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In vitro Susceptibility of Some Gram Positive and Gram Negative Strains of Bacteria and Fungi to Root Extracts to *Acacia modesta*

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Abstract

With the therapeutic concept of using the defensive ability of plants against microbial infection, root extracts of *Acac* *modesta* were tested for their potent antimicrobial action. Root extracts exhibited considerable bacteriostatic activity agai two gram positive and two gram negative strains. The antibacterial action was compared with the effect of streptomyc The maximum zone of inhibition of 11 mm diameter was observed in *β-Streptococcus* while a minimum 3mm zone inhibition was found in *Escherichia coli*. Among the fungal species tested, yeast (*Saccharomyces cerevisiae*) exhibit maximum sensitivity action of the extracts. It is also concluded that by improving extraction method antimicrobial potent of the extracts can be further enhanced.

Introduction

The use of *Acacia modesta* as "miswak" for mouthwash gives an impression that the plant possess some active compounds needed to eliminate oral bacteria. Considering this practice as a hypothesis or a view that could be verified on scientific basis, enabled us to conduct the present study. *Acacia modesta* belongs to family Mimosaceae and is a tree of dry areas. In North Africa eastwards to Sindh the genus is less well represented, mainly due to over exploitation although in Arabia through Iraq and Iran eastwards to Pakistan and India, species of *Prosopis* may replace *Acacia* both ecologically and economically. There are 1250 species of *Acacia*, of which 134 species (represented 170 taxa) are native of Africa, with 20 species (26 taxa) extending into Asia and 6 species (7 taxa) native to East Asia (Lock and Simpson, 1991; Wickens *et al*., 1995).

The *Acacia* species have extensively been utilized for medicinal purposes in many countries (Saleem *et al*., 1998) because of the ability of different plant parts to inhibit microbial growth (Valsaraj *et al*., 1997; Newbold *et al*., 1997). However, data regarding antimicrobial activity of *Acacia modesta* is lacking. In this context, the present study was conducted to find out the biologically active compounds in the root extracts of *Acacia modesta* that can inhibit bacterial and fungal growth.

Materials and Methods

Root samples of *Acacia modesta* were collected from Margalla hills Islamabad. These were cut into small pieces, oven dried at 60° C for two hours and were finely ground to form a powder. Ethanol extracts were obtained by soaking the powdered material in 60 percent ethanol for 72 hours and then filtered (Hakim, 1969). Autoclaved extracts were obtained after centrifugation (800g for 10 minutes) of grounded root pieces followed by autoclaving. For 'Hot extracts' the Soxhlet apparatus was used. For this purpose 50g of finely grounded powder material was taken and distilled water was used as extracting solvent (Schulin *et al*., 1998).

In this study four bacterial and three fungal strains we used. The bacterial strains were *Escherichia coli* (gra positive), *Klebsiella pneumoniae* (gram positive), *Streptococcus* (gram negative) and *Staphylococcus aure* (gram negative). Among fungal strains tested we *Rhizoctonia solani*, *Fusarium* spp. and *Saccharomyc cerevisiae* (yeast).

Antibacterial Test: Antibacterial test was conduct following Well Diffusion method (Rios *et al*., 198 Nutrient agar plates (7.5cm diameter) were prepared havi 0.5 cm thick layer of medium. Each plate was inoculat with one bacterial strain by the help of sterile swab. Pu culture of *Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus* and *Staphylococcus aureus* were used inoculum. Three wells were bored (5mm diameter) in ea plate using cork borer. One well was filled with 20 μg streptomycin (antibiotic) and the other two as 120μl percent ethanol extract and 60 percent ethanol solve respectively. Plates were left in a cooled incubator at 4 for one hour to let the solution diffuse in the agar mediu After that these were incubated at 37 C for 16-18 hou The zones of inhibition were measured in mm diameter.

Antifungal Test: Root extracts (ethanol & water) of *Aca modesta* were added in Potato Dextrose Agar (PD medium at a concentration of 0.25, 0.5 and 1 per cent. T efficacy of the solvents used for extraction was also tes in another set to avoid error in case solvents possess a antifungal property and the values obtained for solve were adjusted from those of extracts. PDA without extra or solvents served as control. The media was poured i petri plates (10 ml in each) and discs of inoculum (5 m diameter) of *Rhizoctonia solani*, *Fusarium* sp, and Yea (*Saccharomyces cerevisiae*) from the edge of activ growing culture, were placed in the center of each pla The plates were incubated at 25 C for 48 hours. Th were three replicates of each treatment. The radial gro of the test fungi was recorded in mm diameter.

