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Phytochemical Analysis and *In-Vitro* Antibacterial Activity of Chloroform, Water and Ethanolic Stem Extracts of *Calliganum polygonoides (Phog)*

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Abstract: Plants have been recognized long ago as rich sources of natural products for the treatment of a wide spectrum of diseases. It has been reported that plant extracts are commonly used in traditional medicine and its contribution with respect to health coverage was estimated for over 80% of the world's population, especially in the developing world. The present investigation, qualitatively evaluated the present of some certain phytochemicals in the crude extract of the macerated plant stem obtained from three solvents (ethanol 90%, distilled water and chloroform). Ethanolic extract shows higher presence of phytochemicals over water and chloroform extract. The in-vitro antibacterial activity of crude extract of the plant's stem was tested on one gram positive bacteria (Staphylococcus aureus) and one gram negative bacteria (Escherichia coli). The test was performed by simple Agar diffusion Assay. Zone of inhibition (mm) of the extracts was determined. Of the various extract that was tested ethanolic extract was the one which shows remarkable effect on the microbes (Escherichia Coli 9.40mm and Staphylococcus Aureus 14.60mm). Based on these discovery it might be concluded that Calligonum polygonoides possessed great potential against these human pathogens. It might also be speculated that these plant extract can be subjected to further chemical and biochemical analysis to characterize their chemical constituents and the chemical compound responsible for the antibacterial activity.

Keywords: Antibacterial, *Calligonum polygonoide*, Methanol, Extract, *E. coli*, *S. aureus*

1. Introduction

Plants have been recognized long ago as rich sources of natural products for the treatment of a wide spectrum of diseases. It has been reported that plant extracts are commonly used in traditional medicine and its contribution with respect to health coverage was estimated for over 80% of the world's population, especially in the developing world [1]. Phytomedicine almost went into extinction during the first half of the 21st century due to the use of the 'more powerful and potent synthetic drug'. However, because of the numerous side effects of these drugs, the value of medicinal plants is being rediscovered as some of them have proved to be as effective as synthetic medicines with fewer or no side effects and contraindications. It has been proved that although the effects of natural remedies may seem slower, the results are sometimes better on the long run especially in chronic diseases [2].

Calligonum Polygonoides Linn belongs to the family Polygonaceae, locally known as phog, phogala or phogaro [3]. It is an endangered plant specie, with both food and medicinal value. Medicinally, *C. Polygonoides* can be used as; the aqueous paste of plant acts as an antidote against the

heavy dose of opium and poisonous effects of *Calotropis procera*. According to Katewa and Galav [4] the plant extract is used in cure for typhoid. Plant decoction is given to animals to cure urinary problems. Floral buds give cooling effect to the body and cure sun stroke [5]. To control sun stroke a dose of 50gm floral buds in 100gm curd is very effective [6]. According to [7] flowers are very nutritious with high amount of proteins, bearing digestive and tonic properties, useful against asthma, cough and cold. Medicinally, it is used for treating eczema and juice of shoot is used for eyes as an antidote to scorpion sting [6, 8, 9, 10]. The decoction of plant after boiling is used as gargle for the sore-gums [11-12].

Methanol extract of the C. polygonoides has been reported to show strong toxicity in brine-shrimp lethality test [13]. Preliminary phytochemicals screening of *C. polygonoides*, shows that flavonoids are present in buds, seeds, flowers and stems. Alkaloids are present in roots, buds, seeds and flowers. Proteins are present in flowers and seed. Tannins, steroids, phenols, carbohydrates and terpenoids are present in roots, stems, buds, flowers and seeds [14]. The essential oil from air dried buds and roots of *C. polygonoides* contain a complex mixture of terpenoids, hydrocarbons, phenolic compounds, acid derivatives and ketones. On the other hand, due to the complex nature of the phytochemicals present in a plant extracts, the extraction solvent system needs to be adequately considered. A recent study provided data on the importance of selection of an appropriate solvent type and concentration and indicated that ethanol extracts of plants can offer significant potential for the development of novel antibacterial therapies [15]. To date, limited information is available on *C. polygonoides* with respect to its potential in the treatment of human pathogens. Therefore, the present study was conducted and the principal aim was to qualitatively analyze the group of phytochemicals present in the stem extract using three set of solvents (ethanol, chloroform and water) and evaluate the antibacterial activity in the stem extracts of the plant against some human pathogenic bacteria due to growing resistance against the prevailing antibacterial agents. Observations are that this pathogen is becoming resistant against gentamicin, ciprofloxacin, tetracycline, chloramphenicol, and norfloxacin. *E. coli* is involved in causing severe infections of the urinary tract (of both community and nosocomial origin), sepsis, meningitis, and *E. coli*-associated diarrheal diseases. *E. coli* resistance against fluoroquinolones, penicillins, cephalosporins, aminoglycosides and sulfamethoxazole are reported. The ability of *E. coli* to resist the activity of some certain antibiotic drugs is in many cases due to the nature of its cell wall, which is characterized by thin layer of peptidoglycan. The outer membrane layer makes gram-negative bacteria resistant to many antibiotics that interfere with cell wall synthesis. This variation in activity which arise as a result of nature of the organisms is why this research involve the antibacterial activity test of both gram negative and a gram positive bacteria to observe the effect of the extract on both latter set of organisms.

