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Investigation of *in-vitro* Antimicrobial activity of *Pterospermum acerifolium* Linn. Fruit

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ABSTRACT

The aim of the present study was to evaluate the antimicrobial potential including MIC of traditionally used and ethnobotanically important tree *Pterospermum acerifolium* fruit. Laboratory isolates of six pathogenic microorganisms, viz., *Staphylococcus saprophyticus*, *Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeruginosa, Aspergillus parasiticus* and *Rhizopus oryzae,* were used in the study for the evaluation of antimicrobial activity. Fruit extracts prepared in different solvents demonstrated a varied level of broad spectrum antimicrobial activity against all microbes. Among all the extracts used, chloroform extract showed maximum antimicrobial activity in the form of zone of inhibition(mm) against *P.aeruginosa* (73.83±1.06mm) and *R. oryzae* (84.6±0.7) followed by *S. saprophyticus* (71.36±1.00) and *A. parasiticus* (57.26±1.6), while minimum inhibitory zone was observed by aqueous extract against *E. coli* (11.59±0.98) and *S. aureus* (16.43±1.20). Remaining concentrates were likewise active and indicated microbial inhibition at different levels. Antimicrobial intensity was compared with that of standard discs, like Amikacin, Kanamycin sulphate and Ketoconazole and was found reasonably well.

Key Words: Ethnopharmacology, *Pterospermum acerifolium* Linn, Antimicrobial activity, antibacterial activity, antifungal activity

INTRODUCTION

Medicinal Plants have been used by people from centuries to cure diseases (WHO, 1996). Knowledge about the use of plants in the treatment of diseases came from close observations and experiences of early human. The gradual accumulation of such knowledge of therapeutics properties of plants helped the primitive culture to provide their medical needs.

Pterospermum acerifolium Linn. (Sterculiaceae) is a large tree, upto 30m tall. It is generally known as Kanack Champa and Moo Chkund. It has gray bark and rusty pubescent young parts. Leaves are oval. Flowers are mostly solitary, 10 to 15cm long, white and fragrant. Capsules are 5 to 10cm long, 5 valves and rusty brown in color. Seeds are compressed, obliquely oval, brown wings and membranous. Blossoming period keeps going from March to May (Abedin & Ghafoor, 1976).

The flowers are considered to be laxative, anthelmintic and stomachic. They are often used in inflammation, blood disorders, ulcers. The tree was used as ayurvedic anticancer medicine (Balachandran, 2005). Blossoms are thought to be diuretic, mitigating, and controls numerous blood issues, cancer and other sickness because of the presence of cell reinforcement substance (Caius, 1990; Mehrotra & Shone, 2008; Sannigrahi *et al*., 2010).

The aim of this study is to evaluate *in-vitro* antimicrobial activity of various extracts of *P. acerifolium* fruit against selected pathogenic microorganisms by agar diffusion method.

MATERIALS AND METHODS

Pods after collection from *Lawrence Garden, Lahore during March-May (2010) were grinded and macerated (4 days) with* petroleum ether (40-60ºC), chloroform, methanol and purified water (Inmaculada *et al*., 2005). The extracts were filtered and concentrated and kept in vacuum desiccators for complete removal of solvent. Percentage Yield (w/w) and physical characteristics of dried powder were noted. For antimicrobial activity, suspensions of dried powder were made with their respective solvents using 0.5mg/ml concentration.

Evaluation of antimicrobial activity of extract of fruit of *P. acerifolium*

Experimental investigation on the antimicrobial properties of plant parts were initially recorded in the late nineteenth century (Cruickshank *et al*., 1975). Antimicrobial activity of extracts against microbes was analyzed by agar well diffusion method according to the protocol of Oretaga *et al*. (1996) and Farreira *et al*. (1996). Four human pathogenic microorganisms, such as *E.coli* (Gram-ive) (ATCC-339), *P.aeruginosa* (Gram-ive)

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(ATCC-321), *S. saprophyticus* (Gram+ive) (ATCC-385), *S. aureus* (Gram+ive) (ATCC-299) and two fungal strains, such as, *A. parasiticus, R. oryzae* were used in the antimicrobial investigation. Strains were laboratory isolates. All the strains were collected from Microbiology Lab. King Edward Medical University and Institute of Industrial Biotechnology, GC University, Lahore. Fluconazole (5mg/ml), Ketoconazole (5mg/ml), Amikacin (30µg), Ampicillin (10µg), Kanamycin sulphate (5mg/ml), Sulfamethoxazole (23.75µg) and Erythromycin (15µg) were used as reference standards discs / drugs.