Results

The ethanol root extract of *Acacia* exhibited a trend of increased antibacterial activity from gram positive to gram negative bacteria. *Escherichia coli* and *Klebsiella pneumoniae* were more resistant compared to gram positive ones while *Staphylococcus aureus* was observed the most sensitive (Fig. 1). It was also observed that both *Escherichia coli* and *Klebsiella pneumoniae* are equally sensitive and in both the cases a 3mm zone of inhibition was produced while the other two gram positives have responded differently in terms of sensitivity. *Staphylococcus aureus* being the most sensitive produced a maximum zone of inhibition of 9mm and in case of *β-Streptococcus*, 5mm zone of inhibition was observed.

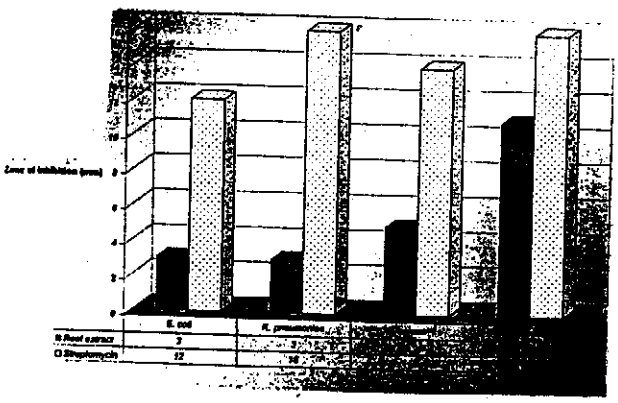

Fig. 1: Comparison of streptomycin with root extract of *Acacia modesta*.

Comparison of zones of inhibition of root extract with that of antibiotic streptomycin showed least difference in case of *Staphylococcus aureus* and a wide difference in case of *Klebsiella pneumoniae* (Fig. 1).

As far as antifungal activity of extracts is concerned, the direct relationship between extract concentration and antifungal activity was observed. In all cases with the increase in concentration of root extracts the antifungal activity increased. All the fungal species tested showed least growth in ethanol root extracts whereas root extracts obtained by Hot method were least antifungal. In the latter, yeast (*Saccharomyces cerevisiae*) exhibited the maximum radial growth of 23 mm when hot-extract was used at a concentration of 0.25 per cent. None of other fungi tested had shown resistant to such an extent. On the other hand the growth of *Saccharomyces cerevisiae* was considerably suppressed (9 mm diameter) by ethanol extracts particularly when used at a concentration of 1 per cent (Fig. 2). This trend was further observed for *Rhizoctonia solani* and *Fusarium* sp. where ethanol extracts at a concentration of 1 per cent showed greatest suppression in the growth of both the fungal species. Another interesting observation for these fungal species is that although ethanol root extracts manifested maximum antifungal activity but the radial growth in 1 per cent autoclaved extract is similar with that of 1 per cent ethanol extracts (Fig. 3 and 4).

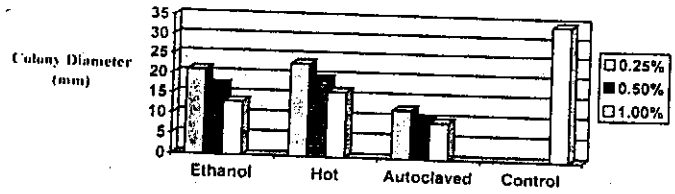

Fig. 2: Effect of root extracts of *Acacia modesta* on the growth of *Saccharomyces cerevisiae*.

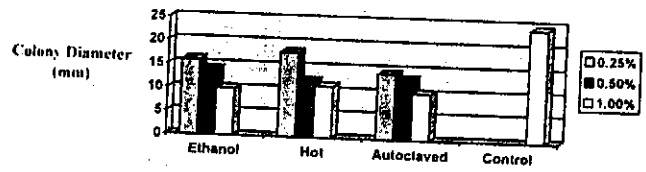

Fig. 3: Effect of root extracts of *Acacia modesta* on the growth of *Fusarium* spp.