2. Materials and Methods

2.1. Sample Collection

Sampling was made at the botanical garden of Jodhpur national university India under the proper guidance of horticulturist Mr. S.S Sissodia then authenticated by the Head, department of botany, Professor N.L.V. Yas.

2.2. Sample Preparation

The stem of the plant was carefully collected, and air dried at normal room temperature, well ventilated, for a period of two weeks. The well cleared stem of the plant was broken into pieces before subjected into mechanical grinding, and transformed into powdery form [16]. The purpose of standardized extraction procedure for crude drugs (medicinal plant parts) is to attain the therapeutically desired portion and to eliminate unwanted material with a selective solvent. Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Which in this study are ethanol, chloroform and distilled water. Ten grams of the powder was soaked in 100 ml of 90% ethanol for 24 hours under room temperature, 22°C. Thereafter, the resultant solutions were filtered through Whattman filter paper No.1 grade. The filtrate was concentrated through evaporation process using water bath at 100°C. The extracts were stored in sterile glass bottles at 4°C until further use. The same procedure applied for the remaining solvents. Stem extract in different solvents was subjected to different chemical tests for the detection of different phytoconstituents using standard procedures.

2.3. Antimicrobial Assay

The antimicrobial assay of crude extracts was performed by disc diffusion method. Suspension or dilution of bacteria were prepared by dipping a loop of bacterium in 10 ml distilled water taken in sterilized labeled test tube. One milliliter dilution was transferred from test tube to the respective sterilized Petri plates by using sterilized pipette. The Petri plates were gently rotated to mix the dilution and medium and allowed to solidify at room temperature.

2.4. Antibacterial Test

For testing the antibacterial activity of crude extracts, uniform filter paper discs (6 mm diameter) were formed, sterilized and dipped in chloroform, ethanol and distilled water crude extracts of stem of *C. polygonoides*. The filter paper discs were placed in Petri dishes at their labelled positions. In second step, commercially available antibiotics reference antibiotic discs were placed on the top of the media at the centre of Petri dishes. All the steps were performed in aseptic area. The plates contained the bacterial culture were incubated at 37°C for 24 hours. After the incubation time, all the plates were carefully examined for presence of zone of inhibition as a property of antimicrobial activity.

3. Results

The phytochemical screening of the various extracts shows remarkable presence of important group of compounds as presented in table 1 below

Table 1. *Results of phytochemical screening of C. polygonoides.*

S/No	Phytochemicals	Distilled water	Ethanol	Chloroform
1	Alkaloids	-	+	-
2	Coumarins	+	+	-
3	Flavonoids	-	+	-
4	Phenols	+	+	+
5	Quinines	+	+	+
6	Saponins	+	+	+
7	Steroids	+	+	+
8	Tannins	+	+	-
9	Triterpenoids	+	+	-
10	Xanthoproteins	+	-	+

*KEY: + = Presence, - = Absence.

Table 2. *Zone of inhibition (mm) of antibacterial activity of stem crude extract of different solvent.*

Bacteria	Ethanolic extract 50mg/ml	Water extract 50mg/ml	Chloroform extract 50mg/ml	Chloramphenicol 50mg/ml
E. coli	9.40	0.00	0.00	30.00
S. aureus	14.60	0.00	0.00	33.00

4. Discussion

The phytochemical screening of stem extracts indicate the presence of very important phytochemicals, with the ethanolic extract having to show higher presence of phytochemicals (alkaloids and flavonoids), over water extract and chloroform. This provide insight on a more suitable solvent to be used for further investigation. The results of this work in respect to phytochemical screening is in tandem with the work of [17-20] whom detected the presence of flavonoids, alkaloids, proteins, tannins, steroids, phenols, carbohydrates and terpenoids in different parts of the study plants.

The comparative zones of inhibition of stem crude extracts (chloroform, water and ethanol) of *C. polygonoides* with reference to control antibiotic. It was observed that ethanol extract exhibit antibacterial activity against (*E. coli*) and (*S. aureus)*, 9.40mm and 14.60mm respectively, while water and chloroform show no activity against the test organism. The result of the biological activity may be related to the amount of phytochemicals present comparatively higher in ethanolic extract over water and chloroform. Further isolation and characterization of those groups of chemicals that shows variation base on dissolution to solvent like (alkaloids and flavonoids) could be done for further drug synthesis and enhancement base on structure activity relation.

