Evaluating the Antimicrobial Activity of Methanolic Extract of *Rhus Succedanea* Leaf Gall

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**ABSTRACT**

**Introduction:** The worldwide increased bacterial resistance to antibiotics and the undesirable side effects associated with constant use of synthetic drugs has prompted the search for novel antimicrobial agents, particularly those manufactured from plants. This study is designed to ascertain the antibacterial potential of *Rhus succedanea* leaf gall extracts on the growth of gram-positive and gram-negative bacteria. **Methods:** The methanolic and hexane extract of different concentrations (100, 250, and 500 µg/ml) were prepared and their antibacterial efficacy was tested against clinical isolates of *Escherichia coli, Salmonella typhi*, *Micrococcus luteus*, and *Staphylococcus aureus* using agar well diffusion method and the size of inhibition zone was measured in millimeters. **Results:** The methanol and hexane extracts differed significantly in their antimicrobial activity with methanol extract showing a potent inhibitory activity in the range of 16±2 to 23±1, which was almost equal to the values of ciprofloxacin (25±3), used as a standard. Further, the methanol extract was mostly potent and effective in inhibiting the growth of gram-negative bacteria, namely, *E. coli*, when compared to gram-positive bacteria stains, which are responsible for antimicrobial activities. The phytochemical screening showed positive results for the presence of steroids, triterpenes, alkaloids, and carbohydrates. **Conclusion:** The potent antibacterial activity of *Rhus succedanea* leaf gall extracts indicates its useful therapeutic application against bacterial infection. Furthermore, this study indicates that the extract might be exploited as natural drug for the treatment of infectious diseases and could be useful in understanding the relations between traditional cures and current medications.
Karkatshringi also represents usage in the treatment of children’s ear infections, suppress haemorrhage from gums and used to suppress nosebleeding. Hakims consider galls useful in pulmonary infections, diarrhoea and vomiting.

Although there is enough information on its use in various diseases’ treatment, no scientific evaluation has been carried out to justify or substantiate Rhus succedanea (Anacardiaceae) leaf gall extracts’ traditional use. Moreover, the biologically active constituents responsible for the observed biological activities have not been well-defined till now. Henceforth, in this study we have attempted to evaluate antimicrobial potential of Rhus succedanea (Anacardiaceae) leaf gall extract and to elucidate its phytochemical constituents for having knowledge on the principal components responsible for its biological activity particularly the antimicrobial activity. This is the first report exploring the biological potential of gall extracts of R. succedanea as antimicrobial agents and providing evidence for exploitation of these extracts for further therapeutic applications.

Materials and Methods

Plant material

Rhus succedanea galls were obtained commercially in Delhi, India and authenticated By Dr. S. Sundararajan at center for advanced studies in biology, Jain University, Bangalore and the voucher specimen (JU-RUV-62) was conserved in the herbarium. The galls were cleaned with distilled water, dried and crushed into fine powder by using electric grinder.

Preparation of extract

The coarsely powdered gall materials were sequentially extracted with hexane and methanol in soxhlet apparatus for 24 h. The extracts were evaporated to dryness under reduced pressure using a Rotavapor (BuchiFlawil, Switzerland) and a portion of the residue was used for the phytochemical analysis.

Phytochemical analysis

The preliminary phytochemical analyses of the extracts were carried out using the methods described.

Microorganisms

The bacterial strains used for study were Escherichia coli (MTCC 723), Micrococcus luteus (MTCC 3160), Salmonella typhi (MTCC 96), and Streptococcus aureus (MTCC 96). The organisms were maintained on nutrient agar slope at 4°C. For the experiments, stock culture was prepared by inoculating each culture from slants to agar slope at 4°C for 24 h. The stock culture was serially diluted by ten-fold and the number of colony forming units (CFU) was calculated from plates of each dilution and thereby the total CFU was calculated in the stock culture. For antimicrobial screening, the stock cultures of 1×10^5 CFU per ml were used.

