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In-Vitro Activities of Nine Ethnomedicinal Plants from Pakistan against Bacteria Causing Infectious Diseases in Human

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Abstract: Medicinal plants have an appreciable value in the development of modern therapeutics. In the present study ethanolic extracts from the leaves of nine selected medicinal plants, including *Calendula arvensis*, *Dodonaea viscosa*, *Olea europaea*, *Ficus carica*, *Otostegia limbata*, *Withania somnifera*, *Eucalyptus camaldulensis*, *Mentha longifolia* and *Cannabis sativa* were assessed against *Escherichia coli*, *Klebsiella species*, *Pseudomonas aeruginosa*, *Salmonella species* and *Staphylococcus aureus* using agar well diffusion method. All the selected bacterial pathogens are the major causative agent of human infectious diseases. Plant extracts used in the present study have shown potential activity against *E. coli*, *P. aeruginosa*, *Salmonella species and S. aureus* while inhibitory activity was exhibited against *Klebsiella species*. In comparison to ampicillin most of the plants showed increased activity against *P. aeruginosa*. Among the nine plant extracts, the *Ficus carica* showed highest activity against all the pathogens while exhibited increased activity (29 ± 1 mm) against *P. aeruginosa* as compared to ampicillin (6 ± 1 mm). The medicinal potential of selected plants was for the first time tested against a set of infectious disease pathogens and have shown remarkable activity. Further phytochemical analysis will be helpful for the future elucidation of novel therapeutic agents from these plants.

Key words: Nine ethnomedicinal plants; Gram negative /Gram positive bacteria; ethanolic extracts; antibacterial activity.

Introduction

Medicinal plants are used since ancient times for the treatment of several diseases (Elisabetsky, 1990). From decades, there is ample interest to unlock the secrets of old herbal remedies (Izzo and Ernst, 2009). Globally ethnobotanical studies of traditional plants are valuable in the

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development of public health care and conservation programs (Balck, 1996).

The majority of the population of developing countries uses herbal medicine due to its comparable toxicity, safety and availability (Res J et al., 2003). Plants produce chemical constituents including alkaloids, flavonoids, glucosides, fatty oils, hydrogen, oxygen, carbon compounds, gums, resins, tannins, essential oils, nitrogen and salts which produce a potent biological and pharmacological response *in vivo* (Cetkovic et al., 2007; Sheeba, 2010). Global burden of infectious diseases caused by bacterial pathogen is a major health concern (Colombo and Bosisio, 1996; Iwu et al,. 1999; Zhang, 2006). Herbal medicines are traditionally used for the elucidation of bioactive molecule which can be used to treat infectious diseases (Res J et al., 2003).

The increasing failure of chemotherapeutic due to emergence of drug resistance by microbial agents has potentiated the screening of new medicinal plants for their antimicrobial activity (Eggleston et al., 2010). Developing countries like Pakistan depend largely on plant resources for agriculture and herbal medicines (Shinwari, 2010; Walter et al., 2011).

The present study was designed to investigate the antibacterial activity of nine naturally growing plants from Khyber Pakhtunkhwa, Pakistan.

Materials and Methods

Collection and processing of plants

Nine plants, including *Calendula arvensis*, *Dodonaea viscosa*, *Olea europaea*, *Ficus carica*, *Otostegia limbata*, *Withania somnifera*, *Eucalyptus camaldulensis*, *Mentha longifolia* and *Cannabis Sativa* were collected from different locations (Malakand, Cherat, Mardan and Kohat) of Khyber Pakhtunkhwa (KPK). Medicinal uses and phytoconstituents of these plants were documented (Table 1). Plant identification was done at the Department of Botany, Kohat University of Science and Technology (KUST), Kohat. All collected plants were first washed, dried and then ground as described (Chitra et al., 2012).

Ethanol extraction

Ethanol extraction of plant materials were performed as described (Biswas et al., 2011). Briefly 10 g of each plant dried powder was soaked in 100 ml ethanol for seven days, and then filtered by a sterilized cotton filter. The plant extract was then kept in a shaking water bath at 45°C for three days to evaporate ethanol from the solution. Then 15 mg of each extract was dissolved in 1 ml DMSO (15mg/ml).

