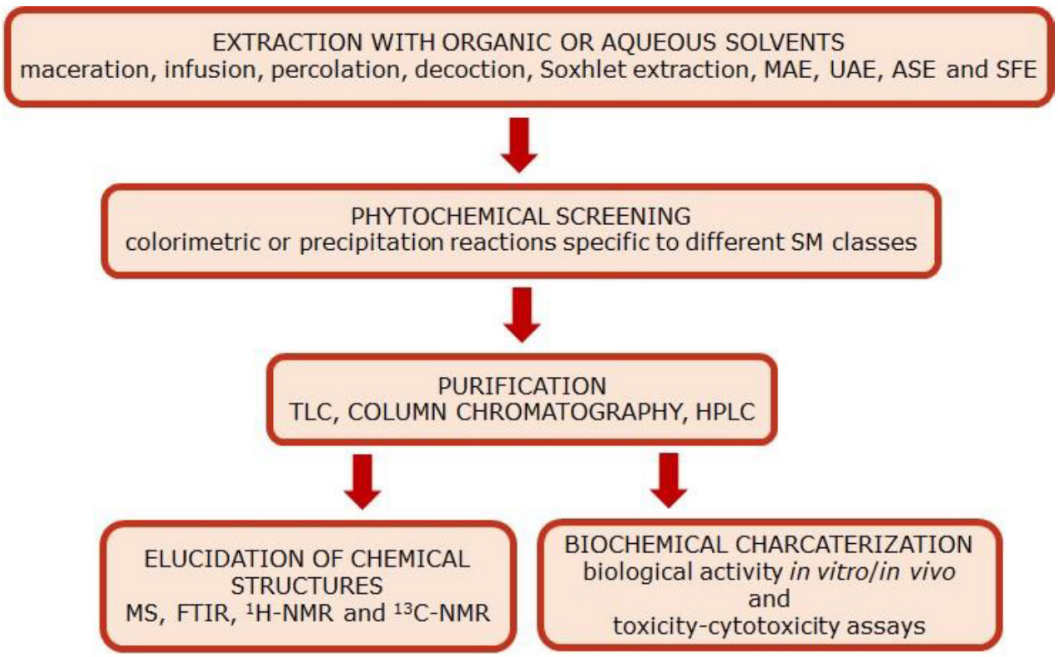


Figure 1. A brief summary of the general methodology for studying bioactive compounds from plants. SM-SM, MAE-Microwave Assisted Extraction, UAE-Ultrasound Assisted Extraction, ASE- Accelerated Solvent Extraction, SFE-Supercritical Fluid Extraction, TLC-Thin Layer Chromatography, HPLC-High Performance Liquid Chromatography, MS-Mass Spectrometry, FTIR-Fourier Transform Infrared Spectroscopy, 1H-NMR-proton Nuclear Magnetic Resonance and 13C-NMR-carbon Nuclear Magnetic Resonance.

method consists of using a solvent or a mixture of solvents having different polarities and a particular affinity with compounds that are going to be extracted. The mixture (plant-solvent) is placed in a container with lid and let it rest for two or three days until the compounds could be transferred from vegetal tissues to the solvent. This method is widely used with soft vegetal material[70]. The infusion is a maceration process too but uses shorter extraction times and the solvent usually is cold or boiling water. This method is used to obtain a diluted solution of compounds that are easily extracted[67]. The decoction is a more convenient method for extracting water-soluble compounds from roots and barks that are stable at high temperatures and usually results in oil-soluble compounds compared to maceration and infusion[71]. The decoction method is carried out boiling the vegetal material in water by 15 minutes, then cooling, filtering and adding water until it reaches the desired volume[67]. Finally, percolation is an extraction method that shares similar fundamentals. The method uses a conical filtration camera open on both sides where the material is placed with the solvent. The camera is connected to a flask and once the material is inside the camera, the system is opened to let it strain. The solvent can be used several times to rinse the material until the saturation point[68]. Another way to conduct the extraction of SM is using a Soxhlet apparatus. In this method, a Soxhlet extractor, a condenser, and a round bottom flask are used. The finely ground vegetal material is loaded into the thimble of a strong paper of cellulose and then placed in the Soxhlet extractor. The solvent goes in the round bottom flask, and it needs to be heated. The solvent vapors go into the thimble and then return to the flask after being condensed. The system is left, at least for sixteen hours[72]. The main advantage of Soxhlet extractor is the use of smaller quantity of solvent compared to maceration. However, the exposure to hazardous and flammable organic solvents, with potential toxic emissions is high[68].

