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Antibacterial and Phytochemical Evaluation of the crude extract and Fractions of *Eremostachys laciniata*.

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Abstract – The aim of the present research was to evaluate the antimicrobial potential and phytochemical profile of *Eremostachys laciniata* plant to look into possible natural therapy agents. The shade-dried whole plant material of *Eremostachys laciniata was* soaked in methanol for 10 days. The powdered drug was extracted with 80 % methanol three times and filtered at room temperature. The filtrate was evaporated in rotary to get a dark-greenish residue (extract), which was further suspended in water and partitioned successively with *n*-hexane, chloroform, distill water and *n*-butanol to obtain *n*-hexane-soluble, chloroform-soluble, distill water-soluble, *n*-butanol soluble and aqueous fractions, respectively. Different fractions (chloroform, n-hexane, butanol, water and ethyl acetate) of the plant were tested against various bacterial strains namely *E.coli, S.aureus, S.typheae, P.auriginosa*.The extracted fractions of the plant exhibit moderate activities showing 9mm to 18mm inhibition against different strains of bacteria as compared to the control, which gave maximum inhibition upto 29mm. The *Eremostachys laciniata* was found to be good source of alkaloids, flavonoids, tennins, resins, carbohydrate, starch, proteins, glycosides, coumarin, terponides, steroids and saponine. Anthranol glycosides were absent in the plant. The above results revealed that Fagonia cretica have an excellent anti-bacterial activity and can be used for disease therapy.

Keywords – *Eremostachys laciniata,* crude extract, phytochemical, antibacterial

1. Introduction

Since disease, decay and death have always co-existed with life; the study of illnesses and their treatment must also have been contemporaneous with the beginning of the individual intelligence. The connection between man and organic items is as old as the history of humanity itself [1]. Vegetation are important source of organic drugs [2].Therefore, therapeutic plants have been in use for the treatment of individual sufferings since olden days [3]. Even today almost 25% of all recommended medications in the third globe contain components based on therapeutic plants. The globe has experienced the growing medical and professional passions in therapeutic plants, mainly due to their tremendous economic potential and extensive social acceptability of plant-based items. According to a WHO calculate, around 80% around the globe depends on natural treatments for their basic medical care needs [4, 5]. It was estimated from the development of the chemotherapeutic techniques that infected illnesses could be absolutely recovered but medication rebellious viruses curved an enormous problem so it is very vital to decide on an appropriate and a protected anti-microbial mediators [6].

Eremostachys laciniata with two subspecies (subsp. laciniata and subsp. Iberica) is one of the 17 Eremostachys varieties from the plants of Iran. In Iranian conventional therapeutic practices this varieties is known as "Chelle Daghi" and its rhizomes are used as emollient to reduce joint disease discomfort. Moreover, decoction of the rhizomes and blossoms of E. laciniata are trusted to cure allergic reactions, frustration and liver organ illnesses. Some therapeutic qualities such as anti-oxidant, anti-bacterial, antidepressant, anti-inflammatory and medication results have been recorded for E. laciniata [7-14]. The plant grows naturally at high altitude of about 2200 m, from Eastern Mediterranean sea area, Main and South-West Japan, Afghanistan and separated areas of Pakistan [15-18]. Past phytochemical research have also revealed the solitude of iridoid, phenylethanoid and flavonoid types from the antenna areas and some iridoid and furanolabdane diterpene glycosides from the rhizomes of these therapeutic varieties [19, 20]. Phytochemicals are chemicals derived from plants and the term is often used to describe the large number of secondary metabolic compounds found in plants. Phytochemical testing analysis is a simple, fast, and affordable process that gives the specialist a fast response to the various types of phytochemical in a

combination and an important device in bioactive substance studies. Vegetation is worked out as medication due to the incident of wide range of normally strenuous elements [21].

The present study is a pace to sort out safe reasons for some anti-microbial broker. Antimicrobial action may be linked to place bioactive substances to make complicated with microbial mobile wall and thus suppressing the microbial growth. The study provides basis for the use of fractions from research plant for the treatment of infections associated with the studied microorganisms.

2. Materials and Methods

2.1. Collection of Plant Material for Biological Activities

Fresh plant of *Eremostachys laciniata* (Fig. 1) was collected from district Karak. The taxonomic identity of these plants was determined by qualified plant taxonomist Dr. Nisar Ahmad, Department of Botany, Kohat University of Science & Technology (KUST), Kohat, and Khyber Pakhtunkhwa, Pakistan. Fresh plant materials were washed under running tap water; air dried and then was homogenized to fine powder and stored in airtight bottles. The Biological activities of these plants were carried out in the laboratory of Department of Microbiology, KUST, and Kohat.

