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# Phytochemical Screening and Antifungal Efficacy of Eremostachys lacinata (L.) Bunge. plant Extract and Potential healthy attribute of Active compounds

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**Abstract**

Genus Eremostachys Bunge is a key medicinal plant grown in Eastern Europe, Central and Western Asia and Middle East. The plants of this genus have numerous secondary metabolites, which exhibit both traditional and pharmacological applications. The species Eremostachys lacinata (L.) Bunge belongs to the family Lamiaceae (Labiatae). The aerial parts (leaves) of Eremostachys laciniata were collected, at altitude range between 800-1300 meter. At the full flowering stage from April 2024 to June 2024. Various locations in the Kurdistan Region of Iraq's MRO (Mountain Rowanduz district), MSU (Mountain Suleimany district), were surveyed to collect plant specimens. A voucher specimen has been deposited in the Herbarium of the University of Garmian. Furthermore, anti-fungal efficacy of Eremostachys lacinata (L.) Bunge in three concentrations (25%, 50%, and 100% mg/ml) by (ethanol 70%) extract against two species of human pathogenic fungi (Microsporum canis and Trichophyton rubrum) were studied. The results showed that the impact of ethanol 70% extracts in Control 2 in Trichophyton rubrum with 48.33mm diameter was the most effective in inhibition fungi compared to all extract concentrations. The average control with 36.66 mm was the most effective to inhibition fungi than average extract with 2.22mm inhibition zone and show the ethanol 70% extract in Eremostachys lacinata had weak affected to against studied fungi.

**Key words:** labiatae; eremostachys lacinata(l.) bunge; qualitative test; anti-fungal efficacy

## 1.Introduction

Eremostachys laciniata (L) Bunge with thick roots and pale purple or white flowers, it is belong to family family (Lamiaceae alt, Labiatae; sub-family: Lamioideae) is a perennial herb used for medicine. It is one of the fifteen endemic species of Eremostachys that are native to Iran; other nations in the Middle East, Western Asia, and Caucasus also grow it [1]. The genus Eremostachys is present as the one of the main genera of the Lamiaceae family, traditional knowledge showed anti-inflammatory and analgesic effects of rhizomes of various species of Eremostachys[2] . Traditionally, a decoction of the roots and flowers of Eremostachys laciniata has been used to treat allergies, headache and liver diseases [1] .This plant is also used to alleviate inflammatory conditions. It is usually given as a remedy in the form of herbal teas, or tisanes of the roots and flowers. The merit of the traditional uses of Eremostachys laciniata has been supported by some previous phytochemical studies on the genus Eremostachys, providing the isolation and identification of several bioactive compounds. Previous phytochemical study on Eremostachys laciniata established the presence of mono- and sesqui -terpenes in its essential oils[i]. The flowers, leaves and roots of Eremostachys angreni and Eremostachys lehmanniana both species have distinctly different flavonoid aglycones and glycoside compositions [ii] . In another study on Eremostachys molucceloides, 9, 10-dibromostearic [3] . Tetrabromostearic acids were isolated from the seed oil [4]. The chemical composition of Eremostachys laciniata oil, the rhizomes of Eremostachys glabra have traditionally been used as a local analgesic. phytochemical investigation on a few other species of Eremostachys revealed the presence of flavonoids [3] . Monoterpene glycosides [4] . Surprisingly, no side effects have been reported so far on Eremostachys laciniata use [iii]. Previous investigations reported the other biological properties such as antioxidant, antimalarial, anti-bacterial, antidepressant, and antinociceptive activities [3] . Phytochemical studies on the extracts of some species of this genus have indicated the presence of different natural compounds. The rhizomes of Eremostachys laciniata were an important source of iridoid glycosides, flavonoids, and phytosterols [4]. Ferulic acid derivatives, furanolabdane diterpene glycoside, iridoid glycosides, and phenylethanoid glycosides have been identified from the rhizomes of Eremostachys glabra . Phytochemical

studies on the essential oil composition of species of this genus have illuminated the presence of terpenoids structures, linear hydrocarbons, and their derivatives[5]. This study concerning the quantification of phytochemical contents of the extracts of their active compounds such as alkaloids, flavonoids, glycosides ,phenols, resins ,saponins ,tannins and terpenoids were detected. In addition, identify the anti-fungal effectiveness of ethanol 70% extracts of Iraqi Eremostachys lacinata (L.) Bunge to against studied pathogenic fungi.