The microwave-assisted extraction (MAE) is another popular and easy technique in which the sample is heated using electromagnetic radiation. This method improves the extraction of intracellular compounds due to the rupture of the cellular wall. Increasing temperature, the humidity inside the cell is transformed into vapor; as a result the intracellular pressure increases and the lysis is provoked. This factor comes together with other effects in the solution that benefit the interaction of the compounds to be extracted with the solvent. The main disadvantage is the possible thermal degradation[73,74]. The ultrasound-assisted extraction (UAE) facilitates partition of analytes with the occurrence the fragmentation of cell wall provoked by the collisions between the electromagnetic waves and the particles. There are two forms of applying it: in direct contact with the sample or using an ultrasound bath, where the contact is given through the walls of the bottle. In the first case the efficacy is 100 times higher than the second one. The procedure is simple, low cost and can be used in both small and larger-scale extraction[75].

In the method called accelerated solvent extraction (ASE), high temperatures and high pressures are applied to the samples. The time required to achieve the extraction is reduced to one hour, which is an advantage in comparison with other methods (48h or 72h). This is a method that separates efficiently analytes from the matrix. Since the nature of the solvent is an important fact in each method of extraction, the solvents used in this method determine the efficiency of the results. The solvents system, temperature, and time of action are determinants in accelerated solvent extraction. In the case of extraction of bixin the most efficient mixture of solvent was cyclohexane: acetone (6:4) at 50°C for 5 minutes[76].

The supercritical fluid extraction (SFE) involve a supercritical fluid. It is a substance that has both physical properties of gas and liquid in its critical point. Pressure and temperature are determinant factors to reach this critical point. The utility of the supercritical fluid is their gas behavior and solvating capacity of liquids. The most used solvent is CO_2

due to its capacity to dissolve nonpolar analytes, it has low cost and low toxicity[77,78]. This method is very selective, very fast, has a high yield percentage and the resultant product has high quality. It has been used in the coffee industry to decaffeinate coffee, or in other industries to extract essential oils[78].

Phytochemical screening

The phytochemical screening is a fast and cheap procedure to determine the main classes of SM or groups of substances that a plant contains. Since each class or group of SM is related to specific biological activities, based on the results obtained in the preliminary phytochemical screening it is possible to guide further research to determine the biological activity of the species in question and the active principles involved. The phytochemical screening consists in executing chemical reactions on aliquots of the plant extracts. The reactions can be based on liquid-liquid partition with solvents, in chemical reactions that produce colorimetric changes, fluorescence, or precipitates of a specific color. Among the SM to be analyzed are alkaloids, anthraquinones, flavonoids, phenols, saponins, sterols, tannins, quinones, coumarins and terpenoids. There are numerous reviews summarizing the principles of chemical reactions and the qualitative changes that can be are observed[68,79,80,81]. A summary of the experimental protocols for the phytochemical screening methods is shown in Table 4.

Secondary metabolite	Name of test	Reactants	Expected result if positive
Alkaloid	Dragendorff's test	Solution of potassium bismuth iodide	A red precipitate
	Wagner´s test	Iodine in potassium iodide	A brown/reddish precipitate
	Mayers test	Potassium mercuric iodide	A yellow coloured precipitate
	Hager ´s test	Saturated picric acid solution	A yellow coloured precipitate
Saponins	Froth test	Water	Formation of 1 cm layer of foam
	Foam test	Water	Produced foam persists for ten minutes
Phytosterols	Salkowski's test	Chloroform, concentrated sulphuric acid,	Appearance of golden yellow color
	Libermann Burchard's test	Chloroform, acetic anhydride, concentrated sulphuric acid	Formation of brown ring at the junction
Phenols	Ferric chloride test	Ferric chloride solution	Formation of bluish black color
Tannins	Gelatin test	1% gelatin solution, sodium chloride	Formation of white precipitate
Flavonoids	Alkaline reagent test	Sodium hydroxide solution	Formation of intense yellow color, which becomes colorless on the addition of dilute acid
	Lead acetate test	Lead acetate solution	Formation of a yellow color precipitate
Diterpenes	Copper acetate test	Copper acetate solution	Formation of emerald green color
Glycosides	Modified Borntrager's test	Ferric chloride, benzene, ammonia solution	Formation of rose-pink color in the ammonical layer
Cardiac glycosides	Legal's test	Sodium nitroprusside in pyridine, sodium hydroxide	Formation of pink to blood-red color
Carbohydrates	Molisch's test	Alcoholic α-naphthol solution	Formation of the violet ring at the junction
	Benedict's test	Benedict's reagent	Orange-red precipitate
	Fehling's test	Fehling's A & B solutions	Formation of red precipitate
Proteins and amino acids	Xanthoproteic test	Concentrated nitric acid	Formation of yellow color
	Ninhydrin test	Ninhydrin reagent	Formation of blue color

Table 4. A summary of the phytochemical screening methods.

