Antibacterial and Phytochemical Evaluation of the crude extract and Fractions of Fagonia cretica.

Rumana Saeed[1], Rashid Iqbal[1], Hameed Ur Rehman[1],Nisar Ahmad[2], Ihsan ul Haq[1], Zubia Masood[3], Salman Khurshid[1],Abid Inayat[1],and M.Mudasar Aslam[2]*

[1]Department of Chemistry, Kohat University of Science & Technology, Kohat, 26000, KPK, Pakistan.
[2]Department of Botany, Kohat University of Science & Technology, Kohat, 26000, KPK, Pakistan.
[3]Department of Zoology, University of Karachi, 75270, Sindh, Pakistan.
Mudasar_kust@yahoo.com

Abstract:

The aim of the present research was to evaluate the antimicrobial potential and phytochemical profile of *Fagonia cretica* plant to look into possible natural therapy agents. The shade-dried whole plant material of Fagonia cretica 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 14mm inhibition against different strains of bacteria as compared to the control, which gave maximum inhibition up to 30mm.The Fagonia cretica was found to be good source of alkaloids, flavonoids, tennins, resins, carbohydrate, starch, proteins, glycosides, coumarin, terponides, steroids and saponine. Anthranol glycosides and starch 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.

Key Words; Phytochemical, Antibacterial activity, Fagonia Cretica,

Introduction:

In past, plants provided a source of motivation for novel medication complexes, as plant-derived remedies made large influences to human health. Their part is two-fold in the progress of new drugs: (1) they may become the plate form for the improvement of a medicine, a natural blue print for the development of new drugs or; (2) a phytomedication to be used for the treatment of syndromes. Customary medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in the developing world [1, 2]. Globalization interferes with infectious disease control at the national level while microbes move freely around the world. Human response to infectious diseases is accustomed by jurisdictional boundaries [3]. In recent years, about 43% of the total deaths that happened in the developed countries are due to infective diseases. The search for new effective antimicrobial agents is necessary. Due to the entrance of microbial resistance and manifestation of fatal opportunistic infections. In epidemic areas, resistance against antimicrobial agents has emerged due to recurrent infections [4]. Antibacterial activity of the plant has been verified by numerous researchers [5-7]. The antibacterial activity of medicinal plants of Khyber Pakhtunkhwa is needed to be done for the identification of candidate plants. Zygophyllaceae is a family of about 25 genera and 240 species and well-known in tropical, subtropical and warm temperate, often in drier areas. Represented by 8 genera and 22 species in Pakistan. It's an annual or perennial herb. Flowers are perfect and regular; sepals imbricate or valvate, free, persistent or deciduous; petals usually free and imbricate. Disk or nectar glands are either present or absent. The ovary is superior, 2 to 5 or 10-lobed, and fruit capsule is often spiny or tuberculate. It is yearly to biennial to glabrous shrub let. It flowers all over the year, allocated in India, Pakistan, Iran, Sudan, Somalia and Kenya. It is commonly known as Azghakhi, Damiya and Dhaman in KPK, Pakistan. It is used in the treatment of piles, urinary disorders, dysentery, stomach ache, typhoid, cancer and as a blood purifier [8, 9], to release constipation and as a laxative [10]. It is well known in scientific littérateur to be a therapeutic plant. It is used as diuretic, analgesic, antipyretic, antihepatotoxic, antidysentric, antidote, antiseptic, tonic, bitter, antiasthmatic, stimulant, stomachic and antitumor [11].

Material and Methods:

Plant Material for Biological Activities: Fresh plants or parts of *Fagonia cretica* **(Fig.1***)* was collected randomly from district Karak, *Khyber Pakhtunkhwa*, Pakistan. The taxonomic identity of the plant was determined by qualified plant taxonomist at *Botany Department, 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.

Figure 1. Pictorial representation of Fagonia cretica with its leaves, fruits and inflorescence

Extraction and Fractionation: The shade-dried whole plant material of *Fagonia cretica* 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 [12].

Antibacterial activity: For antibacterial activity bacterial strains were taken from the Microbiology laboratory of Department of Microbiology, Kohat University of Science and Technology, Kohat KP, Pakistan. These bacterial strains were subculture on the nutrient agar. The nutrient agar plates were prepared and left for solidification. Then wells were formed by using sterile cork borer. The plates were then inoculated by the bacterial cultures using the sterilized swabs. The wells were filled with the plant extracts (100 µl in each well). For positive control chloramphenicol was added to the center of the nutrient agar plate. The Petri plates were incubated for 24 h at 37 °C. After incubation the Petri plates were checked for different zone of inhibition formed by the plant extracts [13].

Preparation of inoculums: A loopful bacterial culture was immersed in the distilled sterile water to form the dilution of inoculums.

