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Phytochemical analysis and biological investigations on Halocuemum strobilaceum



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Abstract

Halocnemum Strobilaceum is a salt-tolerant plant species that grows in arid regions. In this study, we conducted a comprehensive phytochemical analysis of the ethanol extract and methanol obtained from *H. strobilaceum*. The extract was analyzed for the presence of various secondary metabolites, which included alkaloids, flavonoids, tannins, phenolics, saponins and terpenoids.

The biological activities of the extract were evaluated against several microorganisms including bacteria and fungi using agar well diffusion and broth microdilution assays.

Introduction

Throughout the ages, humans have relied on nature for their basic needs, for the production of food, shelter, fertilizers, and medicines). Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies. Although some of the therapeutic properties attributed to plants have proven to be erroneous, medicinal plant therapy is based on the empirical findings of hundreds and probably thousands of years of use.

The knowledge of medicinal plants was gradually developed and passedon from one individual to other, which foundation for traditional medicine throughout the world[1].

Classification

| Kingdom | Plantae |
|----------|------------------------------|
| Division | Magnoliophyta |
| Class | Magnoliopsida (Dicotyledons) |
| Subclass | Caryophyllidae |
| Order | Caryophyllales |
| Family | Amaranthaceae |
| Genus | Halocnemum M.Bieb (8) |



Species *Halocnemum strobilaceum* (Pall.) M.Bieb.

Botanical description

H. strobilaceum is perennial, fleshy, glabrous, yellowish – green shrub and succulent, glabrous shrub or subshrub (height 20-60 cm). The root system is superficial, with a poorly developed central pivot and a lateral superficial depth of 10-35 cm. Old stems are woody, intertwined with brownish bark; stems are jointed and succulent with many branches, and they stand erect to ascend with opposite orbicular buds. The hermaphrodite flowers are placed in inflorescences short, lateral, terminal, sessile, opposite, and cone-like or globular to oblong. The fruit is utricle and seeds are brown, compressed, and smooth to minutely tuberculate [2].

Taxonomic status

H. strobilaceum was formerly placed in the family Chenopodiaceae, but after the complete genome sequencing of its chloroplast and molecular-based APG system, it was transferred to the family Amaranthaceae[2].

Distribution

H. strobilaceum is a halophytic *plant*. This plant population is widespread in areas of high salinity of the world, is distributed in the Mediterranean region, Central and West of Asia, Arabia, Iran, Pakistan, and Mongolia [3]. In Iraq, this plant is found in vast areas of saline flats with high salinity and high ground water level including south of Iraq. This plant grows reaches a height of 150 meters above sea level. It was found to grow in sandy and clay soils.

Ethnobotany and medicinal uses

H. strobilaceum had been used in folk medicine for various medical conditions. It is used to treat fever, jaundice, headache in region of Oued Righ in Algerian Sahara [4]; expectorants, and digestion in Fars province in Iran [5]; pregnancy difficulties, and dysmenorrhea in Arabian Peninsula [6]. Also, it applied to hair loss in Libya [7]. Other uses of *H. strobilaceum* include using its aerial parts extract as probiotics in aquaculture feed [8]. *H. strobilaceum* is a good source of vitamins C and E as well as sodium, potassium, calcium, and magnesium. The green or red-violet shoots are consumed in Tunisia as gourmet vegetables due to their organoleptic properties and are used as preservative in the food and beverage industries.

So, because of the increase in salt tide and desertification areas, it has become beneficial to use plants that are resistant to this environment for medical and non-medical use. Therefore, in this research, we will focus on extracting the plant and examining its effectiveness and what it contains for components that can be used in the future for medical use.

Pharmacological activity

1) Antioxidant and anti-inflammatory activity Extracts of *H. strobilaceum* are rich sources of flavonoids and polyphenols, whichgives them significant antioxidant properties.

H. strobilaceum has large amounts of polyphenol compounds and, consequently, significant antioxidant properties.

- 2) Anticancer and cytotoxic activity *H. strobilaceum* root n-hexane extract has demonstrated strong cytotoxic activity against human cancer cell lines(9).
- 3) Antimicrobial activity the evaluation of antibacterial activity of *H. strobilaceum* shoot fractions against five human pathogenic bacteria showed that it could inhibit bacterial proliferation. *H. strobilaceum* root and aerial part n-hexane extracts exhibited significant effect against Bacillus subtilis and Staphylococcus aureus.
- 4) **Insecticide** and enzyme inhibitory activity.

There are several plants from the Amaranthaceae family known to have medicinal properties.