Pharmacological activities of secondary metabolites

Plants have the ability to synthesize a vast and diverse group of SM. Many of them constitute bioactive substances that plants use as defense molecules. These molecules interact with specific targets in microorganisms or animal cells to exert some biological activity that neutralizes them. On the other hand, the diversity of metabolic pathways that plants use in the production of SM guarantees the existence in these defense molecules of specific structures useful to develop new drugs and medicinal products. That is why plants constitute an important source of substances that can be used for improving health and/or curing diseases. Among the beneficial pharmacological activities of the plants stand out antitumor, antioxidant, and antibacterial and activities[82,83].

Special attention has been devoted to the antitumor activity of SM. According to the World Health Organization, among the causes of death that most affect humanity today cancer is found[84]. Even when there are numerous alternatives for cancer treatment, research is continuing today to find new molecules from natural sources with better treatment effectiveness or able to alleviate the toxic effects of treatments[85]. Examples of isolated metabolites of plants with antitumor activity are lupeol, asiatic acid, celastrol, aurapten, ursolic acid, saidmanetin and indole-3-carbinol and hypericin. These substances have been shown to affect signaling to control cell growth and apoptosis, immune response and stromal microenvironment[85-95].

Another health problem that has been in focus on the action of medicinal plants is the antimicrobial resistance. It is estimated that around 25 thousand patients die per year in the European Union, due to infections caused by resistant bacteria[96]. In the United States, it is estimated that resistant bacteria cause around 77 thousand deaths per year[97]. These estimates give a clear idea that the search for new molecules with antimicrobial activity is a priority in basic research and necessity of the pharmaceutical industry. The antimicrobial activity of many plant extracts has demonstrated to be effective against Gram-positive and Gram-negative[98,99]. Besides, several authors have pointed out the possible synergy between antibiotics and plant extracts[100]. In the case of polyphenols, the antibacterial activity is based on the ability of these compounds to inhibit growth, reproduction, respiration, and any other vital function of microorganisms. This action is performed by the oxidation of specific enzymes, which inhibit some critical functions, such as breathing. It is also reported that polyphenols bind to DNA chains disrupting protein synthesis in microorganisms. Other authors suggest that some polyphenols can break the cell membranes of microorganisms, producing cell apoptosis[101,102]. It is also known that monoterpenes can interact with the phospholipids of cell membranes of many microorganisms due to their lipophilic nature. As a result, the ordered structure of the membranes is interrupted, thus causing cell lysis[103,104].

The antioxidant activity has also been studied from plant extracts. It is mainly related to the presence of polyphenols or phenolic compounds. Flavonoids act primarily as buffers and capture free radicals to generate the flavinic radical, much less reactive since in their structure the missing electrons are more delocalized. Also, flavonols such as quercetin can chelate transition metal ions such as iron or copper, preventing the formation of reactive oxygen species[105,106].

Conclusions

Currently, phytochemical research is aimed at isolating and identifying compounds synthesized by plants with pharmacological activities of importance for the treatment of human diseases. The development of efficient methods of extraction and the battery of methods that exist for the scrutiny of the extracts of these medicinal plants allow more profound studies on the pharmacological activities of metabolites and their potential application in human health.

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Received: 20 September 2019
Accepted: 25 October 2019

NEWS AND VIEWS

Prevalence of Human papillomavirus (HPV) genotypes in Ecuadorian women.

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DOI. 10.21931/RB/2019.04.04.12

Abstract: Infection with the human papillomaviruses (HPV) is now well-established as the leading cause in the development of cancer of the uterine cervix. HPV 16 and 18 are described as the most prevalent high-risk genotypes, involving about 70% of registered cervical cancer cases worldwide. Among women in Ecuadorian population, cervical cancer is the second most frequent malignancy, and about 75% of active sexual women will be infected with at least one type of HPV genotypes throughout their lives. In the present investigation, the prevalence of HPV genotypes was determined from samples collected at Society for the Fight Against Cancer (SOLCA) in Ecuadorian women between 18 to 78 years. The participants underwent a Pap test, biopsy, cervical and vaginal swab for HPV detection and genotype identification. The results show the high prevalence index of HPV genotype 16 and 58, with a higher burden from the second to the fourth decade of life.

KeyWords: Human papillomavirus, HPV 16 and 18, cervical cancer, Ecuadorian women.

Introduction

Epidemiological studies have established human papillomavirus (HPV) infection as the primary cause of invasive cervical cancer (ICC) and a relevant factor in other anogenital diseases[1,2]. Cervical cancer is the second most common cancer and is now the first leading cause of cancer deaths in South America women[2].

