

BIOCHEMICAL SCREENING OF CRUDE EXTRACT AND ITS DERIVED FRACTIONS OBTAINED FROM *CALLIGONUM POLYGONOIDES* AND *TYPHA LATIFOLIA*

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Abstract

The extracts and its derived fractions from two important medicinal plants species *Calligonum polygonoides* and *Typha latifolia* were tested for microbicidy against two bacteria and two fungal pathogens and preliminary phytochemical evaluation. The crude extract and fractions from *C. polygonoides* plant were found to be most potential against *Pseudomonas aeruginosa* compared to *Escherichia coli*. The ethyl acetate and *n*-butanol fractions showed moderate inhibition zones of 8 mm against *P. aeruginosa* followed by crude extract, *n*-hexane and chloroform fractions each giving 7 mm zone of inhibitions. The crude extract and ethyl acetate fraction of *C. polygonoides* revealed 8 mm inhibition against *E. coli* followed by chloroform fraction with 7 mm zone of inhibition. In case of *T. latifolia* plant crude extract and aqueous fractions were found to be the most effective against *Pseudomonas* and *E. coli* giving inhibitions zone of 10 and 11 mm respectively. Chloroform fraction of the plant showed 8 mm while other fractions showed 7 mm zone of inhibition. Both the selected plants were found equally potential against the tested fungi. *n*-butanol fraction of *C. polygonoides* gave 10 mm zone of inhibition against *F. oxysporum*, followed by crude extract of ethyl acetate showing 8 mm. The *n*-hexane and chloroform fractions showed 11 mm of inhibition against *A. alternata*. *n*-hexane fraction and crude extract from *T. latifolia* gave 11 mm inhibition against *F. oxysporum* and *A. alternata*. The selected plant extracts were analyzed for the presence of different bioactive chemical groups. In preliminary phytochemical screening alkaloid, phenol and saponins were found in both plants. After the result and screening of the selected research plants it is concluded that both plants are potentially active and showed good antimicrobial activity and presence of important phytochemicals.

Key words: Medicinal plants, Biological activities, Phytochemical screening, Drug sighting.

Introduction

Plants are used by human as long as when life originates on earth (Shinwari *et al.*, 2009). In earlier times plants were only used for food, shelter and for medicine but with passage of time they were investigated for other purposes. Many people of developing countries nowadays use medicinal plants for the treatment of various disorders, thus medicinal plants provide primary health (Buitron, 1999; Qasim *et al.* 2010). Our country Pakistan is very rich in botanical wealth and a large number of diverse types of plants growing wild in different parts of our country. In Pakistan plants are also used for medicinal and primary health purposes. It is estimated that about 1572 Genera and 5521 plant species are identified in Pakistan (Shinwari *et al.*, 2006). Very few of these plant species are searched and documented for their medicinal properties (Shinwari & Qaisar, 2011). In Pakistan most of urban and rural people use medicinal plants for various diseases on the advice of Hakeems and old peoples Karim & Mahmood (1999). Herbal medicines are used universally for many diseases in non-industrialized societies. Most of modern pharmaceuticals using by physicians now were long ago used as herbal remedies such as opium, Digitalis and Quinine. According to an estimate of the World Health Organization (WHO) about 80 percent population of

some Asian and African countries presently uses various herbal medicines for some aspect of primary health care (Fahd, Toufic, 1996). In the United States according to the World Health Organization, approximately 25% of modern drugs used have been derived from plants (Krek, 1979). We have to paydouble attention in choosing soil and cropping strategies, because in this way we can obtain good yields of high quality and best-priced products, respecting their long-lasting safety and nutritional value (Wainwright, 1989).

The aim of this research is to screen *Typhala tifolia* and *Calligonum polygonoides* following activities and constituents.

A: Antibacterial activity

B: Antifungal activity

C: Phytochemical Screening

Two bacterial strains *Escherichia coli* and *Pseudomonas aeruginosa* were used in antimicrobial activity. Both plants showed good inhibitory action against bacteria. Two fungal strains *Fusarium oxysporum* and *Alternaria alternate* were used in antifungal activity. Both plants also showed good inhibitory actions against fungi.