## 2. Materials and methods:

**Plant collection:**

Plant specimens (plate1) were collected in several localities within the Kurdistan Region of Iraq, including MRO (Mountain Rowanduz district), MSU (Mountain Suleimany district), from April 2024 to June 2024. Eremostachys lacinata (L.) Bunge leaves were collected with the required herbarium data, which included the growth stage, collector name, location and some ecological notes. Specimens were dried and pressed for diagnosis. The plant is refrigerated until needed after drying. Flora of Turkey with Flora Iranica were used for diagnosis the studied species [1].

**Extraction method:**

Extraction ethanol's 70% need to add 10gm of plant powder with 100 ml of ethanol were combined with stirring (weight-to-volume ratio of 1:10). The mixture then left to soak in the refrigerator for 24 hours. The extract was filtered through multiple layers of gauze and Whatman filter papers No. 1 to eliminate any remaining fibers and non-pulverized plant parts, after then the extract was placed in a rotary evaporator to produce a thick layer. It was then placed in a shaker incubator set at a temperature of 25-30 °C. Then extracted components were put in information-labeled, airtight containers that were shielded from light and moisture, and kept at 4°C until further analysis were done[5].

**Phytochemical screening:**

A qualitative analysis using coloring and/or precipitation reactions from various plant extracts is known as phytochemical screening (Figure 1). It intends to focus on the significant families of secondary metabolites that the plant provides [6]. The current study's phytochemical screening is dependent on the application of many reagents (Table 1).

| No. | Reagents/Solutions | Composition |
|---|---|---|
| 1 | Mercuric chloride solution 1% | Stock solution: dissolving 1gm of mercury (II) chloride $HgCl_2$, in 100ml D.W.<br>Working solution: used to detection of alkaloids |
| 2 | Ferric chloride Solution 1% | Stock solution: dissolving 1gm of ferric chloride Iron (III) FeCl3, in 100ml of D.W.<br>Working solution: used for the purpose of detecting glycosides, phenols and tannins in plant samples. |
| 3 | Sodium hydroxide solutions | Stock solution: Dissolving 5.0gm of sodium hydroxide NaOH in 50ml D. W.<br>Working solution: For indication of the presence of flavonoids. |
| 4 | Dragendorff's reagent | Solution A: the first was prepared by adding 2ml of concentrated HCl (37%) to 0.6gm of Bismuth (III) nitrate Bi $(NO_3)_3$+10ml of D.W was added to it.<br>Solution B: adding 6gm of KI to 10ml of water + 7ml of concentrated HCl was added to A and B solutions and they were mixed with each other+ the volume was completed to 400ml of D.W. |
| 5 | Mayer's Reagent | Solution A: dissolving 1.85gm of mercuric chloride in 60ml of D.W.<br>Solution B: dissolving 5gm of potassium iodide KI in 10ml of distilled water.<br>Prepared for the detection of alkaloid compounds. |
| 6 | Wagner's Reagent | Dissolving 6gm of potassium iodide KI in 5ml of D.W + 2gm of iodine $I_2$ to the mixture, mix them well+ complement their volume to 100 ml of D.W.<br>Using of detection of alkaloid compounds. |

**Table 1: Reagent Preparation for Phytochemical Screening:**

Table 1 **Fungal suspension:**

The suspensions made using a normal saline solution and compared to the standard McFarland's solution from a 7-10 day old fungal colony .