HPV genome is a circular double-stranded DNA molecule of 8000 base-pair, which contains an average of eight open reading frames, divided into three regions. The first is the upstream regulatory region (URR), which has the regulatory function of the transcription of the viral genes E6 and E7. The second is an Early region, which consists of six open reading fragments (E1, E2, E4, E5, E6, and E7), which code for nonstructural proteins involved in replication and oncogenesis, but the central molecules in the replication process are the viral proteins E6 and E7, which interact with host cell proteins to induce proliferation and malignant transformation of cells[3]. The third is the late region, which encodes the structural proteins L1 and L2[4].

Over 230 HPV genotypes have been identified, and about 40 of them can infect the anogenital and mucosal region[5,6]. Genital HPV types have been classified into high oncogenic risk groups usually associated with ICC and low oncogenic risk groups which is found mainly in genital warts. However, there are discrepancies related to the categorization of many HPV types with possible oncogenic risk[7]. Nowadays, the International Agency for Research on Cancer (IARC) only recognize 12 genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59) as high risk, from which HPV 16 and 18 are the most prevalent worldwide, involving about 70% of cervical cancer cases[5].

The HPV infection is highly transmissible, usually by skin to skin contact, although not necessarily sexual intercourse[8]. This kind of disease can occur at any age, and it is estimated that more than 50-75% of sexually active women are infected with at least one HPV genotype throughout their[6,9]. Regarding Ecuador, there is no data published by PAHO/WHO on the prevalence and incidence of HPV. Nevertheless, according to the National Institute of Statistics and Censuses in Ecuador (INEC), cervical cancer is the second most common malignancy with a prevalence in younger women than 35-44 years of age, declining in elderly women[7,8].

The objective of this work is to estimate the prevalence of high-risk HPV genotypes in Ecuadorian women, focusing on genotypes 16 and 18 associated with cervical cancer.

Clinical samples

The criteria used for the clinical sample was: adult women between 18 – 78 years, born and living in Ecuador from different urban areas (table 1)[7,8]. The samples were collected at Society for the Fight Against Cancer (SOLCA for its abbreviation, Spanish) Hospital from women diagnosticated HPV, cervical cancer, cervicitis, cervical dysplasia or without any cervical problem[5,8–10].

The women participated in two procedures, including their clinic history through a survey (age, marital status, pregnancies, smoking, previous diagnoses, previous Pap test and number of sexual partners), and physical exams for sample collection[5]. The methods used for sample collection were: Pap test, vaginal swab, cervical swab and biopsy (Table 1). The Pap test, also called Pap smear, consists of a speculum that is inserted into the vagina, and spatula or brush will scrape cell samples from the cervix[5,8,9]. The vaginal and cervical swab method uses three cotton swabs that are rubbed simultaneously in the area of vagina and cervix while the speculum is inside of the vagina[5]. The last way is the biopsy, which consists of removing a sample of tissue from the lower part of the uterus with laser or scalpel. All the samples were analyzed in different laboratories within the country and abroad.

Molecular analysis

The analysis of the samples collected allows to knows the prevalence of HPV and its different genotypes in Ecuadorian women. There are three molecular analysis: DNA extraction, HPV detection, and genotype identification. The various techniques for DNA extraction and purification were Cetyl Trimethyl Ammonium Bromide (CTAB) method, spectrophotometry or commercial methods such as AmpliLute Liquid Media Extraction Kit or kit PVH Fast 2.0[8–10]. The detection and genotyping of HPV were made by conventional PCR, reverse blood strip, genotyping method, linear array

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HPV genotyping test and commercial methods such as HPV GenoArray Diagnostic Kit[5,8-10].

The assays made focuses on the characterization of forty (40) types of HPV that produce infection in the genital tract, especially, on the twelve (12) genotypes considered of high risk because of cause cervical cancer, including 16, 18 y 58.

HPV prevalence

A total of 6 research included in this study. Table 1 shows the percentage and numerical amounts of HPV prevalence of each population, which demonstrates that most of the research exceeds 50% of women with positive HPV.

HPV 16, 58 and 66 were the most commonly detected type (Table 2). In many cases, multiple HPV genotypes were identified, including high and low-risk genotypes.

Conclusions

The overall frequency of high-risk HPV genotypes detected in women population was high for HPV 16 and 58 and low for HPV 18 in Ecuador. Moreover, evidence was found of a high prevalence rate of HPV 66 of low-risk considered as possible oncogenic risk genotype. The data are very similar to those obtained around the world, suggesting once again that strategies on sexual education and prevention methods in adolescents could reduce the incidence and mortality rate of cervical cancer in the Ecuadorian population.