Antibacterial assay

Pure cultures of *E. coli*, *Klebsiella spp.*, *P. aeruginosa*, *Salmonella spp.* and *S. aureus* were obtained from the Department of Microbiology, KUST. Antibacterial activity of plants extract was determined by agar well diffusion method (Bauer et al., 1996). Ampicillin (30 µg), a known potential antibiotic, was used as positive control while DMSO, a universal solvent, was incorporated as negative control.

Data analysis

Each experiment was carried out in triplicates and the results were represented as the mean of the triplicates. An average zone of inhibition was calculated by using Microsoft Excel Software 2010.

Table 1. Ethnomedicinal characteristics of selected plants

S.No.	Botanical name	Family	Local name (Pashto)	Part used	Phytoconstituents	Medicinal uses
1	*Cannabis sativa Linn.*	Canabinacea	Bhang	Leaves	Cannabisativine, cannabinoids, Anhydrocannabisativine (Turner et al., 2009).	Leucoderma, scabies, wounds, inflammation and STDs (Dilara and Nath, 2000).
2	*Withania somnifera (Linn.)*	Solanaceae	Kutilal	Leaves, Roots, Seeds	withanosides I, II, III, IV, V, VI, and VII (Matsuda et al., 2001).	Anti-inflammatory effect (Al-Hindawi et al,. 1992), antioxidant (Bhattacharya et al., 1997).
3	*Otostegia limbata (Benth.)*	Labiatae	Pishkar	Whole plant	Tricyclic clerodane-type diterpenoids (limbatolide D & E) (Khan et al., 2009).	Treating bleeding gums of children and opthalmia (Ahmad et al., 2006).
4	*Mentha longifolia (L.) Huds.*	Labiatae	Villanay	Leaves	Longifone, (longiside-A and -B) and flavanone-glycoside (longitin) tricetin 7-O-methylether 3′-O-glucoside 5′-O-rhamnoside, tricetin 3′-O-glucoside 5′-O-rhamnoside and tricetin 3′-O-rhamnosyl- $1 \rightarrow 4$ – rhamnoside (Ali et al., 2002).	Carminative, diarrhea, dysentery and stomachache (Haq et al., 2011).
5	*Ficus carica Forsk.*	Smoraceae	Inzar	Fruit, Latex/leaves	Steroids, triterpenoids, cumarines, flavanoids and glycoside (Kalaskar et al., 2010).	Treatment of leucoderma ringworm, antipyretic and vermicidal, skin diseases, ulcers and diabetes (Kirtikar and Basu, 1992).
6	*Dodonaea vescosa (L.) Jacq.*	Sapindaceae	Ghwara-sky	Whole plant	Tannins, saponins, flavanoids and terpenoids (Prakash et al., 2012).	Heal simple ulcer, fracture (Veerapur et al., 2004).
7	*Olea europaea*	Liliacesae	Zaitoon	Leaves	Oleuropein, Hydroxytyrosol, Leteoline-7-glucoside (Khan et al., 2007).	Hypertonia, arteriosclerosis, rheumatism, gout, diabetes mellitus, and fever (Al-Azzawie and Alhamdani, 2006; Gonzalez et al., 1992).
8	*Eucalyptus camaldulensis Dehn.*	Myrtaceae	Lachi	Leaves	Ellagitannins, flavonoids, phloroglucinol derivatives and galloyl esters (Singab et al., 2011).	Flu (Sultana et al., 2006).
9	*Calendula arvensis (L.)*	Asteracea	Zyar gulay	Leaves/flower	28-O-β-D-glucopyranoside-3-β-O-(O-β-D-galactopyranosyl $(1 \rightarrow 3)$-β-D-glucopyranoside, 3-β-O-(O-β-D-galactopyranosyl $(1 \rightarrow 3)$-β-D-glucopyranoside (Babadjamiay et al., 1987).	Anti-viral, anti-bacterial and fungicide, skin problems, and anti inflammatory (Hansel et al., 1992).