5. Conclusion

In this investigation, ethanolic extract shows higher presence of phytochemicals over water and chloroform extract. The *In-vitro* antibacterial activity of crude extract of the plant's stem was tested on one gram positive bacteria (*S. aureus*) and one gram negative bacteria (*E. coli*). The test was performed by simple agar diffusion assay. Zone of inhibition (mm) of the extracts was determined. Of the various extract that was tested ethanolic extract was the one which shows remarkable effect on the microbes (*E. coli* 9.40mm and *S. aureus* 14.60mm). Based on these discovery it might be concluded that *C. polygonoides* possessed great potential against these human pathogens. It might also be speculated that these plant extract can be subjected to further chemical and biochemical analysis to characterize their chemical constituents and the chemical compound responsible for the antibacterial activity.

References

[1] WHO 2002. Traditional medicine: Growing needs and potential. WHO Policy Perspectives on Medicines. World Health Organization, Geneva*: 1–6.*

[2] Akunyili DN. The role of regulation of medicinal plants and phytomedicine in socio-economic development, AGM/SC of the Nigerian Society of Pharmacognosy. 13., 2003; 1-7.

[3] Bhandari M. M. (1978). Flora of the Indian Desert (Scientific Publishers) Jodhpur 331-332.

[4] Katewa SS and Galav PK (2005). Traditional herbal medicines from Shekhawati region of Rajasthan. Indian Journal of Traditional Knowledge 4 237-245.

[5] Singh UR, Wadhwani AM and Johri BM (1996). Dictionary of Economic Plants in India. ICAR, New Delhi 37 and 60.

[6] Kumar S, Praveen F, Goyal S and Chauhan A (2008). Indigenous herbal coolents for combating heat stress in the hot Indian Arid Zone. *Indian Journal of Traditional Knowledge* 7 679-682.

[7] Mohil P (2013). Depleting diversity of *Calligonum polygonoides* L. with their importance in the arid region of India. Environmental Consciousness and Human Perceptions (Lambert Academic Publishing) 90-96.

[8] Yawer MA, Ahmed E, Malik A, Ashraf M, Rasool MA and Afza N (2007). New lipoxygenase inhibiting constituents from *Calligonum polygonoides*. *Chemistry & Biodiversity* 7 1578-1585.

[9] Bewal S, Sharma SK, Parida A, Shivam S, Rao SR and Kumar A (2009). Utilization of RAPD analyze natural genetic variation in *Calligonum polygonoides* L. - A Keystone species of Indian Desert. *International Journal of Integrative Biology* 5 148-151.

[10] Singhi M and Joshi R (2010). Famine food of arid Rajasthan: utilization, perceptions and need to integrate social practices by bio-resolutions. *EthnoMed* 4 121-124.

[11] Singh V and Pandey RR (1998). Ethenobotany of Rajasthan, India 4, 64, 80, 237 and 270.

[12] Samejo MQ, Memon S, Bhanger MI and Khan KM (2012). Chemical composition of essential oil from *Calligonum polygonoides* Linn. *Natural Product Research* 27 619-623.

[13] Samejo MQ, Memon S, Bhanger MI and Khan KM (2011). Preliminary phytochemical screening of Calligonum polygonoides Linn. Journal of Pharmacy Research 4 4402-4403.

[14] Wendakoon C, Calderon P, Gagnon D 2012. Evaluation of selected medicinal plants extracted in different ethanol concentrations for antibacterial activity against human pathogens. Journal of Medicinally Active Plants 1(2).

[15] Harborne JB, Baxter H. The handbook of natural flavonoids, Volume 1 and 2. Chichester, UK: John Wiley and Sons, 1999.

[16] Dalal H. M. In-vitro Antibacterial Activity of Ethanol Extract of Calligonum comosum Plant Against four Human Pathogens in Saudi Arabia Department of Biology, Faculty of Science, Nora Bint Abdul-Rahman University, Postal Code 11474, Riyadh, Saudia Arabia. October 2013.

[17] M. Q. Samejo, S. Memon, M. I. Bhanger, K. M. Khan, Nat. Prod. Res. 2013, 27, 619–623.

[18] M. Q. Samejo, S. Memon, M. I. Bhanger, K. M. Khan, Ind. Crops Prod. 2013, 45, 293 –295.

[19] M. Q. Samejo, S. Memon, M. I. Bhanger, K. M. Khan, J. Pharm. Res. 2013, 6, 346–349.

[20] M. Okasaka, Y. Takaishi, K. Kogure, K. Fukuzawa, H. Shibata, T. Higuti, G. Honda, M. Ito, O. K. Kodzhimatov, O. Ashurmetov, J. Nat. Prod. 2004, 67, 1044–1046.