Phytochemical screening assay: After obtaining the crude extract or active fraction from plant material, phytochemical screening can be performed with the appropriate tests. For getting an idea regarding the type of phytochemical existing in the extract mixture or fraction. The test for conforming the presence of alkaloids, anthraquinones, cardiac glycosides, coumarins, flavonoids, saponins, tannins and terpenoids was carried out as per established protocols [14-15].

Result and Discussion:

Antibacterial activity: Total of four extracts of *Fagonia cretica* (aqueous, *n*-butanol, chloroform, *and n*-hexane) were used to evaluate the antibacterial profile of the plant and methanol, aqueous extracts were taken for phytochemical screening of the plant. The antibacterial assay shows that all of the extracts were found to be active against the tested pathogens but all the fractions of our research plant were potentially active against *S. aureus* when compared with positive control, showing almost 100% inhibitions (**Table 1**). The antibacterial disc which was used as positive control in the research work to detect the sensitivity of bacterial strains used also showed zones of inhibition against the four bacterial strains. Positive control has maximum zone of inhibition against *E. coli* i.e. 30mm and minimum zone of inhibition against *S. aurous i.e. 14mm* (**Table 1**). It also reveals that the bacterial strains used for the activity were fully active. The overall activity also shows that aqueous extract of the plant shows maximum activity against all the strains. Graphical representation of various fractions against different bacterial strains is shown in **fig 2**.

Table 1: Antibacterial activity (mm) of different extracts of *Fagonia cretica* against selected bacterial strains

Bacterial strains / **Extracts ↓**	***E.coli***	***S.aureus***	***S.typheae***	***p.auriginosa***
Aqueous	12	14	10	12
n-Butanol	10	11	12	9
Chloroform	10	12	11	10
n-Hexane	10	10	13	9
Positive control	30	14	22	21

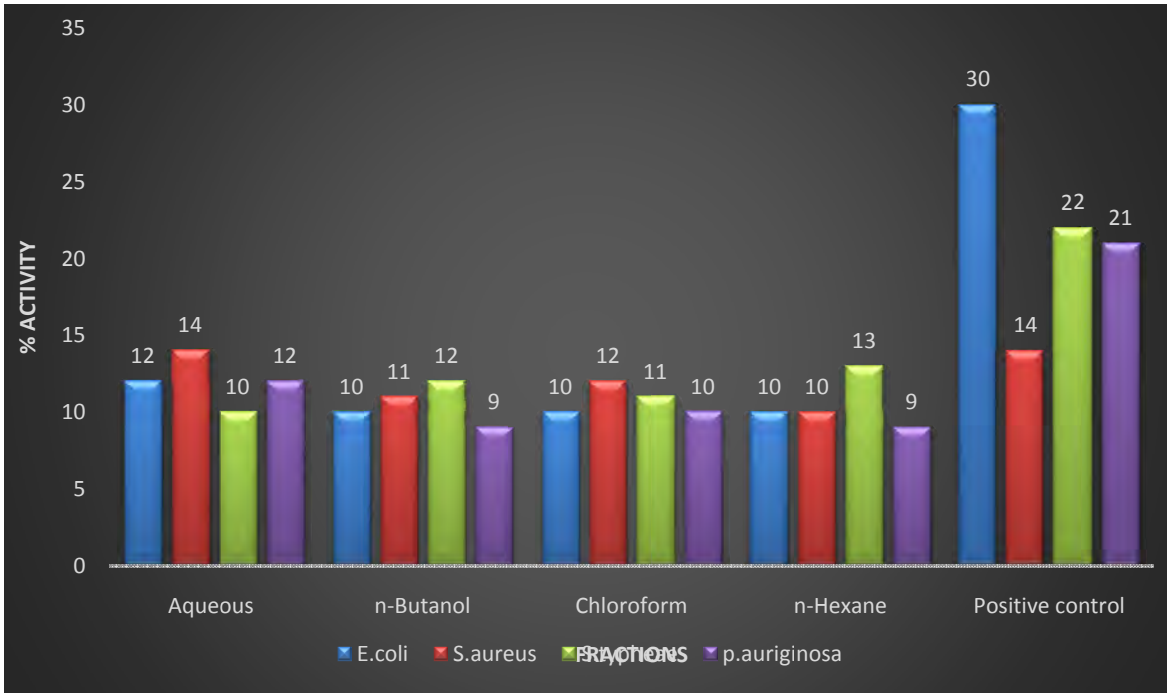

Fig 2: Graph showing the comparative study of various fractions against different bacterial strains.