**Inoculum Preparation of Studied Fungal Suspensions:**

Since the disk diffusion approach used phenotypic susceptibility identification, the following steps were necessary [7]:

1. Prepared an inoculum from a standardized fungal culture:
   - Selected colonies in remote locations.
   - Prepared fungal suspension (inoculum).
   - By utilizing McFarland standards, the fungal solution was standardized.
2. Giving one of the following a fungal suspension inoculation:
   - A specific growing medium is Sabouraud-Dextrose Agar (SDA).
   - Plate incubation.
   - Measured the inhibition zone.

The diffusion agar method was used to observe the sensitivity of two species of pathogenic fungi (Microsporum canis and Trichophyton rubrum) with extract of the studied plant at concentrations (25%, 50 % and 100% mg/ml). Using the streak plate method, the infected Sabouraud-Dextrose Broth (SDB), which contains fungi that grow quickly, must be incubated for two to six hours, to manufacture inoculum from colonies produced within suspensions prepared from 7-10 day old fungal colonies, the "direct colony suspension method" was typically utilized [3]. After that, the growth was put into a sterile test tube with 5 milliliters of sterile normal saline solution in it, the McFarland standard states that the turbidity of the inoculum should be 0.5 [2]. Using a vortex mixer, the fungal suspension in the test tubes was thoroughly and consistently mixed [4]. Make sure there was not an excessive amount of inoculum on Sabouraud-Dextrose Agar (SDA) before inoculating fungal suspension in a growth medium, to do this, 100 Microlliter( µL )of the fungal suspension was applied to the SDA plates. Three agar wells per plate were made using a sterilized cork borer with a 6 mm diameter, using a micropipette with a 50 µL/well tip (for one-time use), three different concentrations (25%, 50%, and 100% mg/ml) of the plant extract solutions were carefully added to the appropriate wells in the plate media, after that at a temperature of 25°C for fungi for a period of six days, and measurements were taken each two days and measurements of the diameter of the inhibition zone was measured by millimeters (mm) using Caliber after incubation [4].

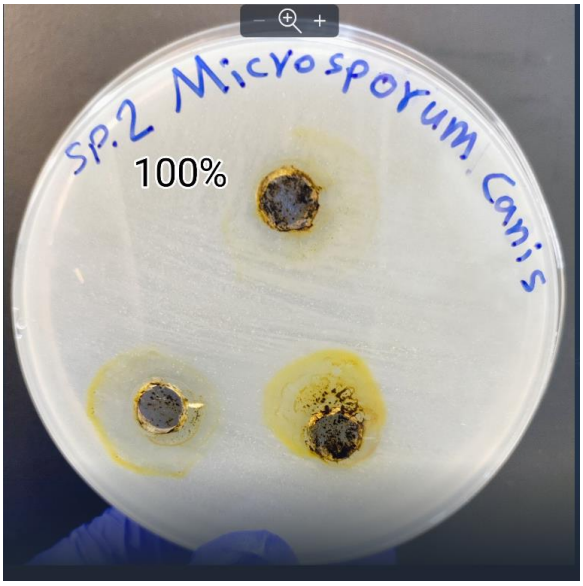
Two controls were prepared as a control treatment (the first positive for fungi and the second negative for fungi). As following: (Figure 3)

1. Taking (50µL/well) of the Clotrimazole (100mg/ml) which acts as an antifungal (positive control of studied fungi). Three replicates for one treatment of studied pathogenic fungi were used (Microsporum canis and Trichophyton rubrum).

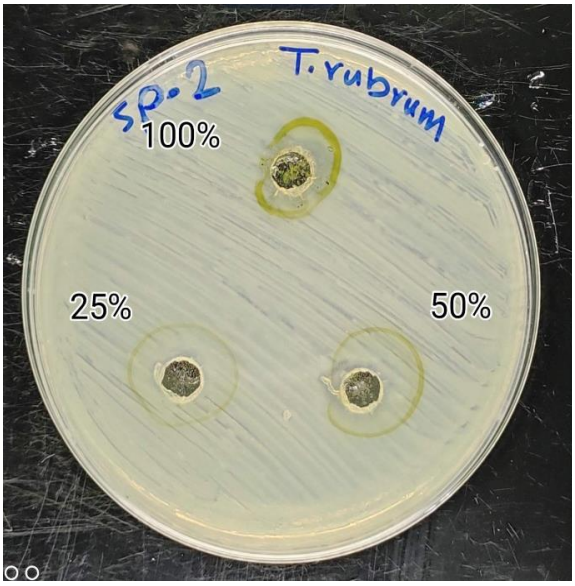
2. In the previous method, made a well and by using a micropipette (50µL/well) of sterilized distillated water (control was negative) put in the well labeled for it. Three replicated for each treatment studied fungal species [iv].