The preliminary phytochemical screening of the selected plants indicates the presence of important phytochemicals in the plants.

Material and Methods

Antibacterial activity

Plant material: The selected plants *Typha latifolia* L. and *Calligonum polygonoides* L. (Fig. 1) were collected from District Bannu Khyber Pakhtunkhwa, Pakistan in the month of June-July 2015. The plants were then properly identified and then after a proper procedure the plant parts were grinded and finally turned into fine powder for extract formation.

Test bacteria and culture media: Fresh media of Muller-Hinton Ager (MHA) were prepared for Bacterial growth in petri dishes. Two Bacterial strains *Pseudomonas aeruginosa* and *Escherichia coli* were used for testing antibacterial activity of extract. Fresh strains of *Pseudomonas aeruginosa* and *Escherichia coli* were taken from Microbiology Department, Kohat University of Science and Technology (KUST) Kohat.

Experimental procedure: Well diffusion method was used for antibacterial activity. 75 mg extract of each solvent were dissolved in 5ml of Dimethyl Sulphoxide (DMSO). The bacterial strains were evenly spread on petri plates with the help of sterile swabs. 7 wells were bored in each plate with cork borer at the tip of 6 mm. An antibiotic “Cefotaxime” are also used and placed in the centre of each petri dish. After all these steps the petri plates were tightly covered and placed in incubator at 37°C up to 24 hours. The inhibitory zones in each plate were examined and measured after 24 hours. The experiment was repeated 2-3 times to minimize mistakes and to obtain correct results (Salim *et al.*, 2017).

Antifungal activity: Media preparation: Fresh media of Potato Dextrose Ager (PDA) was prepared for antifungal activity. 9 gram of PDA was mixed and dissolved in 250 ml of distilled water in conical flask. The media was then autoclaved at 121°C temperature for 15 minute.

Fungal strains used: For antifungal activity two strains of fungi *Fusarium oxysporum* and *Alternaria alternate* were used. The fresh strains of *Fusarium oxysporum* and *Alternaria alternate* were taken from Department of Microbiology, Kohat University of Science and Technology (KUST) Kohat.



Antifungal activity procedure: Same well diffusion method mentioned in antibacterial activity was used for this experiment. Antifungal activity takes 48-96 hours to show its complete results (Salim *et al.*, 2017).

Phytochemical screening: Both the plants were analysed for phytochemical presence. The presence of Alkaloids, Flavonoids, Tannins, Phenols, Saponins and Glycosides were qualitatively analyzed in the phytochemical screening. The tests were conducted according to the standard procedure used for phytochemical analysis (Nisar *et al.*, 2016).

Results

The present study tested the antibacterial and antifungal activities and phytochemical analysis of *Calligonum polygonoides* and *Typha latifolia*. Both of plant's extracts were experimentally analysed for various activities.

Antibacterial activity: In antibacterial activity *Calligonum polygonoides* L. crude extract and its fractions showed powerful inhibitory action against both bacteria *Pseudomonas aeruginosa* and *Escherichia coli*. The Ethyl acetate and methanol fractions of *C. polygonoides* showed maxim activity against both bacteria forming 8mm inhibitory zone (Figs. 2 and 3). The chloroform fraction showed second highest zone (7mm). Moderate inhibition was shown by water fraction (5mm). *n*-Hexane fraction showed less inhibition against both bacterial strains (4mm). *C. polygonoides* plant extract was active against both bacteria *E. coli* and *P. aeruginosa* (Table 1). In case of second selected plant *Typha latifolia* L., the methanol and water fractions show maximum inhibition (Figs. 4 and 5). Against *E. coli* both water and methanol fraction showed 10 mm zone of inhibition, while against *P. aeruginosa* water have 11 mm and methanol have 10 mm of inhibition. *n*-Butanol and chloroform both showed 6 mm of inhibition against *E. coli* while *n*-hexane and ethyl acetate show 7 mm and 9 mm of inhibition respectively. Against another bacterial strain *P. aeruginosa* butanol and chloroform both showed 7 mm of inhibition while *n*-hexane and ethyl acetate both showed 6 mm of inhibition (Table 2).



Fig. 1. *Typha latifolia* and *Calligonum polygonoides*.



