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THE POTENTIAL ACTIVITY OF GALLS OF *QUERCUS INFECTORIA* OLIV. : AS ANTIBACTERIAL AGENT AGAINST DENTAL PATHOGENS.

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Petroleum ether, chloroform, methanol and water extracts of *Quercus infectoria* galls were screened for antimicrobial activity against *Staphylococcus aureus, Streptococcus mutans, Streptococcus sanguis, Streptococcus salivarius* and *Lactobacillus acidophillus* by Cup-Plate method at sample concentration of 200mg/ml. The methanolic gall extract possessed strongest antimicrobial activity against the pathogens tested. *Streptococcus sanguis* was the most susceptible bacterium. The MIC value of the extracts possessing strongest antibacterial activity against most susceptible bacteria were determined using twofold serial micro dilution technique.

Key words: *Quercus infectoria*, antimicrobial activity, dental pathogens.

Quercus infectoria (Fagaceae) is a small tree. The galls arise on young branches of this tree as a result of attack by the gall-wasp *Adleria gallae – tinctoria* (Samuelsson 1999). The galls are known as Mayaphal and Majufal in Hindi. Cowan (1999) reported that, as a result of indiscrimate use of antimicrobial drugs in the treatment of infectious diseases, microorganisms have developed resistance to many antibiotics. There is a need to develop alternative antimicrobial drugs. One approach is to screen local medicinal plants that represent a rich source of novel antimicrobial agents. The present study was carried out to investigate the antibacterial properties of the galls of *Q. infectoria* extracted by four solvents of different polarity against dental pathogens.

Streptococcus mutans, Streptococcus sanguis, Streptococcus salivarius and *Lactobacillus acidophillus* play a major role in dental plaque formation. *Staphylococcus* spp. also ferments many sugars and sugar components that are part of dental plaque or caries. A film of microorganisms on the teeth surface, play an important role in the development of caries and periodontal diseases (Marsh 1992).

MATERIALS AND METHODS

Plant Material -The galls of *Quercus infectoria* used in this study were obtained from the local market and were identified on the basis of physical characteristics. The galls were crushed to small pieces by using pestle and mortar and powdered in an electric grinder.

Extraction of Plant Material-The dried and powdered plant material was extracted with different organic solvents (petroleum ether, chloroform, methanol and water) by Soxhlet apparatus. The crude extracts were obtained by removing the solvent in vacuum evaporator at 30^0C.

Preparation of Plant Extracts-The extracts were prepared by immersing 200 gm of dried powdered material in 600 ml of the following four solvents i.e. petroleum ether, chloroform, methanol and water by Soxhlet apparatus. After removing the solvent, the crude extracts were stored in sterile bottles at 4^0 C until further use.

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Preparation of Extract Solution-The extracts were dissolved in the same solvent with which it has been extracted (petroleum ether, chloroform, methanol and water) to a final concentration of 200 mg/ml.

Microorganisms-The bacterial strains used in this study were *Staphylococcus aureus* MTCC-1144, *Streptococcus mutans* MTCC-890, *Streptococcus salivarius* MTCC-1938, *Lactobacillus acidophillus* MTCC-447 and local clinical isolate of *Streptococcus sanguis.* All of these bacteria are related to dental infections. All the bacterial strains were grown and maintained on nutrient agar slants at 4^0 C.

Antibacterial Activity- The Cup-Plate method was used to evaluate the antibacterial activity. This method depends upon the diffusion of the tested material to such an extent that growth of the added microorganism is prevented entirely in a zone around the hole containing a solution of tested material (Perez *et al.* 1990, Ahmad *et al.* 1998). 0.1 ml of diluted inoculum (10^5 CFU/ml) of test organism was mixed in Mueller Hinton Agar media, shaked and poured in sterilized petridishes. Wells of 8 mm diameter were punched into the agar medium and filled with 45 µl of plant extracts. All the solvents served as negative control. Each extract was assayed in triplicate. The plates were incubated at 37^0C for 24 hrs. The antibacterial activity was interpreted from the size of the diameter of zone of inhibition measured in millimeters (mm).

Determination of MIC-The minimum inhibitory concentration of the most effective extracts (methanolic and aqueous) was determined for *S. aureus* and *S. sanguis* by using the twofold serial micro dilution method at a final concentration ranging from 10 mg/ml to 0.0195 mg/ml. The extracts were added to sterile Mueller Hinton Broth into microtiter plates before bacterial suspension (final inoculum of 10^5 bacteria/ml) were added. Each extract was assayed in triplicate. The extracts in broth were used as positive control and the bacterial suspensions were used as negative control. The turbidity of the wells in the microtiter plate was interpreted as visible growth of the microorganisms. The MIC values were taken as the lowest concentration of the extracts in the wells of the microtiter plate that showed no turbidity after 24 hours of inoculation at 37^0C.

RESULTS AND DISCUSSION

The zone of inhibition of the petroleum ether, chloroform, methanol and water extracts (200mg/ml) from the galls of *Quercus infectoria* against *S. aureus, S. sanguis, S. salivarius, S. mutans* and *L. acidophilus* are shown in Table 1.The methanolic extract showed the maximum zone of inhibition against all the bacteria followed by aqueous extract of the galls of *Q. infectoria*. The methanolic extract is highly effective against all pathogens because more organic compounds were leached in this solvent. The zone of inhibition formed by petroleum ether extract is least effective.