## Analytical Statistics:

R software was used to analyze the data using analysis of variance (ANOVA), and the Tukey test was utilized to determine whether treatments (controls, fungi, and concentrations) differed from each other [5].




*Eremostachys lacinata*




*Eremostachys lacinata*

## Results:

**Chemical detection of bio-active compounds in Eremostachys lacinata :**

The phytochemical screening in (leaf) of Eremostachys lacinata extract by ethanol 70% indicated the presence of studied bio-active compounds (Table 2 and Figure 1).

| No. | Detector type | Test | Procedure | Observations (Indicating Positive Test) | Detection Result | References |
|---|---|---|---|---|---|---|
| 1 | **Alkaloids Detection** | Dragendorff's test | 2 drop of Dragendorff's reagent (potassium bismuth iodide) +2ml of plant sample extract. | Formation of orange or orange red precipitate | + | [7] |
| | | Mayer's test | 2 ml of the plant sample extract +2 drops of Mayer's reagent on the sides of test tube. | Appearance of white creamy precipitate | + | [25] |
| | | Wagner's test | 2 drop of Wagner's reagent (Iodo-potassium iodide) +2ml of plant sample extract. | reddish-brown precipitate | + | [26] |
| 2 | **Flavonoids Detection** | Alkaline reagent test | 1mL extract + 2mL of 2% NaOH solution (+ few drops dil. HCl) | Formation of intense yellow color, which becomes colorless on addition of dilute acid HCL. | – | [4] |
| 3 | **Glycosides Detection** | Killer-killiani test | 1mL filtrate + 1.5mL glacial acetic acid + 1 drop of 5% ferric chloride + 2ml of conc.suiphuric acid $H_2SO4$ (was added carefully along the sides of the test tube). | Appearance of reddish brown colored ring at the junction of two layers. | + | [4] |

| | | | | | | |
|---|---|---|---|---|---|---|
| 4 | **Phenols Detection** | Ferric chloride test | 3 ml of hydro-ethanolic plant extract + 2ml of $FeCl_3$1%solution. | appearance of a bluish green color | + | 4 |
| 5 | **Resins Detection** | Turbidity test | 10ml of hydro-ethanolic plant extract + 20ml of distilled water acidified with HCl 4%. | turbidity appeared | + | 4 |
| 6 | **Saponins Detection** | Foam test | 1ml of the hydro-ethanolic extract of the plant, then adding to 5ml of distilled water and shaking it well and it was inferred from the soaps. | when a thick foam appears in the form of a layer with a diameter of 1 cm that remains visible for (10minute) | – | 4 |
| 7 | **Tannins Detection** | Braymer's test | 2.5ml of plant extract to 10ml of distilled water, then the solution was filtered using filter paper, and then three drops of 1% ferric chloride solution were added to it | bluish-green color | – | 4 |
| 8 | **Terpenoides Detection** | Salkowski test | 5ml of hydro-ethanolic plant extract was mixed with 2ml of chloroform $CHCl_3$ + 3ml of concentrated H2SO4 was carefully added to form a layer. | The interface developed a reddish-brown color | – | 5 |