Ecuador is among countries with the highest incidence of cervical cancer in Latin America with Colombia, Brazil, and Peru[9]. According to INEC in 2017, 755 deaths for cervical cancer were recorded. Therefore, HPV vaccination is the best strategy to prevent and protect Ecuadorian women from ICC. In Ecuador, this vaccine is freely distributed by "Ministerio de Salud Pública".

Besides, the American Cancer Society recommends annual tests such as Pap test, vaginal, and cervical swab, especially in women over 20 years of sexually active and HPV DNA test for women over 30 years.

	Population	HPV +	Prevalence	Age	Collection Methods
1[5]	1,581	689	43 %	20 - 70	Pap test
2[8]	124	84	68%	18 - 55	Pap test / Biopsy
3[9]	302	73	24%	18 - 78	Pap test / vaginal swab /cervical swab
4[10]	164	141	86%	19 - 77	Biopsy
5[11]	63	55	87%	-	Biopsy
6[12]	166	113	68%	-	Cervical swab / Biopsy

Table 1. Numerical and percentage prevalence of HPV, range of ages of each population and methods of collection of clinical samples.

Table 2. Prevalence of HPV genotypes 16, 18, 58 and 66 in Ecuadorian women.

	Gen 16	%	Gen 18	%	Gen 66	%	Gen 58	%
1[5]	87	5.5%	50	3.1%	41	2.6%	32	2%
2[8]	3	2.4%	1	0.8%	6	4.8%	1	0.8%
3[9]	7	2.3%	1	0.3%	1	0.3%	6	2%
4[10]	59	35.9%	4	2.4%	11	7.8%	43	30.5%
5[11]	12	31.7%	2	4.9%	5	12.2%	20	48.8%
6[12]	39	23.5%	4	2.4%	1	0.6%	18	10.8%

Discussion

There are few studies about the prevalence of HPV and its different genotypes in Ecuadorian women. Nevertheless, the data collected showed considerable evidence of the presence of HPV in participants because the incidence is approaching or exceeding 50% in most of the studies.

Based on the results, the twelve genotypes of HPV consider of high risk are present in Ecuadorian women, including genotype 16, 18, and 58 that appear in all research. Most of these genotypes are associated with cervical problems such as cervical dysplasia or cervicitis, and cancer. Each high-risk genotype can produce different cervical risks and can act independently or with multiple genotypes. In most cases, numerous HPV genotypes were detected between low and high risk increasing the probability of developing cervical injuries.

HPV genotypes 16, 18, and 58 are considered the most common high-risk genotypes, so, genotype 16, a viral type with the strong transmission, persistence, and transformation, has a high prevalence index in the studies, followed by genotype 58 considered as oncogenic. However, genotype 18 had a low frequency which is in concordance with previous findings showing low percentages in South America (5% of HPV infections) compared with North America (11%) and Europe (8%). The reason of these differences in geographic distribution of HPV genotypes is unknown, however various authors have proposed complex interactions between HPV types and HLA haplotypes as a possible explanation[10].

Other criteria were the age that showed HPV prevalence in women between 20 – 40 years during their active sexual life. On the other hand, the genotype 66, into the category of low risk, was the most prevalent gen of low risk in Ecuador.

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Received: 20 May 2019
Accepted: 2 September 2019

NEWS AND VIEWS

Therapeutics and prospects of Interleukin 2.

Nicole Lovato and Leandro Santiago Padilla.

DOI. 10.21931/RB/2019.04.04.13

Abstract: Interleukin-2 was discovered back in 1983 as an autocrine growth factor for cultured T cells and was the first biological product created through the use of recombinant DNA. IL-2 tumor immunotherapy performed the first historical clinical demonstration of the possibility to cause an effective anticancer immune reaction, mediated by cytotoxic lymphocytes activated from IL-2 stimulation. The Interleukin 2 receptor is a heterotrimeric protein that is composed of three peptide chains: the alpha chain, the beta chain and the gamma chain of the common cytokine receptor. There are 3 majors' ways of interfering with the IL-2/IL-2R to use it as treatments: Antibodies, Aptamers, and punctual mutagenesis. Recent studies have shown, that Il-2 therapies for cancer, specifically targets restoring the individual's natural antitumor immune response. HIV directed treatments have demonstrated the necessity of introducing the IL-2 complemented with the patient's antiretroviral therapy.

KeyWords: Interleukin-2, cancer, HIV, immunotherapy.