Amaranthus caudatus: has been used traditionally as a medicinal plant in several cultures. The plant contains a variety of bioactive compounds, including antioxidants, saponins, and flavonoids, which may have anti-inflammatory, antibacterial, and antiviral properties. In some cultures, it has been used to treat ailments such as diarrhea, dysentery, and inflammation.[10]

Another plant from the Amaranthaceae family with medicinal properties is Spinacia oleracea, or spinach. Spinach is a nutrient-dense leafy green vegetable that is rich in vitamins, minerals, and antioxidants, which make it great for improving overall health. Spinach has been reported to have a variety of potential health benefits, such as reducing blood pressure, improving eye health, and improving overall bone health. *Chenopodium ambrosioides*: Also known as "epazote," this plant is commonly used in Mexican cuisine and traditional medicine. Epazote has been reported to have antibacterial, anti-inflammatory, and anti- parasitic properties, and it is often used to treat gastrointestinal disorders, such as indigestion and flatulence.[5]

Aerva lanata: This plant, also known as "mountain knotgrass" or "hierba del zorrillo," is widely used in traditional Indian medicine, where it is believed to have diuretic and anti-inflammatory properties. It is often used to treat ailments such as kidney stones, urinary tract infections, and fever.[6] *Alternanthera sessilis*: This plant, commonly known as "sessile joyweed," is valued for its medicinal properties and is often used in traditional medicine to treat a variety of ailments, including diarrhea, dysentery, and inflammation. The plant contains compounds that have been shown to have antifungal, antibacterial, and antioxidant properties.

These are just a few examples of the many medicinal plants from the Amaranthaceae family. It is a diverse and widespread plant family, and many of its members have been used for centuries in traditional medicine.[7]

Method of extraction

We collected the plant from Basrah city / Siba area, in November. Then dried the whole plantand and blend it, then put a certain amount of the plant and divided it into two halves. We will take 40 mg and use 20 of them and put them in filterpaper and leave them in the device and put 200-250 ml of liquid ethanol (as a first extract), then we repeat the same process on the remaining 20 g of material but we use the methanol solvent extract (as a second extract) wait Unital extract complete and finally get solvent material.

Than used these solvent material after dried to identify biological activity and

material extracted with ethanol, we measured its weight and appeared to be 0.2





Detection

Ethanol (solvent) extracts of flavonoids:

First, we add ethanol to the dry ethanol-extracted sample

We withdraw 1 ml of the solution and put it in the test tube, then add a piece of magnesium riboon and concentrated H2so4 acid drop by drop (it is dark red in color, indicating the presence of flavonoids) and the extract contains it.

detection of tannins:

Also, we withdraw 1 ml of the previously prepared solution of the extracted sample and put it in a test tube, then we double it as drops of FeCL3, after adding it, a dark blue color appears, indicating the presence of aptannines in the extract.

Detection of the presence of alkaloids: We withdraw 1 ml of the same solution of the previously prepared extract and put it in the test tube.

We add Dragendorff's reagent to the same test tube drop by drop, ((DR is a solution of potassium bismuth iodide composing of basic bismuth nitrate (Bi(NO₃)₃), tartaric acid, and potassium iodide (KI))) an orange precipitate appears. This extract contains a large amount of alkaloids.

<u>Another detection of alkaloids using Mayer's reagent</u> :We repeat the above same-mentioned steps and test by adding Mayer's reagent to the test tube containing the extract. After the reaction, we did not see a clear result.

Methanol(solvent) extracts:

Detection of flavonoids in a sample extracted by methanol

We add methanol to the extracted dry sample and put 1 ml of the solution in the test tube. Then we add a piece of magnesium and concentrated H2SO4 acid to the solution, drop by drop. At the beginning of the reaction, we will have a bright red colour. This indicates that it contains flavonoids

<u>Detecting tannins</u>: We take 1 ml of the same solution previously prepared for the sample and put it in the test tube and add FeCL3 reagent drop by drop to have a dark blue cloudy solution indicating the presence of tannins

<u>Detection of alkaloids:</u> We take 1 ml of the same previously prepared solution, put it in a test tube, and add Dragindorff's reagent drop by drop . An orange precipitate will form, indicating that the extract contains a large amount of alkaloids.



Antibacterial activity:

We have examined the antibacterial activity of this plant by culturing the bacteria in plates containing the plant extract, and from these bacteria it is. Bacillus, pseudomonas and staphylococcus bacteria, and it has been shown to have good efficacy against diffirnt types of bacteria;



bacillus:





Pseudomonas:

References:

1- Al-Yousef, H. M., Ali, D., Alqahtani, F. Y., Alkhathlan, H. Z., Alaraidh, I. A., Assad, M. R., ... & Alfarhan, A. H. (2016). Antibacterial Activity of Halocnemum strobilaceum Extracts against Selected Pathogenic Bacteria. Evidence-Based Complementary and Alternative Medicine, 1-6. <u>https://doi.org/10.1155/2016/6318360.</u>

2- Nasernakhaei, F. & Zahraei, M. (2022). *Halocnemum strobilaceum* (Pall.) M.Bieb.: a review of its botany, phytochemistry, pharmacology and ethnobotany. Journal of Medicinal Plants 2021; 20(80): 1-12.