**Table 2: Qualitative Tests for Phytochemical Screening**

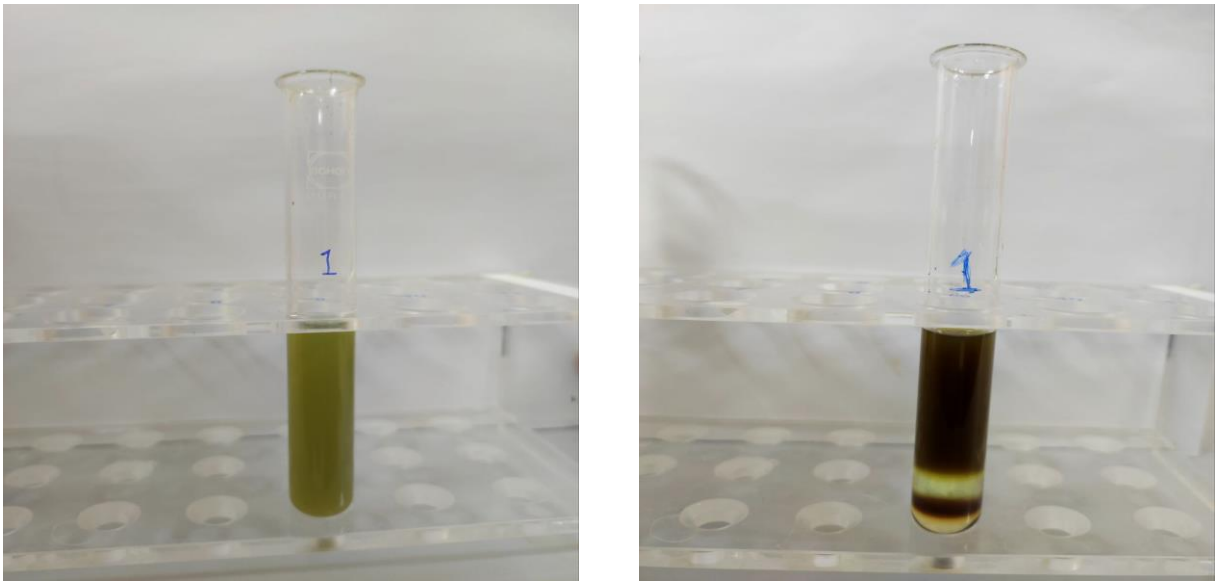



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**Figure 1: All studied Qualitative test A/Alkaloids, B/ Flavonoids, C/ Glycosides, D/Phenols, E/Resins, F/ Saponins G/Tannins, and H/ Terpenoides detections respectively.**

**Antifungal efficacy of Eremostachys lacinata extract:**

(Table 3) indicated to the impact of the ethanol 70% extract with concentrations 25, 50 and 100% mg/ml in the inhibition the growth of Microsporum canis and Trichophyton rubrum, with inhibition diameter (mm) compared to the control 1and 2 . The control treatment 2 in Trichophyton rubrum with 48.33mm inhibition zone was the most effective in inhibition fungi compared to control 1 and all extract concentrations. The concentrations of 25, 50 and100% mg/ml in Microsporum canis with 0mm diameter respectively show no affected studied fungi and there were no significant difference among them. Also at concentrations 25 and 50% mg/ml in Trichophyton rubrum with 0mm inhibition zones respectively there was no significant differences between them and show no affected Trichophyton rubrum. Also at concentration 100% mg/ml in Trichophyton rubrum with 13.33mm diameter was the most effective to inhibition fungi compared to control 1and all extracts concentration. Also noted from (Table 3) the average control with 36.66 mm was the most effective to inhibition fungi than average extract with 2.22 mm inhibition zone and show the ethanol 70% extract in Eremostachys lacinata had weak affected studied fungi.

| Extract | Concentration mg/ml | Fungi | | Average concentration | Average extract |
|---|---|---|---|---|---|
| | | *M.canis* | *T.rubrum* | | |
| Ethanol 70% | 25% | A<br>0<br>ab | A<br>0<br>ab | 0<br>ab | 2.22<br>ab |
| | 50% | A<br>0<br>ab | A<br>0<br>ab | 0<br>ab | |
| | 100% | AB<br>0<br>ab | AC<br>13.33<br>ac | 6.66<br>ac | |
| | Average fungi | AB<br>0 | AC<br>4.44 | | |
| Controls | 1/ D.W | A<br>0<br>ab | A<br>0<br>ab | 0<br>ab | 0<br>ac |
| | 2/ Clotrimazole | AB<br>25<br>ac | AC<br>48.33<br>ad | 36.66<br>ad | 36.66<br>ad |

