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Research Article
Bioactive Molecules and Antimicrobial Studies of Indian Traditional Medicinal Plant *Rhus semialata* Seeds

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Abstract

Background and Objective: Oil extracts from the seeds of *Rhus semialata* were screened and used for antimicrobial activity against bacterial strains-*Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoneae* and fungal strains-*Aspergillus niger* and *Penicillium* sp. Current study was aimed to scientifically validate and document the antimicrobial properties of *R. semialata* seeds. **Materials and Methods:** The plant material extracts were carried out with non polar and highly polar solvents for oil extraction using petroleum ether, chloroform and methanol for phytochemical, antimicrobial and Gas Chromatography and Mass Spectroscopy (GCMS) studies. **Results:** Extracts contains a wide range of medicinally active components includes organic acids, alkaloids, phenols, flavonoids, tannins, fixed oils, fats and terpenoids by phytochemical analysis. GCMS studies showed more than 31 compounds among them the major compounds observed was tridecane (57%), oxalic acid, isobutyl nonyl ester (43%), nonane and decane (57%). Antimicrobial activity of *R. semialata* seed extract exhibited maximum zone of inhibition (25 mm) at 100 µL concentration of chloroform. **Conclusion:** The traditional use of the extracts in infections and inflammatory conditions is rationalized based on the active principles present in the oils and it has been formulated for use in many infections of bacterial and fungal pathogens.

Key words: Bioactive compounds, *Rhus semialata* seeds, plant extracts, antimicrobial, infections, volatile oils

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Indian traditional medicinal plants have been used from ancient time to treatment of various diseases, irrespective of the form of existence, from animals to humans and from the common to the eminent. Medicinal plants have been reported and applications as useful therapeutic benefits from ancient times. Even now they are economically essential, being used in the agri-horticulture, pharmaceutical, cosmetic, perfumery and nutraceutical industries[1,2]. Currently around 30% of medicinal plant species has been discovered, which are potential for effective cures as remedial measure and waiting for required application. Plants which secure medicinal properties were known to be advantage due to presence of chemical diversity of secondary metabolites. Often these are of greater role as natural drugs and if not, of homogeneous attributes as modern synthetic drugs[3]. A wide range of phytochemicals that includes glycosides, flavonoids, anthocyanin, alkaloids, phenols, terpenes, proteins, amino acids, fatty acids etc., have been discovered as a potent natural drugs. These ideas applicable for all generations, some of the applications were very commonly seen and should be cite their literature for effective alternative medicine. Plant chemistry has developed over the history as a specific discipline between natural product chemistry and plant organic chemistry. It is related to the different compounds of organic nature that are possessing and accumulated in plants, deals with the chemical components, structures of these compounds, their biosynthesis, metabolism and biological function specifically antimicrobial agents[4,5].

The North-east region of India is known to have a rich stockpile of plant diversity and is one of the world's "biodiversity hotspots" and supports about 50% of Indian organic biodiversity. It is part of both Himalaya as well as Indo-Burma biodiversity hotspots. More than 200 tribes of different ethnic groups inhibit the region and have rich indigenous traditional knowledge and utilization of local plants for both fodder and medicinal purpose[6]. Many publications and reports on the ethnobotany and traditional knowledge system are studied and published but many are just the listing of the different kinds of plants by different tribes and most of these wild plants, fruits and vegetables are not known or very little known to the outside world[7].

Rhus semialata Murr. is a deciduous tree (syn. *R. chinensis* Mill., *R. javanica* Linn.) found in the outer Himalayan ranges at an altitude of 3,000-7,000 ft, the hills of Assam, Khasia, Naga and Sikkim in India[8,9], upper Burma, China and Japan[2]. The genus *Rhus* belongs to the Anacardiaceae family and comprises over 200 species throughout the world. *Rhus semialata* is one such plant from the Indo-Burma region that has been used since ages to treat various ailments and infections among the Naga tribes of Nagaland[8]. The fruits are edible with sharp acidic taste. The infusion of fruits of *Rhus* plant is traditionally used to control diarrhoea and dysentery, food poisoning, allergies, etc., the fruit contains tannin, gallic acid and the potassium acid salts. Exhaustive literature survey indicated that systemic pharmacological work has so far not been done with regard to this plant[6].