Fig. 2. Extract/fractions of *Calligonum* against *Pseudomonas aeruginosa*.

Fig.3. Extract/fractions of *Calligonum* against *Escherichia coli*.

Fig. 4. Extract/fractions of *Typha* against *Escherichia coli*.

Fig. 5. Extract/fractions of *Typha* against *Pseudomonas aeruginosa*.

Table 1. Microbicidy inhibition (mm) of methanolic Crude extract and its derived fractions of *Calligonumpolygonoides*.

Microbial strains	Crude extract	n-hexane fraction	Chloroform fraction	Ethyl acetate fraction	n-butanol fraction	Aqueous fraction	ANOVA	Antibiotic drug
<i>Pseudomonas aeruginosa</i>	7 ± 1	7 ± 1	7 ± 1	8 ± 1	8 ± 1	6 ± 1	NS	14
<i>Escherichia coli</i>	8 ± 1	5 ± 1	7 ± 1	8 ± 1	6 ± 1	6 ± 1	p<0.01	11
<i>Fusariumoxysporum</i>	9 ± 1	6 ± 1	5 ± 1	8 ± 1	10 ± 1.52	7 ± 1.15	p<0.01	13
<i>Alternaria alternate</i>	8 ± 1	11 ± 1	11 ± 1	6 ± 1	7 ± 1	7 ± 1.52	p<0.01	13

Table 2. Microbicidy inhibition (mm) of methanolic crude extract and its derived fractions of *Typha latifolia* L.

Microbial strains	Crude extract	n-hexane fraction	Chloroform fraction	Ethyl acetate fraction	n-butanol fraction	Aqueous fraction	ANOVA	Antibiotic drug
<i>Pseudomonas aeruginosa</i>	10 ± 1	7 ± 1	8 ± 1	7 ± 1	7 ± 1	11 ± 1	p<0.01	13
<i>Escherichia coli</i>	11 ± 1	7 ± 1	7 ± 1	9 ± 1	7 ± 1	10 ± 2	p<0.01	11
<i>Fusarium oxysporum</i>	7 ± 1	11 ± 1	7 ± 1	8 ± 1	5 ± 1	6 ± 1	p<0.01	14
<i>Alternaria alternate</i>	11 ± 1	7 ± 1	8 ± 1	6 ± 1	8 ± 0.57	6 ± 0.57	p<0.01	13

Table 3. Phytochemical screening of *Calligonum polygonoides*L. and *Typha latifolia* L.

Plant name	Phytochemical	Maximum	Moderate	Minimum	Negative
<i>CalligonumPolygonoides</i>	Alkaloid	+++			
	Saponin		++		
	Flavonoid				-ive
	Glycosides				-ive
	Phenol	+++			
	Terpenoid	+++			
<i>Typhalatifolia</i>	Alkaloid			+	
	Saponin	+++			
	Flavonoid				-ive
	Glycosides				-ive
	Phenol	+++			
	Terpenoid				-ive

+++ =Maximum, ++ = Moderate, + = Minimum, -ive =Not present

Antifungal activity: Both of the selected plants were found best potential against tested fungal strains. Methanol and butanol fractions of *Calligonum polygonoides* showed maximum inhibition of 8mm and 11 mm respectively, other fraction showed moderate inhibition against *Fusarium oxysporum*. Against second fungal strain *Alternaria alternatan*- Hexane and choloroform faction of *Calligonum polygonoides* showed maximum inhibition of 11mm and 10 mm respectively, while other fractions n-butanol, methanol, water and ethyl acetate showed 7mm, 8mm, 7mm and 4mm of inhibition respectively. *Typha latifolia* also showed good inhibition against both fungal strains. N-hexane fraction of *Typhalatifolia* showed maximum inhibition of 11 mm against *Fusarium oxysporum*, while other fractions

showed moderate inhibition of 7mm and 6mm. Methanol fraction showed maximum inhibition of 11mm against *Alternaria alternata* while other fractions showed moderate inhibition of 7mm, 6mm and 8mm (Tables 1, 2).