Introduction

Interleukin-2 (IL-2) was discovered as an autocrine growth factor for cultured T cells and was also the first cloned cytokine in 1983[1]. The primary function assigned to IL-2 was a strong ability to increase the proliferation and in vitro differentiation of T cells[2], and it is for this reason that it was called the T cell growth factor. According to its function in vitro, it was also admitted that IL-2 plays a critical role during the clonal expansion of antigen-directed T cells. These findings led to clinical trials to evaluate the ability of high doses of IL-2 to activate antitumor immune responses in patients with melanoma, renal cancer and other tumors[3]. IL-2 was the first biological product produced through the use of recombinant DNA technology that was applied to humans with cancer or AIDS to increase the number and function of T cells. In this way it was possible to establish the bases of what has become one of the great revolutions both in the field of biomedical and clinical medicine.

Another of the functions of IL-2 is its ability to inhibit the proliferation of pro-inflammatory T helper 17 cells and follicular helper T cells, which are central to the production of autoantibodies[4], may synergize with its effects on Treg cells in the treatment of autoimmune and inflammatory diseases. On the other hand, the challenge of using IL-2 to eliminate pathological immune responses is that the cytokine can activate the effector arm of the immune system, which carries the risk of irritating the disease. Interleukin (IL)-2 cancer therapy could be considered as the first historical immunotherapy against tumors on physio-pathological, which is developed to regulate and guide the cytokine network in an antitumor manner[5] by exogenous administration of antitumor cytokines, whose endogenous production progressively decreases the cancer development[6]. IL-2 tumor immunotherapy performed the first historical clinical demonstration of the possibility to cause an effective anticancer immune reaction, mediated by cytotoxic lymphocytes activated from IL-2 stimulation[5].

Quaternary Structure Receptor

The Interleukin 2 receptor (IL-2R) is a heterotrimeric protein that is composed of three peptide chains: the alpha chain (IL-2Rα), the beta chain (IL-2Rβ), and the gamma chain of the common cytokine receptor (γc) (Figure 1)[1].

When the three chains that make up the IL-2R have been isolated, it was determined that IL-2Rα has a low affinity to the IL-2 receptor (binding affinity kd ~ 10 nM). IL-2Rβ alone has a very low affinity (Kd ~ 100 nM), and γc alone has no detectable binding affinity for IL-2[2]. A complex of IL-2Rb and γc bind with intermediate affinity (Kd ~ 1 nM). A complex with three subunits IL-2Rα, IL-2Rβ, and γc bind with high affinity (Kd~ 10 pM), which shows that the intermediate and high-affinity receptor forms are functional and cause changes in the cell when IL-2 binds to them[3].

Subunit	Binding affinity
IL-2 Ra	10 nM
IL-2 RB	100 nM
Yc	-
IL-2Rb and γc	1 nM
IL-2Ra, IL-2 b, and γc	10 pM

Table 1. The binding affinity of each chain that makes up the IL-2 receptor.

Signaling through the il-2 receptor

The signaling of IL-2R contributes mainly to two of the immune responses of the CD8 + T cells, which are the terminal differentiation of the effector cells in the primary responses and the memory response aspects[2].

The signaling process begins when IL-2 is first captured by IL-2Rα through hydrophobic bonds spanning a relatively weak interaction. The IL-2Rα-IL-2 complex results in a minimal conformational change in IL-2 that drive the association with IL-2Rβ and the binary complex IL-2Rβ-IL-2 is formed through polar interactions. It is important to emphasize that the extracellular dominium IL-2Rα does not interact with IL-2Rβ[1]. Once the IL-2Rα-IL-2Rβ-IL-2 complex has been formed it is recruited to γc through a very weak interaction to IL-2

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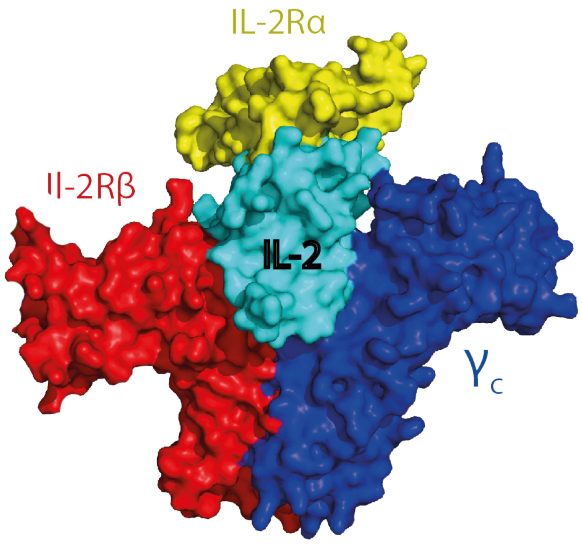

Figure 1. Surface structure of the Interleukin 2 receptor complex and its subunits. Imaged obtained using PyMOL.

and a much stronger interaction to 2Rβ to produce an IL- 2R Quaternary that is stable and of high affinity.