R. semialata is a subtropical plant found in India (Northeast India), commonly called Thumpak in local languages. It is a deciduous, spreading, often multi-branched tree up to ca. 25 m tall. These plants have ovate leaves with small panicle flowers that eventually produce fruits which are in the category of berry. The fruits are edible, a decoction of the fruit is employed against diarrhoea. Study of ethnomedicinal plants for active phytochemical compounds may lead to the discovery of new ground-breaking drugs. In the current investigation, a screening of petroleum ether, chloroform and methanol extracts of *R. semialata* seeds against pathogenic bacteria and fungi to know their antimicrobial properties was performed. Aim of the study was to validate further scientific study to explore the potent extract for secondary metabolites based on the antimicrobial properties.

MATERIALS AND METHODS

Sample collection: Plant seed samples were collected during March-April, 2018 from wild forest of Kohima District, North Eastern India. The plant and seeds were authenticated by Dr. Shiddamallayya Mathapathi, Research Officer (Botany), Regional Ayurvedic Research Institute, CCRAS, Itanagar, Arunachal Pradesh, India. Collected seed material was initially sprayed with ethanol to cause enzymatic degradation of secondary metabolites. The seeds were shade dried, powdered manually inside the laboratory within 10-15 days at room temperature (28-30°C).

Soxhlet extraction of seeds: The shade dried, powdered 100 g seed material was soxhleted with petroleum ether, chloroform and methanol in a soxhlet extractor for 48 h separately.

Preliminary phytochemical screening: The plant extracts were subjected to identify for the presence of various chemical compounds. Stored vacuum dried extracts were screened for the presence of saponins, tannins, alkaloids,

flavonoids, triterpenoids, steroids, glycosides, carbohydrates, amino acids and fatty acids as described by Harborne[10], Kokate[11] and Bhalodia *et al.*[12].

Test microorganisms and growth media: The culture *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoneae* and fungal strains *Aspergillus niger*, *Penicillium* sp. were selected for the studies. These-strains were obtained from National Collection of Industrial of Microorganisms (NCIM), Pune and used for evaluating antimicrobial studies. Both the stock cultures were incubated for 24 h at 37°C on nutrient agar and potato dextrose agar (PDA) medium (Hi-Media Pvt. Ltd, Mumbai, India), respectively, following refrigeration storage at 4°C. The bacterial strains were grown in Mueller-Hinton agar (MHA) plates at 37°C (the bacteria were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C) and the stock cultures were maintained at 4°C as described by Bhalodia and Shukla[13].

Antimicrobial activity-determination of zone of inhibition method: The antibacterial activity of petroleum ether, chloroform and methanol extracts of the sample was evaluated by using agar well diffusion method which is the most widely used method for identifying the antimicrobial activity, which exploits diffusion of antimicrobial compounds through MHA media and PDA media to demonstrate inhibition of bacteria and fungi, respectively. Sterilized Mueller-Hinton Agar was swabbed with the different selected strains of bacteria, it was allowed to dry for some time, then wells were bored using a 3 mm cork-borer. The extracts were added into the respectively labelled well using sterile micropipette. The plates were incubated at 37°C for 24-48 h. The plates were observed for the zone of inhibition (ZOI) around the wells. Similarly, it was done to test antifungal activity, here a suspension of fungi was swabbed into PDA as described by Selvamangai and Bhaskar[14].

Preparation of extract: One milliliter of the chloroform extract of *Rhus semialata* seeds were employed for GCMS analysis. GCMS analysis of the volatile components of *R. semialata* resulted in the identification of more than 31 compounds which constituted the total compounds as described by Harborne[10] and Kokate[11].

Instruments and chromatographic conditions: GCMS analysis was carried out on a GC Clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer (GCMS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30×0.25 mm ID×1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV, helium (99.999%) was used as carrier gas at a constant flow of 1mL min^{-1} and an injection volume of 0.5 EI was employed (split ratio of 10:1) injector temperature 250°C, ion-source temperature 280°C as described by Selvamangai and Bhaskar[14]. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10-200, then 5-280°C min^{-1}, ending with a 9 min isothermal at 280°C as described by Selvamangai and Bhaskar[14]. Mass spectra were taken at 70 eV, a scan interval of 0.5 sec and fragments from 40-550 Da as described by Usharani and Chitra[15]. The plant extract was dissolved in methanol and filtered with polymeric solid phase extraction (SPE) column and analysed in GCMS for different constituents. Using computer searches on a NIST REFPROP Version 9.1 database and comparing the spectrum obtained through GCMS compounds present in the plants sample were identified as described by Santhikumari *et al.*[16].

