REPORT

Studies on antibacterial activity of some traditional medicinal plants used in folk medicine

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Abstract: Ethanolic extracts of eight medicinal plants commonly used in folk medicine were tested for their antibacterial activity against four Gram positive strains (*Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis* and, *Streptococcus pneumoniae*) and six Gram negative strains (*Escherichia coli, Proteus vulgaris, Proteus mirabilis. Salmonella typhi para A, Salmonella typhi para B* and *Shigella dysenteriae*) that were obtained from different pathological laboratories located in Karachi, Pakistan. Disc diffusion method was used to analyze antibacterial activity. Out of eight, five medicinal plants showed antibacterial activity against two or more than two microbial species. The most effective antimicrobial plant found to be *Punica granatum* followed by *Curcuma zedoaria Rosc*, *Grewia asiatica L* and *Carissa carandas L, Curcuma caesia Roxb* respectively. From these results, it is evident that medicinal plants could be used as a potential source of new antibacterial agents.

Keywords: Medicinal plants, Folk medicine, Antibacterial activity, disc diffusion method.

INTRODUCTION

The significance of medicinal plants for prevention, treatment and cure of diseases are always recognized. History revealed that plants have been a valuable source of natural products for maintaining human health at all the times. Their importance is continuously increasing day by day. Most of the people now prefer natural therapies to overcome serious side effects of some of the present day medication (Owolabi *et al*., 2007). According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs (Nascimento *et al*., 2000).

Therapy with antimicrobial drugs, such as clindamycin, that perturb the intestinal flora but fail to inhibit growth of other microorganisms can permit the proliferation of *Clostridium difficile* and the elaboration of exotoxin.. The essential oil of *Cinnamomum zeylanicum* bark enhanced the bactericidal activity of clindamycin and decreased the minimum inhibitory concentration of clindamycin required for a toxicogenic strain of C.difficile (Shahverdi, *et al*., 2007). Curcumin is a hydrophobic polyphenol derived from rhizome of the herb *Curcuma longa*. Extensive research over the last half century has revealed important functions of curcumin, such as anti-inflammatory, cytokines release, antioxidant, immuno-modulatory, enhancing of the apoptotic process, and anti-angiogenic properties (Bar-Sela *et al*., 2010). Recently Pomegranate extracts have been used as anticancer agents and they contain a large number of potentially bioactive substances (Grossmann *et al*., 2010). Beside these, numerous studies are available in the literature that highlights importance of medicinal plants. It is estimated that phytochemical, pharmacological and antibacterial activity of plant extracts present a unique challenge to strike new source of traditional medication.

In Pakistan, most of the people prefer to take traditional drugs for cure of their diseases. Keeping in mind the increasing importance of medicinal plants to present day requirements to get new valuable, and most effective sources of active pharmaceutical ingredients, present work was designed with the objective to study the antibacterial activity of various plants of Pakistani origin, which are commonly used in eastern medicine and are not well explored. During present study, eight traditional medicinal plants (table 1) were studied to determine their antibacterial activity against standard culture of four Gram positive and six Gram negative strains.

MATERIAL AND METHOD

Plant materials

Eight medicinal plants used in the present study are listed in table 1. The plants materials were purchased from the local market and were identified by an expert. Selected parts of medicinal plants were washed and dried (table 2). The dried part of the plant material was grinded mechanically in a grinder, soaked in 50% ethanol / water

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Table 1: Traditional medicinal plants selected for antibacterial study