Results

Before documented the antibacterial activities of selected plants, a comprehensive review about the ethnomamedicinal studies is listed in Table 1. About the antibacterial activities, ethanolic extract of studied plants showed variable inhibitory zones against the selected pathogens. *Calendula arvensis* extract showed potential antibacterial activity against all the isolates. Increased activity was conferred against *S. aureus* (20 ± 1 mm) and *E. coli* (18 ± 0.5 mm) while zone of inhibition was exhibited against *Salmonella spp.* (15 ± 1.5 mm), whereas minimum zone of inhibition was noted against *P. aeruginosa* (13 ± 1 mm) *and Klebsiella spp.* (10 ± 0.0 mm) (Table 2).

It was found that extract of *Dodonaea viscosa* showed increased activity against *E. coli* (17 ± 1 mm) and *S. aureus* (16 ± 1 mm), while a zone of inhibition was noted against *Salmonella spp.* (13 ± 1 mm) and *P. aeruginosa* (13 ± 1.5 mm), whereas the minimum activity was conferred against *Klebsiella spp.* (10 ± 1 mm). The extract of *Olea europaea* showed highest activity against *E. coli* (18 ± 0.5 mm) while less activity was noted against *Klebsiella spp.* (10 ± 0.0 mm). *Ficus carica* ethanolic extract exhibited potential activity against *P. aeruginosa* (29 ± 1 mm) *and E. coli* (23 ± 0.5 mm), however a zone of inhibition was shown against *Salmonella spp.* (20 ± 1.0 mm) and *S. aureus* (20 ± 1 mm), minimum activity was conferred against *Klebsiella spp. (*10 ± 0.5 mm).

The *Otostegia limbata* plants extract was evaluated for antibacterial activity. It was observed that ethanolic extract of *Otostegia limbata* showed inhibitory potential against two bacterial pathogens including *S. aureus* (19 ± 1 mm) and *Salmonella spp.* (18 ± 0.0 mm) while minimum inhibitory zone was shown against *Klebsiella spp.* (11 ± 1 mm). The ethanolic extract of *Withania somnifera* leaves showed significant biological activity against the selected bacterial isolates. Increased zone of inhibition was observed against *S. aureus* (21 ± 0.5 mm) and *Salmonella spp.* (20 ±1.5 mm). The ethanolic extract of *Eucalyptus camaldulensis* and *Mentha longifolia* showed potent activity against *E. coli* (18 ± 0.5 mm) *and Salmonella spp.* (18 ± 0.0 mm), while the *Cannabis sativa* plant extract showed antibacterial activity against *S. aureus* (20 ± 2 mm) and *E. coli* (20 ± 0.5 mm).

The ethanolic plant extract of *Withania somnifera, Eucalyptus camaldulensis*, *Mentha longifolia and Cannabis sativa* plants showed decreased activity against *Klebsiella spp.* (10 ± 0.5 mm) (Table 2). In the current study it was observed that *F. carica* showed antibacterial activity as compared to other plants which is almost similar with the zone exhibited by ampicillin, a known antibiotic. *F. carica* showed excellent activity against *P. aeruginosa* and *E. coli*. Interestingly all the plants showed inhibitory activity against *P. aeruginosa* as compared to ampicilin.

Table 2. Antimicrobial activity of plant extracts against pathogenic bacteria

Plants	Mean of average zone of inhibition (mm) of plant extracts relative to ²DMSO against bacterial pathogens				
	E. coli	*Salmonella spp.*	*P. aeruginosa*	*S. aureus*	*Klebsiella spp.*
C. arvensis	18 ± 0.5	15 ± 1.5	13 ± 1.0	20 ± 1.0	10 ± 0.0
D. viscose	17 ± 1.0	13 ± 1.0	13 ± 1.5	16 ± 1.0	10 ± 1.0
O. europaea	18 ± 0.5	14 ± 0.5	14 ± 1.0	16 ± 0.5	10 ± 0.0
F. carica	23 ± 0.5	20 ± 1.0	29 ± 1.0	20 ± 1.0	10 ± 0.5
O. limbata	13 ± 1.0	18 ± 0.0	13 ± 0.5	19 ± 1.0	11 ± 1.0
W. somnifera	18 ± 1.0	20 ± 1.5	16 ± 1.0	21 ± 0.5	10 ± 0.5
E. camaldulensis	18 ± 0.5	18 ± 1.0	15 ± 1.0	13 ± 2.0	10 ± 0.0
M. longifolia	18 ± 1.5	18 ± 0.0	12 ± 0.5	12 ± 1.0	10 ± 0.5
C. sativa	20 ± 0.5	18 ± 0.5	14 ± 1.0	20 ± 2	11 ± 0.5
[1]Ampicillin	38 ± 2.5	27 ± 1.5	6 ± 1.0	20 ± 2.5	10 ± 1.0

Values are mean inhibition zone (mm) ± S.D of three replicates, [1]Ampicillin was incorporated as positive control, while [2]DMSO was incorporated as negative control.