All the bacteria *E.coli* (Gram-ive) (ATCC-339), *P.aeruginosa* (Gram-ive) (ATCC-321), *S. saprophyticus* (Gram+ive) (ATCC-385), *S. aureus* (Gram+ive) (ATCC-299) were cultivated on nutrient agar medium (Cruickshank *et al;* 1975). 6ml of nutrient agar medium was added in test tubes. These tubes are sterilized and placed in slanting position to solidify. Bacteria were transferred into these slants.

Sterilized Petri plates (180ºC for 2 hrs) were divided into 4 sets and labeled with the name of microbes (bacteria and fungi) crude extracts and medicines. The sterilized melted nutrient agar (for bacterial culture) and potato dextrose agar for fungal culture was poured into these plates (Jett *et al;* 1997). Antifungal activity of extracts against *A. parasiticus, R. oryzae* was also examined. These strains were maintained on potato dextrose agar (Johansen, 1940). 10ml of sterilized distilled water was poured in 3 to 5 days old slant for inoculum preparation (Qadeer *et al*., 1990). Spread plate method was used for distributing microorganism evenly over the surface of agar plates according to the protocol of Wise (2010).

Antimicrobial Screenig Protocol

Antibacterial activity of extracts and Amikacin (30µg) were performed against selected bacterial strains. In the same way antifungal activity of extracts, Ketoconazole (0.5mg/ml) and Kanamycin sulfate (0.5mg/ml) was measured against selected fungi. Microbial colonies were appeared on agar plates. Resistance to these colonies to a certain distance is a zone of inhibition and was calculated (Koch, 1994).

RESULTS AND DISCUSSION

The coarse powder of the shade dried fruit was subjected to successive extraction. The plant material ware treated with solvents with increasing order of polarity, to isolate all possible kinds of phytoconstituents present in the fruit of *Pterospermum acerifolium*. Of the different fractions highest yield of chloroform fraction was obtained that was 1.49%, followed by methanol and water fraction that was 1.47%, and petroleum ether fraction that was 1.46% (Table-1).

Percentage yield at room temperature may be due to different solubility rates of organic compounds in polar and non-polar solvents, similarly, colour variation and change in texture may be due to the presence of Proteins, Sugars, Lipids, Fiber and Vitamin C. Flavonoids, Saponins (Phytohormones) and Alkaloids. Present studies of these physicochemical properties, will help to highlight the importance of these valuable organic compounds found in these plant species and their demand in the market will increase in the near future (Wilfred *et al*., 2010).

Table I: Physical properties of different solvent extracts of *P. acerifolium* fruit

Physical properties	**EXTRACTS**			
	Petroleum Ether	**Chloroform**	**Methanol**	**Water**
Yield (%)	1.46	1.49	1.47	1.47
Color	Olive	Green	Reddish purple	Dark red
Physical state	Powder	Dense	Resinous	Dense

Inhibition zones (mm) by solvents (petroleum ether, chloroform, methanol, water) against microbes are shown in Table II. Of all the solvents used, methanol and chloroform showed maximum inhibition zones to microbes. Inhibition zones (mm) by standard discs (ampicillin,

carbenicillin, trimethoprim, amikacin) against bacteria is shown in Tables III. Results revealed that Amikacin is strongly active against all the tested bacterial strains. Inhibition (mm) by medicines (fuconazole, kanamycin sulphate, ketoconazole) against fungi are shown in Table IV, and Ketoconazole indicated most extreme action against *A. parasiticus* while Kanamycin sulfate being reactive against *R.oryzae*.

Table II: Inhibition zones (mm) by solvents against microbes

Microbes	Solvents			
	Petroleum Ether	**Chloroform**	**Methanol**	**Water**
E.coli (G-)	9.94±0.24	10.36±0.47	10.51±0.35	3.17±0.95
P. aeruginosa (G-)	8.6±1.05	20.4±1.09	19.76±1.47	2.11±0.17
S.saprophyticus(G+)	8.79±1.48	9.09±1.04	20.4±1.66	3.27±0.98
S. aureus (G+)	10.25±0.61	15.14±1.02	15.51±1.22	4.41±1.37
A. parasiticus	10.25±0.61	10.14±1.04	20.51±1.79	2.74±0.79
R. oryzae	10.25±0.61	15.14±1.02	15.51±1.22	4.41±1.37