Identification of bioactive constituents novel extract by GCMS: Interpretation on Mass-Spectrum of GCMS was carried out by using computer searches on database of National Institute Standard and Technology (NIST) and comparing the spectrum obtained through GCMS, compounds present in the plants sample were identified by comparing with the spectrum of known components stored in the NIST library as described by Ghosh *et al.*[17]. Identified bioactive constituents of the *R. semialata* seeds compound name, molecular formula, molecular weight and chemical structure of the component materials were ascertained.

Statistical analysis: The experiments have been conducted in 3 times and the average value of the data has presented.

RESULTS

The results of the tests of antimicrobial activity in the *Rhus semialata* seed extracts examined against *Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli, Aspergillus niger* and *Penicillium* sp. in nonpolar to polar solvents. The plant extracts were found to be active against at least one of the test organisms. Further, to identify secondary metabolites among the three extract, qualitative phytochemical study were carried out and which was found maximum antimicrobial property and also studied for GCMS analysis.

Phytochemical analysis: The results of qualitative analysis of petroleum ether, chloroform and methanol extract contains a

Table 1: Phytochemical analysis of *R. semialata* seed extract

	Soxhlet extracts		
Phytochemical test	Petroleum ether	Chloroform	Methanol
Alkaloids	+ve	+ve	+ve
Carbohydrates	-ve	+ve	-ve
Flavonoids	+ve	+ve	+ve
Steroids	-ve	-ve	-ve
Saponins	-ve	-ve	-ve
Tannins	+ve	+ve	+ve
Phenolic compounds	-ve	+ve	+ve
Fixed oils and fats	+ve	+ve	+ve
Phytosterols	-ve	-ve	-ve
Terpenoids	+ve	-ve	-ve

+ve: Present, -ve: Absent

wide range of medicinally active components includes organic acids, alkaloids, phenols, flavonoids, tannins, fixed oils, fats and terpenoids by phytochemical analysis (Table 1).

Antimicrobial activity of *R. semialata* seeds of various extracts: Antimicrobial test was performed using different concentrations of chloroform extracts (10, 30, 60, 100 µL) for bacterial strains (*Klebsiella pneumoniae, Escherichia coli* and *Staphylococcus aureus*) and fungal cultures (*Aspergillus niger* and *Penicillium* sp.). *Klebsiella pneumoniae* exhibited maximum zone of inhibition (19 mm) compared with other bacterial and fungal cultures (Table 2).

Antimicrobial test was performed using different concentrations of chloroform extracts (10, 30, 60, 100 µL) for bacterial strains (*Klebsiella pneumoniae, Escherichia coli* and *Staphylococcus aureus*) and fungal cultures (*Aspergillus niger and Penicillium* sp.). *Staphylococcus aureus* exhibited maximum zone of inhibition (25 mm) compared with other bacterial and fungal cultures (Table 3).

Both bacterial and fungal cultures were tested for antimicrobial activity in presence of methanol extract at four different concentrations (10, 30, 60, 100 µL). The result showed the extract having significant effect in controlling the growth of bacterial strains. *Klebsiella pneumoniae* showed highest zone of inhibition of 29 mm compared with other bacterial and fungal cultures (Table 4).

GCMS analysis: Chromatogram of the chloroform extract of *Rhus semialata* seeds showed the presence of more than 31 bioactive constituents. The active principles with their retention time (RT), REV, molecular formula, molecular weight, concentration (peak area %) and chemical structures were presented in Table 5. The total number of compounds identified in chloroform extract were the GCMS retention time

Table 2: Antimicrobial activity of *R. semialata* in presence of petroleum extract

Organisms	Concentration (µL)	ZOI (mm)
Klebsiella pneumoniae	100	19
	60	13
	30	12
	10	07
Staphylococcus aureus	100	16
	60	15
	30	14
	10	09
Escherichia coli	100	10
	60	07
	30	06
	10	05
Aspergillus niger	100	08
	60	04
	30	02
	10	00
Penicillium sp.	100	06
	60	02
	30	01
	10	00