Phytochemical screening: Both the selected plants were qualitatively screened for the presence of phytochemicals. In *Calligonum polygonoides* L. Alkaloids, phenols, and terpenoids were present. Saponins were found in moderate concentration while flavonoids and glycosides were not found. In *Typha latifolia* L. saponins and phenol were found in maximum concentration while alkaloids were found to be minimum. Other phytochemicals such as terpenoid, flavonoids and glycosides were negatively reported (Table 3).

Discussion

The selected research plants showed strong biological activities according to ethnobotanical approaches and the positive results of samples assured strong antimicrobial and antifungal activities (Gul *et al.*, 2012). The plant samples showed potential antimicrobial activities due to the presence of various bioactive groups of compounds such as alkaloids and phenols as reported in phytochemical screening. According to some authors the plants active compounds make complex structure with bacterial cell wall (Cowan, 1999) and thus, inhibit the growth of bacteria. The present study of *C. polygonoides* provided basis for further use of its bioactive fractions against infections of related bacterial strains. The antimicrobial activity of *Typha latifolia* was also illustrated by Londonkar *et al.*, (Agrios, 1997), where they showed maximum inhibition then our current results against same bacterial strains. Fungi are ubiquitous in nature, found in all environments and many of them cause serious diseases in plants, animals and also in human beings. It is reported that 100,000 species of fungi cause various diseases in plants; about 50 species of fungi cause disorders in human beings and also in animals (Agrios, 1997). In present studies, crude extract and its various fractions *n*- hexane, *n*-butanol, methanol, ethyl acetate, chloroform and water of two selected plants *Calligonum polygonoides* L. and *Typha latifolia* L., were experimentally tested against two fungal strains *Fusarium oxysporum* and *Alternaria alternata*. Both the plants were found to have best potential against tested fungal strains. Fungi cause serious diseases in plants and cause economic losses at a large scale and also cause pathological disorders in humans and animals. For the prevention and controlling of these plant diseases much amount of toxic fungicides is used throughout the world, and these fungicides have dangerous side effects on environment by causing soil and water pollution (Borris, 1996). In this way medicinal plants having such important phytochemicals and antimicrobial properties are widely used against such pathogens (Edeoga *et al.*, 2001). So on the basis of these lines the plant extracts of selected research plants were experimentally evaluated against two fungal strains *Fusarium oxysporum* and *Alternaria alternata*.

Both the plants were qualitatively screened for the presence of phytochemicals. Color tests carried for both the plants were of prime importance because prior to go in detail for phytochemical studies of a medicinal plant, it is very necessary to find that the plant is active or not and then it can be selected for further phytochemical screening, biological or pharmacological activities. In *Calligonum polygonoides* L. Alkaloids, phenols, and terpenoids were present. Saponins were found in moderate concentration while flavonoids and glycosides were not found. In *Typha latifolia* L. saponins and phenol were found in maximum concentration while alkaloids were minimum. Other phytochemicals such as terpenoid, flavonoids and glycosides were negatively reported (Table 3). The detected phytochemicals above have various pharmacological importances. For example, alkaloids have anti-inflammatory and analgesic properties as reported by Edeoga & Enata (Ashwani & Ashish, 2012). Saponins play important role in maintaining balance in Na and Ca ions in

cardiac muscles (Mungole *et al.*, 2012). In recent studies saponins are reported that it plays against cancer and used as preventive of cancer (Miller, 1996). Flavonoids show anti-inflammatory, antiallergic and vasodilatory activities (Victor *et al.*, 2004). All these phytochemicals are found in plants so these plants are pharmacologically used for curing of various disorders.

Plants are the cheap source of important phytochemicals detected in our extract and thus it can be easily available and can be used without any harmful effects (Shinwari *et al.*, 2013). Synthetic drugs are more expensive and also have some side effects also. Therefore, the development of new high effective drug is much needed for control and preventing of various diseases.

Conclusion and recommendations: In the present study it was concluded that all the crude fractions of *Calligonum polygonoides* and *Typha latifolia* were active against selected bacterial and fungal strains. The extracts also showed good antimicrobial activity and presence of important phytochemicals. From the above biochemical screening it is clear that the selected medicinal plants contain important chemical classes of drugs and that's why these plants are locally used as medicinally on large scale.

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