Antibacterial assay

Bacterial susceptibility testing

The selected strains of bacteria, grown on nutrient broth were swabbed on the surface of sterile nutrient agar plates using a sterile cotton swab. Agar wells were prepared with the help of sterilized cork borer with 10 mm diameter. Using a micropipette, 100 μl of different concentrations of gall extracts (100, 250 and 500 μg/ml) were added to different wells in the plate. Pure DMSO was taken as the negative control and 100 μg/100μl Ciprofloxacin as the positive control. The plates were incubated in an upright position at 37°C for 24 h. The diameter of inhibition zones was measured in mm and the results were recorded.

Statistical analysis

Statistical analysis was done using SPSS version 10.0.1(Chicago, IL) using a one-way student’s t-test. The value of p < 0.05 was considered statistically significant, when comparing with relevant controls. All results refer to mean ± SD.

Results and Discussion

The present scenario of development of widespread antibiotic resistant pathogens and the undesirable side effects associated with continuous use of synthetic drugs have stimulated alternative therapeutics, particularly plant-based therapeutics. In these lines, the R. succedanea gall extract was tested for its antibacterial potency against gram positive and gram negative bacteria. The result of antimicrobial activity of hexane and ethanolic extract is shown in Table 1. It is observed that the methanol and hexane extracts differed significantly in their antimicrobial potency. It was observed that the antibacterial activity was dependent on solvent used for the extraction and that the methanol extract showed more significant antibacterial activity (16±2 to 23±1), which was almost equal to the values of ciprofloxacin (25±3) that was used as a more standard (Table 1) than the hexane extract. It was found that the methanol extract of galls of R. succedanea was most effective against the gram-negative bacteria (E. coli), when compared to all the other bacterial strains used. The results of previous studies on the antimicrobial activity of extracts of galls of Q. infectoria, P. integerrima and R. succedanea have also shown that gram positive bacteria were more susceptible than the gram negative bacteria.

Fig.1 shows antibacterial effect of methanol extract of R. succedanea gall on Staphylococcus aureus. In an earlier study by Kumar et al., it was observed that the aqueous extract of R. succedanea showed antibacterial activity, which was in the range of 9.6-28.6 at 1000 μg. Therefore, in comparison with earlier studies, in our study the inhibition of microorganism by methanolic extract of R. succedanea (which was in the range of 16-23 at 500 μg) is much more potent and is a better antibacterial agent as per...
In the Phytochemical screening, the methanol extract of gall showed positive indication for the presence of steroids, triterpenes, alkaloids, flavonoids and carbohydrates (Table 2). Therefore the observed higher antibacterial potency of methanol extract can be attributed to two reasons: firstly, to the nature of biologically active components (alkaloids, flavonoids, sterols, quinine, tannins, phenols etc.) which might be enhanced in the presence of ethanol. It has been documented that alkaloids, flavonoids, tannins and phenols are plants metabolites, well known for their antimicrobial activity. Secondly, the stronger extraction capacity of ethanol could have produced a large number of active constituents responsible for antibacterial activity, which have been found to be present in large quantity in the methanol extract as per our study. Our study contradicts other studies in that in comparison with other solvents, methanol is a good solvent for extraction of antimicrobial substances from medicinal plants.

**Conclusion**

In conclusion, this study substantiates the scientific basis of therapeutic potency (antibacterial) of *R. succedanea* galls that is used as a source of drug - karkatasringi, which is widely used in many preparations of Ayurveda and Siddha systems of medicine to treat various diseases. It may be noted that the potency of the extract may be more accurately evaluated in terms of MIC values, as the zone of inhibition might be influenced by solubility and diffusion rate of the phytocompounds. Further, it is necessary to carry out in vivo studies to determine the toxicity of active constituents, their side effects, circulating levels, pharmacokinetic properties and diffusion in different body sites, for their therapeutic application. Further studies on purifying the active components will be helpful in developing this as a drug for therapeutic application. The encouraging result from our study indicates that this extract might be exploited as natural drug for the treatment of infectious diseases and could be useful in understanding the relations between traditional cures and current medications.

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**Ethical Issues**

There is none to be applied.
Conflicts of interest
None to be declared.

References