BioImpacts, 2013, 3(4), 195-198
doi: 10.5681/bi.2013.035
http://bi.tbzmed.ac.ir/

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

Savitri Shrestha[1], Sundara Rajan Subaramaihha[1], Sujan Ganapathy Pasura Subbaiah[1], Ravi Shankara Birur Eshwarappa[2*], Dhananjaya Bhadrapura Lakkappa[3,4*]

[1]*Centre for Advanced studies in Biosciences, Jain University, Chamrajpete-560019, Bangalore, Karnataka, India*
[2]*Department of chemistry, School of graduate studies, Jain University, Bangalore-560002, India*
[3]*Toxinology Lab, School of Chemical and Biotechnology (SCBT), SASTRA University, Tanjavur-613401, Tamilnadu, India*
[4]*Center for Emerging Technologies, Jain University, Ramanagara, Bangalore 562112, India*

ARTICLE INFO

Article Type:
Short communication

Article History:
Received: 28 Aug. 2013
Revised: 21 Nov. 2013
Accepted: 03 Dec. 2013
ePublished: 07 Dec. 2013

Keywords:
Antimicrobial
Rhus succedanea
Gall extracts
Phytochemicals
Drugs
Traditional medicine

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.

Introduction

Since a long time ago, the use of herbs and plant-derived products has been recognizes as part of our traditional health care system. The World Health Organization estimates that 4 billion people (80% of the World's population) use herbal medicines in some aspects of primary healthcare and there is a growing tendency to "Go Natural".[1] Infectious diseases are threatening millions of people around the world and the recent upsurge in widespread antibiotic resistance among pathogens[2-4] and the undesirable side effects associated with constant use of synthetic drugs have stimulated the need for alternative therapeutics,[3] particularly plant-based ones. Of the advantages of plant-based therapeutics, it could be stated that they are natural products, non-narcotic, and easily bio-degradable. They also pose minimum environmental hazards, have less adverse effects, and are easily available and affordable.[5,6] Medicinal plants mentioned in ancient texts and traditional knowledge systems have been a great source of many potent and powerful drugs.[7] Although most of the plants used in the traditional medicine have been identified and their applications well-documented, the antimicrobial efficacy of many plants is yet to be verified.[8,9]

Many plant drugs that are mentioned in Ayurveda, Siddha and Unani systems of medicine are screened for new antimicrobial compound. In this context, *Rhus succedanea* (Anacardiaceae) leaf galls, commonly known as Karkatshringi in Sanskrit are one of the appendages of plant formed due to the invasion of insect -psyllids. Karkatshringi is used in indigenous systems of

***Equal corresponding authors:**
1. B.L. Dhananjaya, Email: chandu_greeshma{at}rediffmail.com
2. Ravi Shankara BE, Email: beravish{at}gmail.com

medicine (Ayurveda, Unani and Siddha) as a remedy in cough, asthma, fever, respiration and liver disorders.[10-12] Karkatshringi also represents usage in the treatment of children's ear infections, suppress *haemorrhage* from gums and used to suppress nosebleeding[13,14] Hakims consider galls useful in pulmonary infections, diarrhoea and vomiting.[15]

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.[16] 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 antibacterial assay.

Phytochemical analysis

The preliminary phytochemical analyses of the extracts were carried out using the methods described.[17-20]

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 flask in sterile nutrient broth and incubated at 37°C for 24 h. The stock culture was serially diluted by ten-fold with sterile peptone water and 0.1ml of each dilution was spread over nutrient agar plates and incubated at 37°C for 24 h. The number of colony forming units (CFU) was counted 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 37ºC for 24 h.[21-23] 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.[3] 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.[24-26] Fig.1 shows antibacterial effect of methanol extract of *R. succedanea* gall on *Staphylococcus aureus*. In an earlier study by Kumar *et al.*,[26] 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

Table 1. Antibacterial activity of gall extracts of Rhus succedanea

Bacterial strains	Zone of inhibition (mm)						
	Hexane Extract			Methanol Extract			Ciprofloxacin
	100 µg	250 µg	500 µg	100 µg	250 µg	500 µg	50 µg
Eschericia coli	12±3	15 ±2	19±6	14±3	18±1	23±1	25±3
Salmonella typhi	11±2	14±2	17±3	15±1	16±3	21±1	23±6
Micrococcus luteus	10±2	11±3	14±2	12±1	16±1	19±2	22±2
Staphylococcus aureus	9±1	9±1	12±2	11±2	13±1	16±2	20±1

The test was done in triplicate. Diameter of the zone of inhibitions is given here as mean ± standard deviation.

our observation in this study.

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.[27] It has been documented that alkaloids, flavonoids, tannins and phenols are plants metabolites, well known for their antimicrobial activity. [27] 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.[27,28]

Table 2. Preliminary phytochemical analysis of methanol and hexane extracts of *Rhus succedanea* leaf gall.

Chemical constituents	Methanol extract	Hexane extract
Phenols	+	-
Flavonoids	+	+
Steroids	+	+
Triterpenes	+	+
Tannins	+	-
Saponins	-	-
Alkaloids	+	+
Glycosides	+	-
Carbohydrates	+	+

Note: + denotes the presence

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.

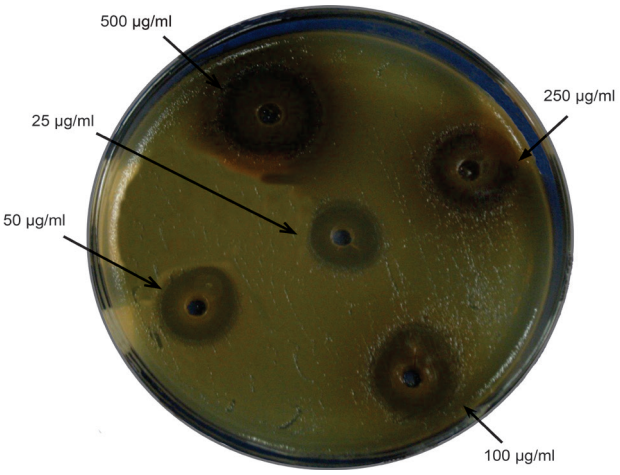

Fig. 3. Agar diffusion assay. Effect of Methanol extracts of galls of *Rhus succedanea* on growth of *staphylococcus aureus*.

Acknowledgements

We acknowledge Dr. R. Chenraj Jain, President, Jain University Trust., Dr. N Sundararajan, Vice Chancellor, Jain University and Prof. K.S. Shantamani, Chief Mentor, Jain University, Bangalore for their kind support and encouragement and for providing facilities for carrying out this work. DBL thanks SASTRA University Management, and the financial support by SASTRA University to BLD in the form of TRR research grants is gratefully acknowledged.

Ethical Issues

There is none to be applied.

Conflicts of interest

None to be declared.

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