S. No.	Botanical Name and Medicinal parts	Common Name.	Family	Origin	Medicinal use to treat/activity	References
1	*Curcuma caesia Roxb* Dried rhizome and leaves	Black turmeric	Zingiberaceae	North-East and Central India	Piles, leprosy, asthma, cancer, wounds, fever, impotency, fertility, tooth ache, vomiting, allergies	Syamkumar *et al*., 2007 Ravindran *et al*., 2007 Amalraj *et al*., 1989
2	*Curcuma zedoaria Rosc* Rhizome	White turmeric	Zingiberaceae	India and Indonesia	Digestion, colic, blood purifier, anti-venom	Wilson *et al*., 2005 Daduang *et al*., 2005
3	*Grewia asiatica L* Fruit, barks and leaves	Falsa	Malvaceae	Indian and Southeast Asia	Demulcent, febrifuge, diarrhea, rheumatism, antibiotic action	Hays, 1953 Chundawat *et al*., 1980 Morton, 1987
4	*Carissa carandas L* Fruit, leaves and roots	Kerenda	Appocynaceae	India, Burma, Malacca and Sri Lanka	Astringent,antiscorbutic, vermifuge, biliousness, Intermittent fever, diarrhea, oral inflammation, earache	Morton, 1987
5	*Tamarindus indica L* Flower, fruit and leaves	Tamarind	Leguminosae	Sudan and tropical Africa	Anthelmintic, antimicrobial,antiseptic,antiviral, asthma, astringent bacterial skin infection	Doughari, *et al* 2006. HealthOnline
6	*Punica granatum* L Fruit, barks and leaves	Pomegranate	Lythraceae	Persia and India.	Leprosy, dyspepsia, antipasmodic, anthelmintic	Morton, 1987 Leslie, 2007
7	*Cinnamomum zeylanicum Blume* Barks	Ceylon cinnamon	Lauraceae	Sri Lanka and India	Colds, diarrhea antioxidant, antimicrobial, foods preservative	Henriettesherbal. Com Shan, *et al* 2005 Mancini, *et al* 1998 Lopez, *et al* 2005
8	*Prunus domestica* L Fruit and barks	Plum	Rosaceae	Armenia.	Antioxidant, laxative, digestive system problem	Jason Zasky. failuremag.com

for 5-8 days. The extract was filtered through Watmman filter paper No.1 and than concentrated in a Rotary evaporator. The concentrated extracts were dissolved in (5mg/ml) distilled water and aliquots were used to test antibacterial activity.

Micro-organisms used

Antibacterial activity was tested against four Gram positive and six Gram negative strains of bacteria (table 2). Seed culture was prepared in Mueller Hinton broth, prepared according to manufacturer's instructions (Merck), and incubated at 37°C for 24 hours.

Antibacterial assay

Mueller Hinton Agar was prepared according to manufacturer's instructions (Merck) and was poured into Petri plates (10x10cm) and incubated at 37°C for 24 hours. Fresh culture was taken by using sterile cotton swab and a lawn was made on nutrient agar plates. Plates were than dried at 37°C for 30 minutes with lids partly opened. Wells of 6 mm diameter were cut in the inoculated agar with the sterile cork borer were and filled with 50% ethanolic extracts.

The plates were incubated for 24 hours at 37°C and inhibition zones were measured to nearest millimeter using a vernier caliper.

RESULTS

During present study, eight traditional medicinal plants were considered to determine their antibacterial activity

against standard culture of four Gram positive and six Gram negative strains (table 1). Results are summarized in table 2 and fig. 1. Out of eight plants material, five showed antibacterial activity for 2 or more bactria were *Carissa carandas* L, *Curcuma caesia Roxb*, *Curcuma zedoaria Rosc* and *Punica granatum* L. No activity was observed for *Tamarindus indica* L, *Cinnamomum zeylanicum Blume* and *Prunus domestica* L against microbial strains tested in the present study.

Table 2: Antibacterial activity of traditional plant extracts against bacteria (zone of inhibition in mm)