Figure 1. *Eremostachys laciniata*

2.2. Extraction and Fractionation

The shade-dried whole plant material of Eremostachys laciniata was soaked in methanol for 10 days (Fig. 2). The powdered drug was extracted with 80 % methanol three times and filtered at room temperature. The filtrate was evaporated in rotary to get a dark-greenish residue (crude extract), which was further suspended in water and partitioned successively with n-hexane, chloroform and n-butanol to obtain n-hexane-soluble, chloroform-soluble, ethyl acetate-soluble, n-butanol-soluble and aqueous fractions, respectively [6].

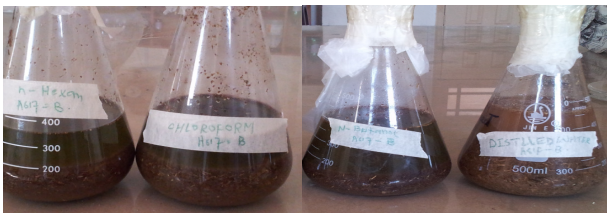

Figure 2. Plant material of *Eremostachys laciniata* soaked in different solutions.

2.3. Preparation of stock solution from different fraction of plant materials

The stock solution was prepared from each 5 fractions i.e. methanol, *n*-hexane, chloroform, *n*-butanol and aqueous fraction. Each fraction was dissolved in the distilled water at the dose rate of 0.025µg/ml and 0.050µg/ml respectively to conduct the antibacterial activities.

2.4. Biological Activities

Application of valuable and negative consequences of a medicine in living organisms can be brought about by phytochemical and biological evaluation/screening of plants. The antibacterial bioassays were carried out, which helped in the selection of appropriate plants fraction for bioassay guided isolation of the dynamic compound.

2.5. Antibacterial Analysis

For antibacterial assess agar diffusion technique as established by Khan, *et al.,* 2011 was adopted with trivial alteration. In this scheme, wells were prepared in petriplates and the solution of veteran plants extort were decanted in these wells and after incubation of 24h, the precincts made around these wells were calculated and evaluated with the zones made around the customary antibiotic used.

2.6. Preparation of Stock Solution

The stash solution was primed by adding 150 mg of the plant mine in 10 ml of the untainted autoclaved refined water to obtain 15mg/ml.

2.7. Pathogenic Microbes Used

Four bacterial species *Pseudomonas aeruginosa, Escherichia coli, Salmonella typhii* and *Staphylococcus aurius* were used for antibacterial analysis. All the pathogens were obtained from Microbiology Department, Kohat University of Science and Technology, Kohat, Pakistan. The microbes were kept at 4 C^o on nutrient agar media.

2.8. Preparation of Inocula

2 or 3 camps of bacteria were taken from 24h aged culture and the colonies were assorted in 10 ml nutrient broth media in a test tube. The test tubes were sited in incubator at 37C^o for 24h. An autoclaved solution was sundry in the test tubes including bacterial cultures and their turbidity were accreted until harmonized with turbidly of McFarland 0 .5 BaSO4 typical. This inoculum's was set for seeding nutrient agar plates.

2.9. Preparation of the seeded agar plates

20g Nutrient agar was taken in one liter distilled water and autoclaved to organize nutrient agar media. The media was then permitted to chill up to 45^{o}C. 75 ml media was dispensed into each petriplate and allowed to congeal. Bacterial strains from broth media were scrubbed by an inoculating sphere or swab. Fissure or wells were ended in each plate by a cork borer (8mm).

2.10. Pouring of ordeal solutions, incubation and measurement of precinct of reticence

100µl of the ordeal solutions, and identified concentration of antibiotic (chloramphenicol) were placed in their relevant

holes. Antibiotic was used as positive control. The plates were then incubated at 37°Cin paraffin oven. The measurement of the span of the zones around holes were done and then evaluated with the diameter of the zones produced by antibiotic.

2.12. Phytochemical screening assay

Phytochemical screening of extracts was performed in order to squared the presence of alkaloids, carbohydrate, glycosides, coumarin, flavonoids, saponins, tannins and triterpenoids steroid etc. [22, 23].