Discussion

Plants are the potential source of antimicrobial agents (Dzoyem et al., 2014). In the present study ethanolic extracts of nine medicinal plants traditionally used for the treatment of several disease, were evaluated for common bacterial pathogens.

The leaves extract of *Olea europaea* showed inhibitory activity against *E. coli* (18 ± 0.5 mm) and *S. aureus* (16 ± 0.5 mm), while the previous study revealed that the fruit and leaves of the *Olea europaea* contain a series of compounds that represent multichemical mechanisms of defence against microbe and insect attacks (Kubo et al., 1985). Similarly *Dodenaea viscose* extract showed optimal activity against *S. aureus* (16 ± 1 mm) and *P. aeruginos*a (13 ± 1.5 mm). The ethanolic extracts of *Dodenaea viscose* also report the activity against the *S. aureus* and *P. aeruginos*a which reflect our present work (Thring et al., 2007).

When *Ficus carica* extract was evaluated, it showed maximum inhibitory zone against *P. aeruginosa* (29 ± 1 mm) and *E. coli* (23 ± 0.5 mm). Some of the results of *Ficus carica* were same with other plants against the tested microorganisms, while *Klebsiella spp.* was minimally inhabited by *Ficus carica*. A previous study observed that microorganisms were sensitive against the extract of *Ficus carica L* (Hiba and Hamid, 2012). Our findings may suggest *Ficus carica* for further elucidation of antibacterial molecules.

The ethanolic extract of *Otostegia limbata* showed zone of inhibition against *E. coli* (13 ± 1 mm), however, increased zone of inhibition was documented against *Salmonella spp.* (18 ± 0.0 mm) and *S. aureus* (19 ± 1 mm), which reflects the use of *Otostegia limbata* extract against human diseases caused by *Salmonella spp.* and *S. aureus*. Similarly extract of *Eucalyptus camaldulensis* showed antibacterial activity against all the tested pathogens which is already reported (Karunaratne et al., 2010).

Withania somnifera showed increased zone of inhibition against *S. aureus* (21 ± 0.5 mm) among all the selected plants, however minimum activity was observed against *Klebsiella spp.* (10 ± 0.5 mm), Our findings are in line with a previous work which reported that ethanolic extract of *Withania Somnifera* leaves showed antibacterial activities against *S. aureus* (Chandoria et al., 2012).

When the ethanolic extract of *Calendula arvensis* was tested against the selected bacterial strains, it showedincreased activity of 18 ± 0.5 mm against the *E. coli*, Antibacterial activity of the leaf extract of *Calendula arvensis* are also previously reported by (Bissa and Bohra, 2011).

The extract of *Mentha longifolia* showed increased activity against *E. coli* (18 ± 1.5 mm) and *Salmonella spp*. (18 ± 0.0 mm) however previously ethanolic extract of *Mentha longifolia* showed lowest activity against *E. coli* (Mabrouk, 2012). The possible explanation of variable findings might be due to climate change, plants and microbial diversity of the area. The *Cannabis sativa* showed zone of inhibition against *P. aeruginosa* (14 ± 1 mm) and their activity was observed against other tested microorganisms; however one study found that a n-hexane extract of leaves of *Cannabis Sativa* were inactive against *P. aeruginosa* (Nasrullah et al., 2012). The differences in results against *P. aeruginosa* may be due to different organic solvent extract of plants used in the cited study.

Conclusion

Our results indicated that selected medicinal plants showed remarkable antibacterial properties against known bacterial pathogens. Finding of the current study will be helpful for further elucidation of phytochemicals and bioactive molecules of these plants.

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