Table III: Inhibition zones (mm) by standard discs against bacteria

Bacteria	Standard discs			
	Ampicillin (AM 10)	**Carbenicillin (Py 100)**	**Trimethoprim (SXT 25)**	**Amikacin (AK 30)**
E. coli (G-)	1.49±0.52	0.78 ±0.52	1.93 ±0.06	20.28 ±0.68
P. aeruginosa (G-)	1.39±0.43	1.67 ±0.51	1.84 ±0.24	23.35 ±1.35
S. saprophyticus (G+)	1.65±0.45	1.53 ±0.48	1.67 ±0.48	29.89 ±0.61
S. aureus (G+)	3.54±0.51	1.32 ±0.39	3.02 ±0.13	16.50 ±1.54

Table IV: Inhibition zones (mm) by antifungal medicines against fungi

Fungi	Standard medicines(5mg/ml)		
	Fluconazole	**Kanamycin sulphate**	**Ketoconazole**
A. parasiticus	30.45±0.61	2.14±1.02	72.81±0.62
R. oryzae	2.02±0.95	49.17±1.00	13.13±0.93

Antimicrobial figures for zones of inhibition by extracts of *Pterospermum acerifolium* fruit

Results revealed that among all the four extracts of *P.acerifolium* fruit (Fig., 1-6), chloroform extract showed maximum antimicrobial activity in the form of zones of inhibition against *P. aeruginosa* (Fig., 2) (73.83±1.06 mm) and *R. oryzae* (Fig., 6) (84.6±0.7) followed by *S. saprophyticus* (Fig., 3) (71.36±1.00) and *A. parasiticus* (Fig., 5) (57.26±1.6), while minimum inhibitory zone was observed by aqueous extract against *E. coli* (Fig., 1) (11.59±0.98) and *S. aureus* (Fig., 4) (16.43±1.20). Remaining extracts were likewise dynamic and demonstrated microbial inhibition at different ranges. Antimicrobial activity was compared with that of standard discs, like Amikacin, Kanamycin sulphate and Ketoconazole and was found significantly well. The antimicrobial strength of *P. acerifolium* may be due to the presence of bioflavonoid, phenolic compounds, unsaturated fat,

amino acids, putranjivadione (triterpene) that actively retard the growth of these microorganisms and cause their death. (Rajos *et al*., 2006, Sharma & Kumar, 2009; Khond *et al*., 2009).