Plant (parts used)	Antibacterial activity in mm (Mean ± S.D) and n=3									
	Gram positive				Gram negative					
	B. subtilis. (ATCC 6051)	*Staph. aureus* (ATCC 2593)	*Staph. epidermidis* (ATCC 12228)	*S.. pnuemoniae* (ATCC 33400)	*E. coli* (ATCC 8739)	*Proteus mirabilis* (ATCC 29906)	*Proteus vulgaris* (ATCC 29905)	*Sal. typhi. para.A* (ATCC 9150)	*Sal. typhi. para B* (ATCC 11511)	*Shigella dysenteriae* (ATCC 29026)
Curcuma caesia Roxb (Rhizome, leaves)	0	0	5.50 ± 0.4082	0	6.67 ± 0.4714	0	0	0	0	0
Curcuma zedoaria Rosc (Rhizome)	0	0	6.50 ± 0.4082	0	10.50 ± 0.4082	0	5.16 ± 0.6236	0	5.33 ± 0.4714	0
Grewia asiatica L (Bark, fruit)	0	6.33 ± 0.8498	0	0	6.33 ± 0.4714	0	7.33± 0.8498	0	0	0
Carissa carandas L (Fruit)	0	4.50 ± 0.4082	0	0	4.93 ± 0.0942	0	0	0	0	0
Tamarindus indica L (Leaves, Fruit)	0	0	0	0	0	0	0	0	0	0
Punica granatum L (Fruit)	4.90 ± 0.0816	7.93 ± 0.0942	0	6.43 ± 0.3299	11.13 ± 0.2624	0	0	6.76 ± 0.6128	5.80 ± 0.6164	10.46 ±0.6847
Cinnamomum zeylanicum Blume (Bark)	0	0	0	0	0	0	0	0	0	0
Prunus domestica L (Fruit)	0	0	0	0	0	0	0	0	0	0

*All the tests performed in duplicates

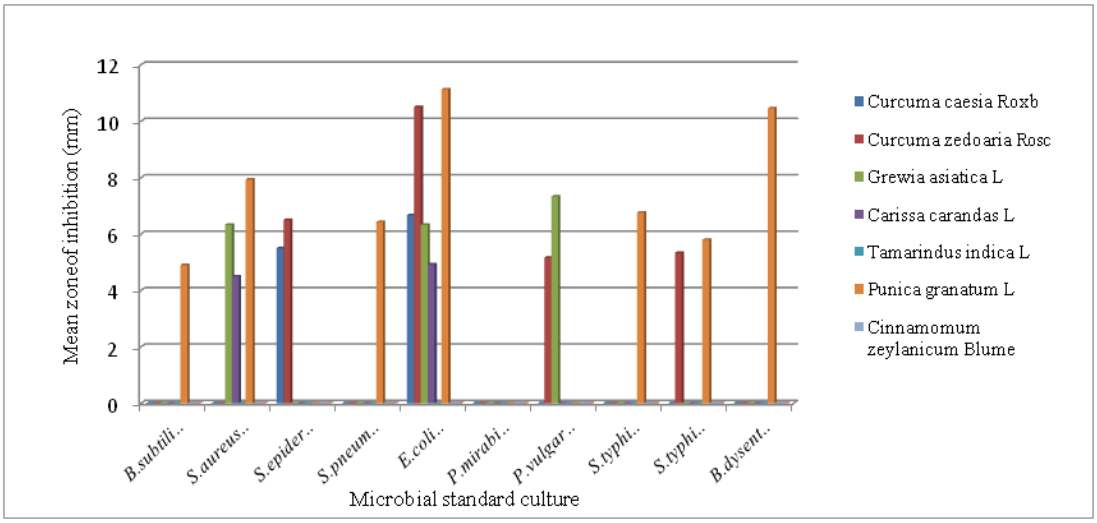

Fig. 1: Antibacterial activity of Medicinal plants against standard culture

The sensitivity of the standard microbial culture used in this study also compared with reference antibiotics of Levofloxacin and Moxifloxacin in table 3. Results show that Levofloxacin found to be most effective as compare to Moxifloxacin.

Table 3: Zone of inhibition (mm) using discs of different antibiotics

S. No.	Name of Organism	Levofloxacin 5 µg	Moxifloxacin 5µg
1.	*B. subtilis.* (ATCC 6051)	16	0
2.	*Staph.aureus* (ATCC 2593)	28	30
3.	*Staph. epidermidis* (ATCC 12228)	16	7
4.	*S.pnuemoniae* (ATCC 33400)	25	0
5.	*E. coli* (ATCC 8739)	15	19
6.	*Proteus mirabilis* (ATCC 29906)	0	0
7.	*Proteus vulgaris* (ATCC 29905)	15	16
8.	*Sal. typhi. para. A* (ATCC 9150)	0	0
9.	*Sal. typhi. para B* (ATCC 11511)	0	0
10.	*Shigella dysenteriae* (ATCC 29026)	9	8