**Table 3: Antifungal activity of extracts of Eremostachys lacinata by (mm)**

| Species | Partes used for treatment | Traditional uses | Ref. |
|---|---|---|---|
| *E. glabra* | Rhizomes | As a native used as a natural analgesic and anti-inflammatory agent | [27] |
| *E. laevegata* | Whole plant | Used as a food preservative, a remedy against several infectious diseases, a shawn insectidical, and a component of cosmetics and home goods | [28] |
| *E. laciniata* | Rhizomes, rose and flower | Flowers and roses have been used orally to cure allergies. Lever illness and headaches. | [19] |
| *E. macrophylla* | Aerial and Rhizomes | Applied a traditional remedy instead of joining paints | [2] |
| *E. superba* | Whole plant | used as an antioxidant and antidepressant. It is used to treat mastitis and restore cattle mulching. | [29] |
| *E. vecaryi* | Whole plant and seeds | used to contaminate seafood. Additionally, seeds are used as coolants to reduce fever | [29] |

**Table 4: Traditional uses of some species of genus Eremostachys**

## Discussion:

According to the results there are differences between the effects of the extracts on the growth of studied fungi species. Furthermore this study confirmed previous observations of the highly different resistant of various fungal species to bioactive compounds. Additionally, the studied extract inhibition fungicidal effects against studied fungi. Flavonoids,Tannis and Terpenoids which support the plant's anti-fungal activity were not present in the plant extract used in this investigation. The studied results considered that the extraction method using ethanol 70% had weak anti-fungal activities. Lamiaceae and the most prominent genus Eremostachys were some of the richest sources of antimicrobial [8] . Results demonstrated that the average control with 36.66 mm was the most effective to inhibition fungi than average extract with 2.22 mm inhibition zone and show the ethanol 70% extract in Eremostachys lacinata had weak affected studied fungi (Table 3). These results may belongs to the fact that the examined phytochemical bio-active compounds of Eremostachys lacinata lack Flavonoids, Saponins ,Tannis and Terpenoids in current Qulalitative test (Table 1). May be due to essential parameters that can affect the ethno pharmacological of the extract, including the age of the plant , the plant part (leaf) used for extraction and types of solvent [9] . The results in our study agree with the results of this investigation Eremostachys lacinata contains alkaloids, saponins, flavonoids, and tannins [10]. Additionally, the focus on the phytochemical studies of a number of species of genus Eremostachys showed the presence of various chemidical structures iridoid glycosides, flavonoids, phenylethanoid glycosides and phytosterols were reported from the rhizomes or aerial parts of Eremostachys lacinata[7] .Ferulic acid derivatives, diterpenoids, and phenylethanoid glycosides were identified from the rhizomes of Eremostachys glabra [11]. Furthermore, several other studies reported iridoid glycoside, flavonoid, phenylethano, fatty acid and steroid structures from the rhizome or aerial parts of Eremostachys azerbaijanica,[12]. iridoids and flavonoids from Eremostachys loasifolia, iridoid glycosides from the aerial parts of Eremostachys molucceloides[v].and various flavonoid derivatives from Eremostachys vicaryi [13]. The present study has shown that the aerial parts of Eremostachys macrophylla are a source of iridoid, phenylethanoid and flavonoids. Since the anti-oxidant and anti-inflammatory effects of phenyl ethanoids and flavonoids [14].analgesic, anti-inflammatory and anti-arthritic properties of iridoid glycosides have previously been confirmed by several in vitro studies [15]. The results considered all extractions method use ethanol 70% had not antifungal activities, with the variation of

these activities between extraction and fungal species, which indicated that these extracts contain antifungal substances that the responsed of fungal species differ according to the quantity and type of these materials. Therefore, this paper can be a guideline for researchers in the field of pharmacology to make more investigations about these plants from other points of view.