The creation of the high affinity IL-2-IL-2R quaternary complex leads to signal transduction, which is mediated by tyrosine kinases named Jak1 and Jak3, which in turn are associated with IL-2Rβ and γc respectively[4]. There are three tyrosine residues within the cytoplasmic tail of IL-2Rβ that are phosphorylated to promote the Shc adapter, then the activation of the pathways of other kinases called MAPK and PI-3K will be carried out and also the activation of a transcription factor called Stat5 that is responsible for the regulation of genes dependent on Stat5[5]. Finally, the quaternary complex of IL-2-IL-2R is internalized briefly in the cell where IL-2, IL-2Rβ, and γc are degraded while IL-2Rα is recycled to the cell surface.

IL2 R α regulation

Cellular expression of the alpha subunit is tightly regulated by three main enhancers located either upstream or downstream from the transcription unit[6]. Generally, resting T and B lymphocytes do not express the α subunit until they are stimulated by adjuvant cytokines. There is only a specific set on pathological conditions, like autoimmune diseases or certain hematological malignancies, that will keep the α subunit to remain expressed on the cell surface of the Lymphocytes[7]. In some cases of T-cell mediated diseases the pathology is limited to only a specific area, and nowadays there are several ways to selectively target and inhibit the high-affinity IL-2 receptor in the focalized region so other IL-2R α expressing cells remain unharmed by the treatment[7]. We will discuss this article three of the primary ways of doing so.

Antibodies. Currently, a variety of monoclonal antibodies are commonly utilized to downregulate CD25 Lymphocytes clonal expansion[8]. Basiliximab and daclizumab are two anti-IL-2Rα mAbs that have already completed phase 3 trials for preventing acute rejection in renal transplants. Daclizumab is a humanized immunoglobulin mAb, while basiliximab is a chimeric antibody. They both block IL-2Ra by competitive antagonism, thereby inhibiting the high-affinity receptor-mediated signaling while leaving unchanged IL-2 signaling by the intermediate-affinity B and Y receptors[9,10].

Aptamers. These novel molecules are similar to antibodies when comparing their recognition capabilities in both affinity and specificity, yet these single-stranded DNA or RNA molecules elicit a much lower immunogenic response, are less toxic, and are cheaper to produce. So far, aptamer has been produced against a wide range of target molecules, including proteins or even cells, where they can act as either stimulating or inhibiting ligands[11]. Shahdordizadeh *et al.* reported, for the first time, the development of a synthetic anti-human Interleukin-2 receptor alpha (CD25) DNA aptamer (Apt51) and its functionality as a CD25 inhibitor. This study, along with the fact that aptamers have a more efficient tissue penetration, showcases and forecast the possibility of using aptamers as more efficient alternative to antibodies on IL-2 based clinical therapies[10].

Mutagenesis. By introducing a particular set of mutations along the binding interface of IL-2 with the alpha subunit it is possible to disrupt their interaction. This new IL-2 mutein can, therefore, cause a decrease in CD25+ cells while still expanding CD8+ and Natural Killer cells[12]. A highly efficient strategy for creating this new muteins is through the recently awarded Novel Prize technique, Phage Display. Using this technique, it is possible to create enormous and diverse IL-2 mutein libraries that can, later on, be used as a selection method based on the desire ligand-receptor affinity[13]. There other several techniques used for the creation of new ligand mutants like protein-protein docking bioinformatics; or for creating different sets of libraries like MutS bacterial strains or error-prone PCR[14,15].

Cancer

The immune system can recognize and destroy cancer cells or inhibit tumor growth by eliciting responses from both the innate and adaptive immunity. Innate immune responses are not based antigen specificity and tend to develop relatively quickly. On the other hand, responses elicited by the adaptive immune system are antigen-specific and develop more slowly. The adaptive response is mediated by both T (cellular response) and B cells (humoral response), providing the organism not only with a "fighting" mechanism but with an immune memory as well. Therefore, the rationale thinking in immune therapies against cancer is to enhance or utilize the adaptive response since it will be the one to provide a stronger and durable response[16].

Cancer cells massive ability for proliferating is, in one

way, due to the absence of an antitumor immune response. The tumor itself can produce a subset of chemical agents that will actively affect several biological pathways, inducing, at last, a lower immune response. This is why immunotherapies currently represent one of the most efficient and promising strategies against cancer when compared to chemotherapy[17].