Discussion

Both antifungal and antibacterial actions of root extracts have proved the use of *Acacia modesta* in antiseptic terms.

The antimicrobial action of root extracts of *Acacia* further revealed that the extracts are bacteriostatic and fungistatic not bactericidal or fungicidal. This finding is in accordance with most of other reports in which bacteriostatic and fungistatic action of the plant extracts was observed more frequently (Tirillini *et al.*, 1996) in contrast to complete elimination of microorganisms.

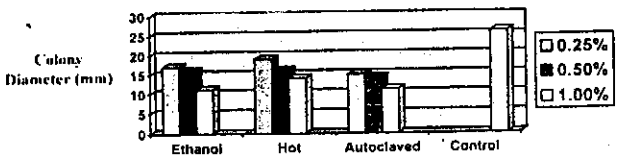

Fig. 4: Effect of root extracts of *Acacia modesta* on growth of *Rhizoctonia solani*

Out of four bacterial strains tested the two gram negative i.e., *Escherichia coli* and *Klebsiella pneumoniae* showed more resistance as compared to other two gram positive ones i.e., *Staphylococcus aureus* and *β-Streptococcus*. The antibacterial action of extract is obvious but differences in the sensitivity of gram positive and gram negative bacteria can be explained in terms of their wall structure. Being simple in nature, gram positive couldn't resist much and exhibited sensitivity. On the other hand, relatively complex nature of wall in gram negative bacteria enabled them to resist comparatively more (Nikaido and Vaara, 1985). We know that the cell envelope surrounding the cytoplasm of bacteria is provided with a cytoplasmic membrane (also termed as cell membrane or plasma membrane) and a rigid cell wall. The gram negative bacteria have an additional outer membrane and a periplasmic space (including cell wall) between the two membranes which are absent in their gram positive counterparts. The periplasmic space is a sheltered environment inside which a large number of enzymes may break down the numerous molecules introduced from outside. Moreover, the outer membrane of gram negative bacteria is known to represent the barrier to the penetration of numerous antibiotic molecules. The ce wall of gram negative organisms also usually hav protruding protein rods (fimbriae) while gram positives hav protruding chains of teichoic acids (Sutherland, 1985). Th protruding protein rods and an additional outer membran is believed to play major role in wall's antigenic specificit particularly in gram negative bacteria (Lugtenberg and Van 1983; Bernard *et al.*,1990). The results of this stud indicates that root extracts of *Acacia modesta* are slight more sensitive to gram positive bacteria while wa characteristics of *E. coli* and *K. pneumoniae* had result in increased strength and evidently they have survive better. This is probably the reason that zone of inhibitio was observed less among these two gram negativ bacteria.

In our results, the highest antifungal activity wa manifested in ethanol extract. Considerably good antifung activity occurred in other extracts as well. From a ecological viewpoint the presence of antifungal activity especially in roots, has great importance (Basile *et al* 1997), in relation to the possible role that the substance responsible for such activity could play in plant's natur defense mechanism. Soil borne fungi has the abilities t cause devastating damage to plants and plant roots (Rou and Wingfield, 1997). The inhibitory effect of roots o *Acacia modesta* against fungi reveals that plant has th natural ability to survive against fungal diseases becaus both *Rhizoctonia solani* and *Fusarium* sp. are soil born pathogenic fungi (Olszak, 1994). In this context, it seem appropriate to believe that plant has the natural ability t survive and to a great extent resist against soil born fung pathogens.

In addition to that maximum antifungal activity in ethan extract further indicates that ethanol is most accepted an widely used solvent for plant extraction. This finding proved by many other workers who conducted th antibacterial assay in ethanol extracts (Maximo Siquiterpenoid *et al.*, 1997).

Reports on variable efficacy of biologically activ compounds of same plant species collected from variou sites (Ermel *et al.*, 1986; Kasmi *et al.*, 1995) stresses th need for the evaluation of extracts of *Acacia* speci collected from different areas. Geographical distribution *Acacia modesta* might reveal different results compared present one because the impact of geographical conditio has already been demonstrated by observing significa differences in composition of plant extracts (Sing Sangwan *et al.*, 1994).

The root extracts of *Acacia modesta* definitely conta active microbial compounds. Future research sho emphasize on isolation, identification and characterizat of the biologically active compounds. In addition to t purification of these active ingredients can be helpful drug formation.

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