DISCUSSION

Herbal plants have been a source of medicinal compounds since times immemorial. Plant extracts are used in different systems of medicine for the treatment of various human ailments, and for treatment of viral and fungal infections. The effects of plant extracts on bacteria have been researched in different parts of the world. It has been suggested that aqueous and ethanolic extracts from plants are a potential source of antiviral, anticancer and antimicrobial agents. In the present work, we studied the antibacterial activity of some medicinal plants that are commonly used in folk medicine but are not explore well.

The chemical nature of pharmaceutically active constituents of these plants were determined by many scientific researches such as volatile oil of *Curcuma caessia* containing camphor, ar-tumerone, borneol, bornyl acetate and curcumene as a major component (Amalraj *et al*., 1989). Volatile oil of *C.zedoaria* obtained from leaves and rhizome contain curzerenone, cymene, monoterpene, sesquiterpene and isoborneol constituents have antimicrobial and antifungal activity (Makaba, 2006). Fruit of *Grewia asiatica* produce yellow oil that have tetratricontane and unsaponfiable components (Mortan, 1987). Bark, leaves and fruit of *Carissa caranda* contain an unnamed alkaloid used as a remedy for biliousness (Mortan, 1987). The juice of *Punica granatum* contains antioxidant polyphenols as a major component (Seeram *et al*., 2006).

Punica granatum L was found to be most effective against most of the strains tested. This is in accordance with previous works reported by other workers (Choi *et al*., 2009; Al-Zoreky *et al*., 2009; Naz *et al*., 2007 and Prashanth *et al*., 2001). These workers indicated antibacterial activity of *Punica granatum* L against many species of Gram positive and Gram negative bacteria.

In case of *Curcuma zedoaria Rosc*, we found activity against *S. epidermitis, E. coli, Proteus vulgaris* and *Salmonella typhi para B* but no activity against *S. aureus*. This collaborates with Wilson *et al.,* 2005 work who also reported that *Curcuma zedoria* is ineffective against *Staph. aureus*.

Similarly *Grewia asiatica* is reported to posses antibacterial activity against *Staph. aureus, E. coli* and *Proteus vulgaris*. Duraipandiyan *et al* (2009) and Zaidan *et al* (2005) had reported strong activity o some of the related species of Grewia asiatica against *Staph. aureus and E. coli*, but to the best of our knowledge, no work has been reported in the literature that has shown the antibacterial activity of *Grewia asiatica* L.

Other plants found to be effective were; *Carissa caranda* L that showed activity against *Staph.aureus* and *E. coli* but to the best of our knowledge no work has been reported in literature survey that has reported such antibacterial activity. *Curcuma caesia Roxb* extract confirm the result of Ray *et al* (2008) who showed that this plant has slight antibacterial activity and present work support this fact by showing activity against *Staph. aureus* and *E. coli.*

Three plants species showed no antibacterial activity in present study. In previous studies, Al-Fatimi *et al* (2007) showed antibacterial activity of *Tamarind indica* flower (Yemeni origin), and Melendez *et al* (2006) found activity against *Staph.aureus* and *E.coli* of *Tamarind indica* (Caesalpiniaceae) spp.

In another study conducted by Watt *et al* (2007) *Cinnamomum zeylanicum* did not appear to be anitmicrobial. Recently antifungal activity of *Prunus domestica* has been detected by Mahmood *et al* (2010).

CONCLUSION

Medicinal plants are valuable source of therapeutic agents and have great potential to treat infectious diseases caused by resistant microrganisms. *Carissa caranda* and *Grewia asiatica* showed antibacterial activity beside *Punica granatum, Curcuma zedoria* and *Curcuma caesia Roxb*.

These plants, should be further explored especially *Carissa caranda* and *Grewia asiatica*, against other microrganisms. Present finding might be beneficial for future studies to learn more about antibacterial activity of herbs commonly use but still unknown regarding their composition, effectiveness and scientific use for treatment of various diseases especially those whose cure is still unknown.

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