Functional foods and Traditional Uses of Eremostchys contain bioactive compounds, which provide health benefits beyond basic nutrition. These bioactive compounds interact with living tissue and are derived from plant, animal, or microorganisms [16]. They have antioxidant, anti-inflammatory, antidiabetic, anticancer, antiviral, and antitumor activities, protecting the body from free radicals and reactive oxygen species [17].

Phytochemicals, found in plants like vegetables, fruits, cereals, legumes, and nuts, are chemical compounds produced through primary and secondary metabolism, possessing biological activities.

Before recommending the scientific dietary standards, nevertheless, more research is required. Even yet, there is sufficient evidence to back up consuming foods high in bioactice compounds [9].

The consumption of foods high in antioxidant-active compounds, such as phenolic compounds like flavonoids, stilbenes, lignans, tannins, and carotenoids, has been linked to improved human wellbeing, according to epidemiological data [18]. These compounds may lower the risk of a number of diseases, including diabetes, Alzheimer's disease, cancer, cataracts, and age-related disorders.

Traditionally, West Asian and South Asian nations have utilized the genus Eremostchys to cure various illnesses. Eremostachys has been treated topically to treat bruises and localized pain and swelling. It has also been used as an anti-inflammatory and analgesic[19] . Traditionally, E. laciniata has been used to cure many ailments, including headaches, asthma, colds, and allergies. It is also used as a herbal tea (made from the root and flower) [20]. Table 4 briefly describes the several plants in this genus that are also utilized in traditional and folk medicine to treat various illnesses. In India, goats, cows, and other livestock are fed the genus Eremostachys superba Royle ex Benth mixed with bovine feed to restore mulching [21]. One of the significant plant genera with a wide range of pharmacological and therapeutic uses is Eremostachys (figure 4). A few numbers of these species' plants, such as E. laciniata, E. loasifolia, E. glabra, E. macrophylla, E. laevigata, E. azerbaijanica, E. labiosa, E. labiosiformis, E. pulvinaris, etc., have been extensively investigated [18]. In terms of their secondary metabolites and pharmaceutical uses, the majority of the species still require investigation. The genus Eremostachys is considered to be important in Ayurvedic and Unani medicine because of its abundance of chemically reactive secondary metabolites. Since every section of the plant contains some essential secondary metabolites, the entire plant is significant for therapeutic purposes[22].