3. Results and Discussion

3.1. Antibacterial activity of selected plant samples

The crude, n-hexane and chloroform fractions of chosen plant samples were veteran beside four bacterial species and zone of inhibition were calculated. The inhibition region for Ampicillin (antibiotic as control) was also noted as standard. The extracts were subjected to four bacterial pathogens namely *E.coli, S.aureus, S.typhii and P.auriginosa* to evaluate the antibacterial potential of the plant. It was observed that all fractions of the plant were potentially active against S. aureus when compared with standard, showing 98% to 80% inhibitions while the same fractions were found to be poorly active against other three tested pathogens (Table 1). Graphical representation of various fractions against different bacterial strains is shown in Fig 3.

3.2. Phytochemical Results

The *Eremystachyes laciniata* is found to be good source of alkaloid, flavonoids, tennins, resins, carbohydrates, starch, proteins, glycosides, coumarin, terphenoids steroids and saponine. Anthranol glycosides were found to be absent in the plant. The presence of phytochemicals in plant is the possible answer to its active antimicrobial profile. The bioactive extract/fractions of the plant can be further used for isolation of natural products from the plant and to add a number of valuable compounds to Phytochemistry and pharmaceutical industries (Tables 2).

Table 1: Antibacterial (mm) profile of the *Eremostachys laciniata*

Bacterial strains	***E.coli***	***S.aureus***	***S.typheae***	***p.auriginosa***
Extracts ↓				
Aqueous	9	11	10	12
n-Butanol	10	15	11	9
Chloroform	11	13	10	10
n-Hexane	18	12	11	10
Positive control	30	16	20	23

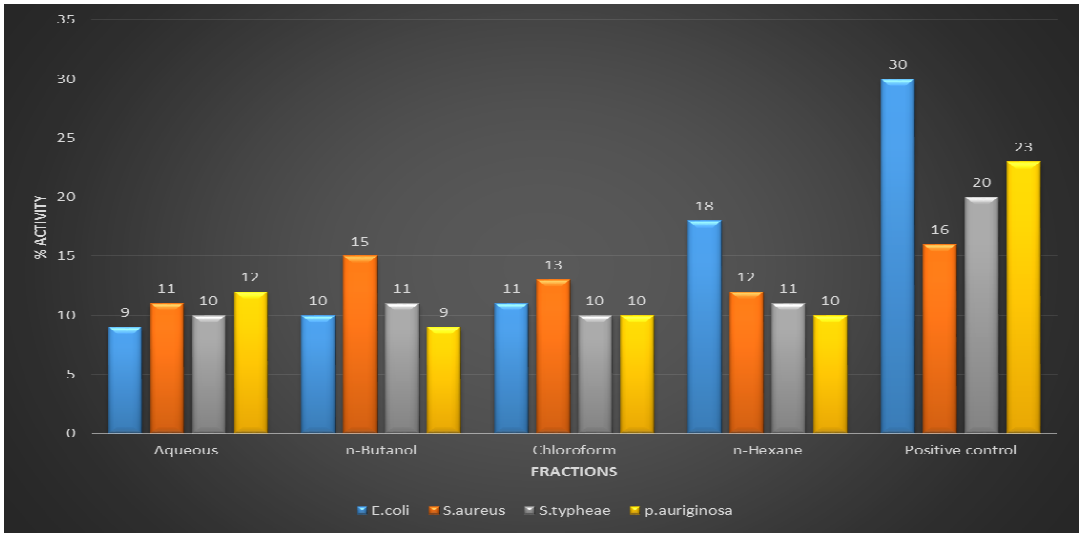

Figure 3. Graph showing the comparative study of various fractions against different bacterial strains.

Table 2: Phytochemicals evaluation of *Eremostachys laciniata*

Indications Used for presence or absence of a compound:
Absence (-); Presence (+)

Phyto chemicals	Inference	Phyto chemicals	Inference
ALKALOIDS		**Resins:**	
Mayer's Test	+	Acetone test	+
CARBOHYDRATES		**Proteins:**	
Molish's Test	+	Biuret's test	+
Benedict's Test	+	**Glycosides:**	
Fehling's Test	+	Keller Killiani Test	+
FLAVANOIDS		**Anthranol glycosides:**	
Shinoda test	+	Borntrager,s test	-
TRITERPENOIDS & STEROIDS		**Saponine:**	
Libermann-burchards test	+	Foam test	+
Tennins		**Coumarin:**	
$FeCl_3$ test	+	NaOH test	+
Starch:			
NaCl test	+		

4. Conclusion

The presence of different phytochemicals in the plant is the possible answer for its active antimicrobial profile. The bioactive fractions can be further use for isolation of natural products.