Currently, one of the main anticancer cytokines in humans is IL-2, which represented the first historical clinical demonstration of the possibility to generate an effective anticancer immune reaction, mediated by cytotoxic lymphocytes activated from IL-2 stimulation. Using IL-2 it was able to generate LAK cells capable of destroying tumoral tissue[18].

Historically interleukin-2 has gone through numerous clinical trials, varying from high-dose treatment to low-dose treatments. High-dose treatment case studies usually had to be held on special facilities on intensive care units due to their massive side effects including capillary leak syndrome. Another side effect was cardiovascular toxicity, but the issue was fixed by using the low-dose treatment. The optimal prognosis using IL-2 obtained during trials initially, was low subcutaneous doses for 2 weeks cycles, since there wasn't any Lymphocyte count increase after this time frame, limiting the treatment application to specific metastatic tumoral growths[19].

Interleukin 2 based immunotherapeutic, has faced several bumps along the way. Not only the well-known side effects of the initial trials hold it back, but the differential stimulation pathways caused by the interaction with its receptor made the scientific community to tag it as Treg Lymphocyte growth factor that may mediate the suppression of the anticancer immunity, despite its stimulatory effects on most other important immune cells[20]. This issue can be addressed in many ways by preventing Treg cells activation based on the previously talked mechanisms or by IL-12 and IL-21[17,21].

The new understandings regarding the antitumoral immune cytokine network are what have brought a new light into the matter. IL-2 remains as the only cytokine capable of activating a set of TH1 lymphocyte functions that are essential for anticancer immunity in humans, which highlights the importance of using it to restore human's inborn ability to prevent tumoral cells formation and/or growth. Since IL-2 immunotherapies aren't based on the molecule's specific ability to produce lymphocyte proliferation, but on restoring the natural antitumor immune response that was previously affected by an IL-2 deficiency or imbalance, the response to the treatment will be absolutely intrinsic to the individual understudy and prior/later monitoring is necessary for ensuring the treatment efficiency[22,23].

Human immunodeficiency virus (VIH)

The Human Immunodeficiency Virus (HIV) is a retrovirus that attacks and destroys CD4 lymphocytes, which are a type of cells that are part of the immune system and are responsible for the production of antibodies to fight infections caused by these external agents[24]. There are antiretroviral drugs that have helped improve the quality of life of HIV positive individuals. One possible strategy is the use of interleukin-2 (IL-2) in combination with antiretroviral therapy (ART) because, without ART, IL-2 can increase the viral load up to six times its level before treatment[25]. These increases in viral load disappear within a month. Then, IL-2 should not be used unless it is combined with ART.

According to the Central Register of Cochrane Controlled Trials (CENTRAL), they identified 25 eligible trials to determine the effect of IL-2 in patients with HIV. Interventions included the use of IL-2 in combination with ART compared to ART alone. Seventeen of the 21 trials reported an increase in the CD4 cell count with the use of IL-2 compared to the control with different measures. However, IL-2 has no significant effect on other clinically important positive outcomes, such as mortality rates, reduction in viral load, and opportunistic infections[26].

On the other hand, in laboratory investigations, IL-2 is used to encourage the reproduction of cells infected with HIV. Then, IL-2 induces the infected cells to produce new HIV particles in large quantities. That is why some researchers were hesitant to administer this medicine to humans because the least desired is to increase the production of HIV. However, they decided to carry out the investigation. The results showed that the groups taking IL-2 combined with anti-HIV therapy (ART) and those receiving only the latter revealed similar changes in HIV levels. After a round of IL-2 treatment an explosion of HIV activity was detected, but it is not maintained, and there is no evidence that this transient rise in HIV levels is harmful[27].

Although the last study was the first to reveal a favorable effect of IL-2, the differences observed between the two groups were not very large, and it is possible that the beneficial effects of IL-2 are partially eclipsed by the effect of the TAR. Moreover, both results do not support the use of IL-2 as a complement of TAR-positive because there is no improvement in the health of people infected with HIV.

Conclusions

Interleukin 2 therapeutics have proven to be efficient only under a particular set of conditions. Since the molecule's affinity for its receptor, regulates the cytokine biological effect over the lymphocytes, most of its therapeutically approach rely on interfering with the IL-2/IL-2R complex formation. When it comes to cancer, clinical studies have shown the importance of not only using a low dose subcutaneous methodology for diminishing the downsides of the treatment but also improving the previous controls for determining precise dosing. On HIV therapeutics, IL-2 therapies must be integrated with antiretrovirals therapeutics; otherwise the treatment would backfire and cause a more drastic HIV proliferation.

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Received: 2 September 2019
Accepted: 10 October 2019

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