Phytochemical Results:

The *Fagonia creticais* found to be good source of alkaloid, flavonoids, tennins, resins, carbohydrates, starch, proteins, glycosides, coumarin, terphenoids and steroids. Antrhraqunine glycosides and triterphnoids were found to be absent in the plant. 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 **(Table 2)**

Table 2: Phytochemicals evaluation of *Fagonia cretica*

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

Phyto chemicals	Inference	Phyto chemicals	Inference
ALKALOIDS		**Resins:**	
Mayer's Test	+	Acetone test	+
Wagner's test	+	**Bufadinoloids:**	
Hager's test	+	Libermann test	+
CARBOHYDRATES		**Proteins:**	
Molish's Test	+	Millon,s test	+
Benedict's Test	+	Biuret's test	+
Fehling's Test	+	**Glycosides:**	
Deoxysugar		Baljet,s test	+
Killer-killani test	+	Legal,s test	+
FLAVANOIDS		**Anthranol glycosides:**	
Lead acetate test	+	Borntrager,s test	-
Shinoda test	+	**Saponine:**	
TRITERPENOIDS & STEROIDS		Foam test	+
Salkowshi test	-	**Coumarin:**	
Libermann-burchards test	+	$FeCl_3$ test	+
Tennins		**Starch:**	
lead acetate test	+	NaCltest	-
$FeCl_3$ test	+		

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.

Acknowledgment: The authors are grateful to Dr. M. Mudasir Aslam for his support and guidance. This research was supported by Kohat University of Science & Technology (KUST), Kohat.

References:

[1] G. Dastagir, F. Hussain and A.A. Khan "Antibacterial activity of some selected plants of family Zygophyllaceae and Euphorbiaceae",J.Med Plants Res, 6, 5360. (2012),
[2] O.O. Igbinosa, O. Igbinosa and O.A. Aiyegoro "Antimicrobial activity and phytochemical screening of stem bark extracts from Jatropha curcas (Linn)", Afr. J. Pharm Pharmacol, 3, 58. (2009),
[3] S. Stepanovic, N. Antic, I. Dakic and M.S. Vlahovic "In vitro antimicrobial activity of propolis and synergism between propolis and antimicrobial drugs",J. Microb. Res, 158, 353. (2003),
[4] L.J. Carballo, L.Z. Hernandez-inda, P. Perzer and M.D. Gravalos "A comparison between two brine shrimp assays to detect in vitro cytotoxicity in marine natural products", Biol. Med. Cent, 2, 1. (2002),
[5] N.M.A Chaudhry and P. Tariq "Anti-microbial activity of Cinnamomum cassia against diverse microbial flora with its nutritional and medicinal impacts", Pak. J. Bot, 38, 169. (2006),
[6] S. Saeed and P.Tariq, "Antibacterial activity of Oregano (Origanum vulgare Linn) against gram positive bacteria", Pak. J. Pharm. Sci,22, 421. (2007)
[7] S. Tauseef et al. "Studies on antimicrobial activity of extracts and fractions of Tagetes erecta"Proc. 6th Int. Conf. Pak. Soc. Microbiol, 3. (2007),
[8] N. Akhtar and S. Begum "Ethnopharmacological important plants of Jalala, District Mardan, Pakistan",Pak. J. Plant Sci,15, 95. (2009),
[9] S.K. Marwat, M.A. Khan, M. Ahmad, M. Zafar and F.U. Rehman "Ethnophytomedicines for treatment of various diseases in D. I. Khan District", Sarhad J. Agric,24, 305. (2008),
[10] S.M. Wazir, S. Saima, A.A Dasti, and M. Subhan "Ethnobotanical importance of salt range species of district Karak, Pakistan", Pak. J. Plant Sci, 13, 29. (2007),
[11] B. Sajid, E. Alia, K. Rizwana, S. Uzma , Alamgeer andM. Hafiz "Phytochemical screeningand antimicrobial activity of Fagonia cretica plant extracts against selected microbes",Journal of Pharmacy Research,4,962. (2011),
[12] A.M.Khan., R.A.Qureshi, S.A.Gillani and U. Faizan,Antimicrobial activity of selected medicinal plants of Margalla Hills, Islamabad, Pakistan"Journal of Medicinal Plants Research, 5,4665. (2011),"
[13] M.S.Rahman and M.A.Rashid,"Antimicrobial activity and cytotoxicity of Eclipta prostrate",Oriental Pharmacy and Experimental Medicine, 8, 47. (2008)
[14] K.R. Khandelwal "Practical Pharmacognosy techniques and Experiments", 20th ed., Pune: Nirali Prakashan, 25, 1. (2010),
[15] B. Shah and A. Seth "Textbook of Pharmacognosy and Phytochemistry", 1st ed., New Delhi: Elsevier India Pvt. Ltd, 233. (2010),