The methanol and aqueous extracts were highly active against *S. sanguis* followed by *S. aureus, L. acidophillus, S. mutans* and *S. salivarius* as compared to the other solvents. Chloroform is more effective in comparison to petroleum ether. Chloroform and petroleum ether extracts showed maximum inhibition against *S. sanguis* followed by *S aureus*. The basis for their difference in susceptibility might be due to active constituents present in the extract.

The extent of antimicrobial activity of the

Table1: The zone of inhibition of *Quercus infectoria* extracts (200 mg/ml) in different solvents in mm.

S. No.	Plant Extract	*S. aureus*	*S. salivarius*	*S. mutans*	*L. acidophillus*	*S. sanguis*
1.	Petroleum Ether	13	10	12	11	16
2.	Chloroform	18	15	16	16	20
3.	Methanol	25	23	23	24	26
4.	Water	24	18	21	23	25

*The entire test is done in triplicate and mean of the results are shown in table

Table 2: MIC values of extracts of galls of *Quercus infectoria* against *S. aureus and S. sanguis*

Concentration (mg/ml)	*S. aureus* Methanolic	*S. aureus* Aqueous	*S. sanguis* Methanolic	*S. sanguis* Aqueous	Control Positive	Control Negative
10.0000	-	-	-	-	+	-
5.0000	-	-	-	-	+	-
2.5000	-	-	-	-	+	-
1.2500	-	-	-	-	+	-
0.6250	-	-	-	-	+	-
0.3125	-	-	-	-	+	-
0.1563	-	-	-	-	+	-
0.0781	+	+	-	+	+	-
0.0391	+	+	+	+	+	-
0.0195	+	+	+	+	+	-

-Absence of growth
+Presence of growth

extracts based on inhibition zone diameter has been described as low (12-18 mm), moderate (19-22 mm), strong activity (23-38 mm) (Ahmad *et al.* 1999). Lin *et al.* (1999) reported that plant extracts are usually more active against gram-positive bacteria than gram-negative bacteria. *S. sanguis* was the most susceptible gram-positive bacteria amongst all the bacterial strains.

Successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Traditional healers use primarily water as the solvent (Ahmad *et al.* 1998) but in our studies we found that plant extracts in organic solvent (methanol) provided more consistent antimicrobial activity as compared to those extracted in water. These observations can be rationalized in terms of the polarity of the compounds being extracted by each solvent and, in addition to their intrinsic bioactivity by their ability to dissolve or diffuse in the media used in the assay

The galls of *Q. infectoria* have also been pharmacologically documented to possess astringent, anti-diabetic, antitremorine, local anaesthetic, antiviral, antibacterial, antifungal, larvicidal and anti-inflammatory activities (Basri and Fan 2005). The main constituents found in the galls of *Q. infectoria* are tannin (50-70%) and small amount of free gallic acid and ellagic acid (Ikram and Nowshad 1977, Evans 1996, Wiart 2001 and Basri and Fan 2005). It is well known that tannin is a phenolic compound that is soluble in water, alcohol and acetone and gives precipitates with protein (Leach 1986). The similarity in the antimicrobial activity of both the aqueous and methanolic extracts suggests that these extracts may have high total tannin content. The antimicrobial activity seemed to depend on the contents of tannin in the plant extracts (Djipa *et al.* 2000).

High amounts of tannin present in the galls of *Q. infectoria* (Ikram and Nowshad 1977, Evans, 1996, Wiart, 2001 and Basri and Fan, 2005) implied that tannin might be the active compound, which may be responsible for the antibacterial activity in this study. Tannin in plant extracts was found to possess antibacterial activity (Irobi *et al.* 1994).

The MIC values of the methanolic and aqueous extract from the galls of *Q. infectoria* against *S. aureus* and *S. sanguis* are shown in Table 2.The MIC values of the methanolic and aqueous extracts were the same (0.1563 mg/ml) against *S. aureus,* whereas the MIC values of methanolic and aqueous extracts against *S. sanguis* were 0.0781 mg/ml and 0.1563 mg /ml. The MIC value of both the extracts against *S. sanguis* in comparison to *S. aureus* suggests that *S. sanguis* showed greater sensitivity towards the methanolic extracts of the *Q. infectoria* galls. The methanolic and aqueous extracts displayed similarities in their activity on the bacterial species. In conclusion, the extract of the galls of *Q. infectoria* have high potential as antibacterial agent. This finding provides an insight into the usage of the galls of *Q. infectoria* as traditional medicine in dental powder and in the treatment of toothache and gingivitis (Kottakkal 1995 and Bhattacharjee 2001). The result of the present study suggest that plant extracts possess compounds with antibacterial properties that can be used as antimicrobial agents in new drugs

for the therapy of infectious diseases caused by pathogens. The most active extracts can be subjected to isolation of the therapeutic antimicrobial and undergo further pharmacological evaluation.

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