*Microsporum canis* *Trichophyton rubrum*

**Figure 4: All studied fungal controls.**

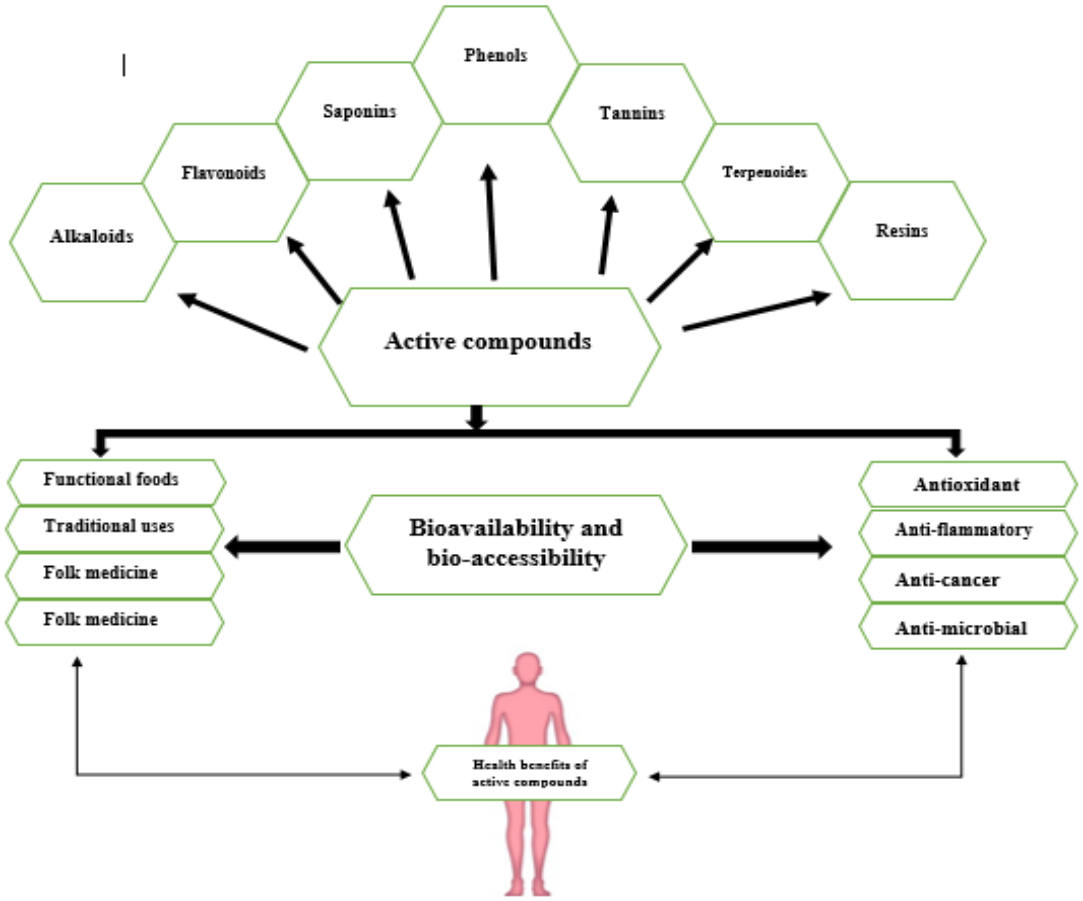


**Figure 5: Potential healthy attribute of Active compounds in *Eremostachys* toward human health and application**

However; most of the species are still need to be explored with respect to their pharmacological applications and secondary metabolites. From a medicinal point of view, genus Eremostachys is playing a key role in Ayurvedic and Unani medicine due to the presence of the number of chemical reactive secondary metabolites [23]. The whole plant is important for medicinal purposes as all parts of the plant contain some vital secondary metabolites [24].

**Conclusions**

The work demonstrates the physiologically relevant roles of Eremostachys lacinata and provides some insights into its chemical makeup, which is currently mostly unknown. Our study found that 70% of the ethanol was recovered from the leaves of the Eremostachys lacinata aerial plant component.

The extracts had no effect on the two fungi that were being studied, Microsporum canis and Trichophyton rubrum. Numerous compounds may account for the plant's potent antifungal properties. The distinct chemical composition of Eremostachys lacinata is primarily rich in phenols and glycosides. The results of the investigation showed that the 70% ethanol extract of Eremostachys lacinata leaves lacked antifungal activity.

The negative results of the present qualitative test for flavonoids, saponins, tannins, and terpenoids may help to explain some of the findings of the inquiry into the antifungal activity. Thus, the focus of future study should be on determining active compounds and comprehending the processes behind their antifungal effects. Therefore, our study suggests that future investigations concentrate on the method of removing bioactive chemicals from the leaves of Eremostachys lacinata. The tabular form compiles the traditional usage and pharmaceutical applications of this genus Eremostachys that have been documented in the literature. It is referred to as High Performance Liquid Chromatography (HPLC) technology.

**Plate 1: Images of the studied species Eremostachys lacinata in their nature**

## Conflict of interests

None

## Author contribution

Dina , Sirwan and Khalid did field trips, collecting and classifying the sample. In addition, Dina analyzing the anti-fungal efficacy of ethanol 70 % extract of Eremostachys lacinata leaves. The Qualitative test was done by Ramal. All authors have read and agreed to the published version of the manuscript.

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