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References

[1] A. Rehman and M.I. Chaudhry, "*Sharing Innovative Experiences*", 8. 2002.
[2] M. Ali and S. I. Azhar, "*Hamdard Medicus"*, 72, 2000
[3] W. Sneader, "Drug Discovery: The Evolution of Modern Medicines", 78, 1985.
[4] C.D. Leak, and C.C. Thomas, "A Historical account of pharmacology to 20th century." III, 1975.
[5] P. Cosa, A.J. Vlietinck, D.V. Berghe and L. Maes (2006), "Anti-infective potential of natural products: How to develop a stronger in vitro 'proof-of-concept*", Journal of Ethnopharmacology*, 106, 290.
[6] Z.K. Shinwari, I. Khan, S. Naz and A. Hussain, "Assessment of antibacterial activity of three plants used in Pakistan to cure respiratory diseases", *African Journal of Biotechnology,* 8, 7082, 2009
[7] M.R. Delnavazi, "Essential Oil Composition, Antioxidant Activity and Total Phenolic Content of Some Lamiaceae Taxa Growing in Northwest of Iran", *Pharmaceutical sciences*, 20, 22, 2014
[8] A.Delazar, "Rhizomes of *Eremostachys laciniata*: Isolation and Structure Elucidation of Chemical Constituents and a Clinical Trial on Inflammatory Diseases", *Advanced Pharmaceutical Bulletin*, 3, 385, 2013
[9] O. Said, K. Khalil, S. Fulder and H. Azaizeh, "Ethnopharmacological survey of medicinal herbs in Israel, the Golan Heights and the West Bank region", *Journal of Ethnopharmacology*, 83, 251, 2002.
[10] N. Erdemoglu, N.N. Turan, I. Cakoco, B. Sener and A. Aydon, " Antioxidant activities of some Lamiaceae plant extracts", *Phytotherapy Research*, 20, 9, 2006.
[11] M. Modaressi, (2009), "Antibacterial iridoid glucosides from *Eremostachys laciniata*", *Phytotherapy Research*, 23, 99, 2009.
[12] M. Nisar, "Antidepressant screening and flavonoids isolation from *Eremostachys laciniata* (L) Bunge", *African Journal of Biotechnology*, 10, 1696, 2013.
[13] S. Khan, M. Nisar, W. Rehman, R. Khan, F. Nasir, "Anti-inflammatory study on crude methanol extract and different fractions of *Eremostachys laciniata*", *Journal of Pharmaceutical Biology*, 48, 1105, 2010
[14] A. Delazar, "Evaluation of analgesic activity of *Eremostachys laciniata* in mice", *Journal of Natural Remedies*, 9, 1, 2009
[15] J.L. Stewart, "Notes on the flora of Waziristan", *Royal Geographical Society*, 32, 316, 1862.
[16] IC, "Labiatae of South-west Asia: Diversity, distribution and endemism", *Proceedings of the Royal Society of Edinburgh*, 23, 1990.
[17] Z. Jamzad, M. Ingrouille, M.S.J. Simmonds, "Three new species of Nepeta (Lamiaceae) from Iran", *Taxon*, 52, 93, 2003.
[18] M. Nasir, "Antidepressant screening and flavonoids isolation from *Eremostachys laciniata* (L) Bunge", *African Journal of Biotechnology*, 10, 1696, 2011
[19] I. Cali, "Secondary metabolites from *Eremostachys laciniata*", *Natural Product Communications,* 3,117, 2007.
[20] A. Delazar, "Furanolabdane diterpene glycosides from *Eremostachys laciniata*" *Natural Product Communications*, 3, 873, 2008.
[21] Z.K. Shinwari, T. Watanabe, M. Rehman and T. Youshikawa, "*A Pictorial Guide to Medicinal Plants of Pakistan*, Kohat University of Science &Technology, Kohat", 247, 2006.
[22] K. Prabhu, "Pharmacognostic and preliminary phytochemicals investigations on the leaves of *Viburnum punctatum*", *Journal of Pharmaceutical Sciences* and *Research*. 1, 43, 2009.
[23] J.U. Bhat, Q. Nizami, M. Aslam, S.A. Parray and S.T. Ahmad, "Pharmacognostical and phytochemical evaluation of Nepeta bractaeta and HPTLC finger printing of its extracts", *International Journal of Universal Pharmacy and Life Sciences* 2, 147, 2012.