- 3- Missouri Botanical Garden SL, MO & Harvard University Herbaria, Cambridge, MA. eFloras 2008.
- 4- Lakhdari W, Dehliz A, Acheuk F, Mlik R, Hammi H, Doumandjid B, Gheriani S, Berrekbia M, Guermit K and Chergui S. Ethnobotanical study of some plants used in traditional medicine in the region of Oued Righ (Algerian Sahara). J. Med. Plants. Stud. 2016; 4(2): 204-211.
- 5- Zali SH and Tahmasb R. Medicinal plants of Farashband tribe's winter pastures and their traditional uses. J. Adv. Health. Med. Sci. 2016; 2(1): 18-27. doi: 10.20474/jahms-2.1.3.
- 6- Heneidy SZ, Halmy MWA and Bidak LM. The ethnobotanical importance and conservation value of native plants in eastern Arabian Peninsula. Feddes. Repert. 2017; 128(3-4): 105-128. doi:10.1002/fedr.201600024.
- 7- Louhaichi M, Salkini A, Estita H and Belkhir S. Initial assessment of medicinal plants across the Libyan Mediterranean coast. Adv. Environ. Biol. 2011; 5(2): 359-370.
- 8- Messina CM, Renda G, Laudicella VA, Trepos R, Fauchon M, Hellio C and Santulli A. From ecology to biotechnology, study of the defense strategies of algae and halophytes (from Trapani Saltworks, NW Sicily) with a focus on antioxidants and antimicrobial properties. Int. J. Mol. Sci. 2019; 20(4): 881. doi: 10.3390/ijms20040881.

9- Boudjelal, A., Siracusa, L., Henchiri, C., Sarri, M., Abderrahim, B., & Baali, F. (2013). Protective effect of Halocnemum strobilaceum against acetic acid- and ethanol-induced colitis in rats. Journal of Medicinal 13

Plants Research, 7(9), 496-505. <u>https://doi.org/10.5897/JMPR2012.0201</u>

Amaranthus caudatus: [4]

- "Evaluation of Antioxidant and Antimicrobial Activities of Love-Lies-Bleeding (Amaranthus caudatus) Extracts" (<u>https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6310445/</u>)

- "Medicinal plants used by traditional healers for the treatment of malaria in the Chipinge district in Zimbabwe" (https://link.springer.com/article/10.1007/s13197-018-3293-7)

10- Spinacia oleracea:

- "A systematic review on the health effects of spinach (Spinacia oleracea L.)" (<u>https://link.springer.com/article/10.1007/s11130-015-0496-2</u>)

- "Spinach, a green leafy vegetable to prevent chronic diseases: a review of the epidemiological and biochemical evidence" (https://link.springer.com/article/10.1007/s00394-015-0926-3)

11- Chenopodium ambrosioides:[5]

- "A review on the traditional uses, phytochemistry, pharmacology and quality control of Chenopodium ambrosioides L." (<u>https://www.sciencedirect.com/science/article/pii/S0378874119335552</u>)

- "Medicinal Plants from Northeastern Mexico Against Cancer and Chronic Diseases"

(https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5628631/)

12- Aerva lanata:[6]

- "Aerva lanata (L.) A. Juss.: A review on phytochemistry, pharmacological properties and toxicology"

(https://www.sciencedirect.com/science/article/abs/pii/S0378874117327 372)

- "Ethnobotanical survey of folkloric plants used in the management of HIV/AIDS opportunistic infections among the communities around R. Nile in Kamuli District, Uganda."

(https://www.ncbi.nlm.nih.gov/pubmed/31416434)

13- Alternanthera sessilis:

- "Alternanthera sessilis: a review of its phytochemical, pharmacological, and ethnomedicinal properties" (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6269257/)

- "Medicinal plants of Myanmar (Burma) (https://www.tandfonline.com/doi/full/10.1080/14786410801915331)

8 - Mozaffarian V. plant taxonomy. Second book: dicotyledones. Tehran: Amir Kabir press. 2004: 71-91.

9 - Razek MA, Moussa A, El-Shanawany Mand Singab A. Comparative chemical and biological study of roots and aerial parts of Halocnemum strobilaceum growing wildly in Egypt. J. Pharm. Sci. Res. 2019; 11(9): 3289-3296.

10-Introduction

[1]-Cragg, G.M. and Newman, D.J. 2004. A tale of

two tumor targets: topoisomerase 1 and tubulin. The Wall and Wani contribution to cancer chemotherapy. J. Nat. Prod. 67 (2):232-244.

Cragg, G.M. and Newman, D.J. 2005. Biodiversity: A continuing source of novel drug leads. Pure Appl. Chem. 77 (1):7-24.

https://repository.up.ac.za/bitstream/handle/2263/24934/01chapter1.pdf? sequence=2&isAllowed=y

[2]-Nasernakhaei F, Zahraei M. Halocnemum strobilaceum (Pall.)

M.Bieb.: a review of its botany, phytochemistry, pharmacology and ethnobotany. J. Med. Plants 2021; 20 (80) :1-12

URL: http://jmp.ir/article-1-3201-en.html

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https://books.google.iq/books?id=fWqxEAAAQBAJ&printsec=frontcov er&dq=inauthor:%22Volkan+Altay%22&hl=ar&newbks=1&newbks_re dir=0&source=gb_mobile_search&sa=X&redir_esc=y#v=onepage&q&f =false