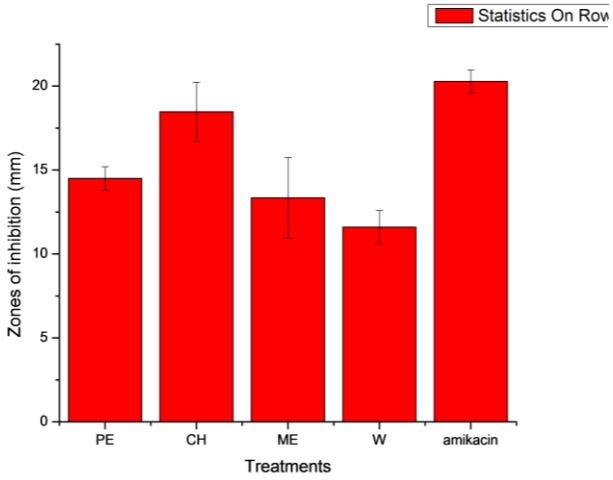

Fig., 1: Inhibition against *E. coli*

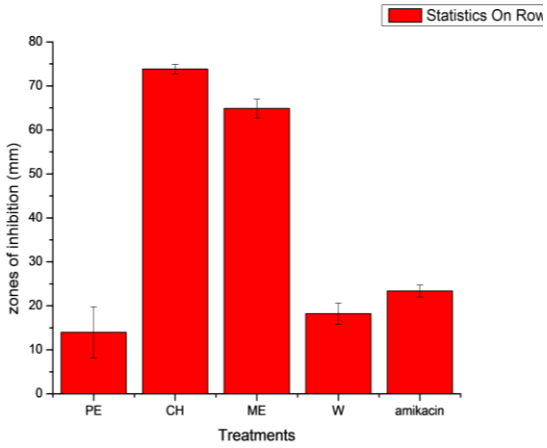

Fig., 2: Inhibition against *P. aeruginosa*

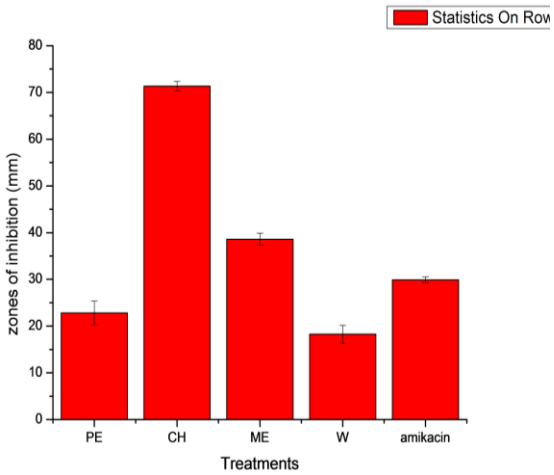

Fig., 3: Inhibition against *S. saprophyticus*

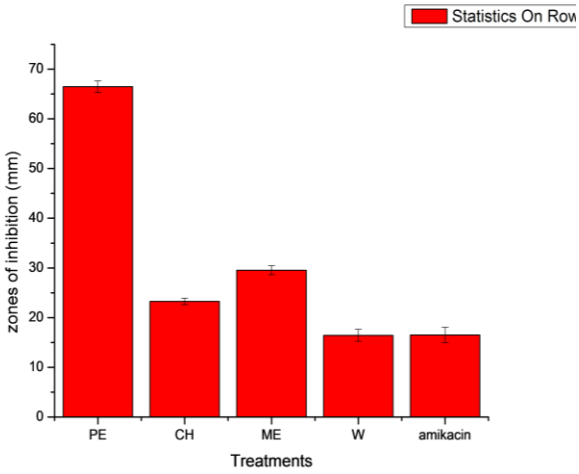

Fig., 4: Inhibition against *S. aureus*

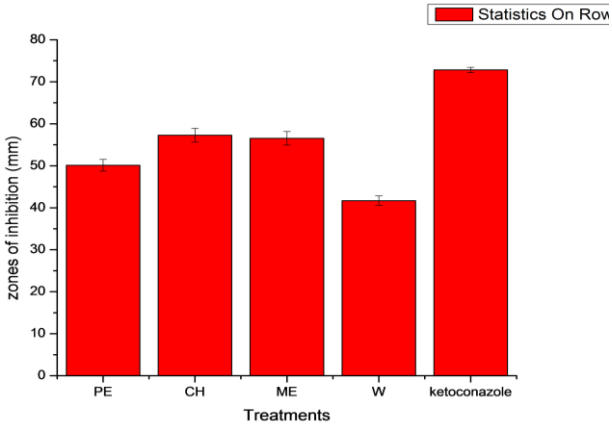

Fig., 5: Inhibition against *A. parasiticus.*

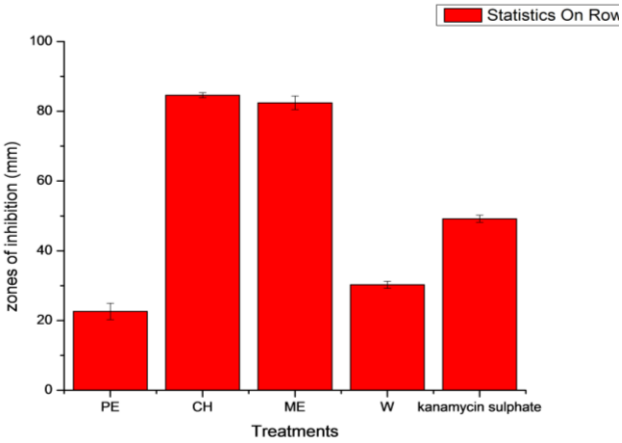

Fig., 6: Inhibition against *R. oryzae.*

CONCLUSION

On the basis of the results obtained in the present work, antimicrobial activity of the ethnobotanically important tree *P.acerifolium* fruit is confirmed against selected microbes. However, further research can be useful in setting up pharmacognostic benchmarks of medication based exploration by *P. acerifolium*. Higher antimicrobial activity of extracts may be attributed to a single antimicrobial compound or may be synergistic effect of many naturally occurring antimicrobial compounds in them. Further studies may help to screen them and can help to make a profile of antimicrobial natural compounds.

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