ZOI: Zone of inhibition

Table 3: Antimicrobial activity of *R. semialata* in presence of chloroform extract

Organisms	Concentration (µL)	ZOI (mm)
Klebsiella pneumoniae	100	18
	60	10
	30	11
	10	09
Staphylococcus aureus	100	25
	60	13
	30	08
	10	06
Escherichia coli	100	08
	60	04
	30	01
	10	00
Aspergillus niger	100	00
	60	00
	30	00
	10	00
Penicillium sp.	100	00
	60	00
	30	00
	10	00

ZOI: Zone of inhibition

(RT) and percentage peak of the individual compounds. The results revealed that tridecane (57%), oxalic acid, isobutyl nonyl ester (43%), nonane and decane (57%) were found as the major constituents covering higher concentration of area in the chloroform extract. The retention time of the bioactive constituents shown the 10 hits during analysis, in that lowest was 6.941 and highest was 27.819 with the presence of tridecane, decane, oxalic acid, isobutyl nonyl ester, undecane and 9, 19-Cyclolanostan-3-Ol, Acetate, (3. Beta)-, β-sitosterol acetate, pseduosarsasapogenin-5, 20-dien methyl ether, β-Sitosterol compounds, respectively. The remaining constituents covered moderate concentration of area of the concentration.

The study of GCMS data suggests that, possible presence of unsaturated hydroxy fatty acids in the sample under investigation. From the above data one can anticipate presence of mixture of tridecane, anethole, phenol,2,4-Bis (1,1-Dimethylethyl)-, n-hexadecanoic acid, (Z)6, (Z)9-pentadecadein-1-Ol, diisooctyl phthalate, squalene, 4H-1, 3,2-dioxaborin, 6-ethenyl-2ethyl-4-methyl-4-(2-methylpropyl)-, cyclohexane, 1-(1,5-dimethylhexyl)-4-(4-methylpentyl)- and 9, 19-cyclolanostan-3-Ol, acetate, (3. Beta)-in the structure of these molecule are in accordance with the proposal made further possible mixture of fatty acids were present in the isolated sample (Table 5).

Table 4: Antimicrobial activity of *R. semialata* in presence of methanol extract

Organisms	Concentration (µL)	ZOI (mm)
Klebsiella pneumoniae	100	29
	60	20
	30	06
	10	04
Staphylococcus aureus	100	08
	60	07
	30	06
	10	06
Escherichia coli	100	15
	60	12
	30	10
	10	07
Aspergillus niger	100	00
	60	00
	30	00
	10	00
Penicillium sp.	100	07
	60	02
	30	01
	10	01

ZOI: Zone of inhibition

Table 5: GCMS analyses of different components in chloroform extract of *Rhus semialata* seeds

Name of bioactive constituents	Retention time (min)	REV	Molecular formula	Molecular weight (g moL^{-1})
Tridecane	6.941	930	$C_{13}H_{28}$	184.3
Decane		927	$C_{10}H_{22}$	142.2
Oxalic acid, Isobutyl nonyl ester		926	$C_{15}H_{28}O_4$	272.3
Undecane		921	$C_{11}H_{24}$	156.3
Anethole	9.631	895	$C_{10}H_{12}O$	148.2
Estragole		893	$C_{10}H_{12}O$	148.2
Phenol,2,4-Bis (1,1-Dimethylethyl)-	12.467	763	$C_{14}H_{22}O$	206.3
N-Hexadecanoic acid	17.228	887	$C_{16}H_{32}O_2$	256.4
Pentadecanoic acid		849	$C_{15}H_{30}O_2$	242.4
Octadecanoic acid		848	$CH_3(CH_2)_{16}$ COOH	284.4
N- Decanoic acid		846	$C_{10}H_{20}O_2$	172.2
(Z)6,(Z)9-Pentadecadien-1-ol	18.195	937	$C_{15}H_{28}O$	224.3
2-Octylcyclopropene-1-Heptanol		926	$C_{18}H_{34}O$	266.5
13-Tetradece-11-Yn-1-ol		912	$C_{14}H_{24}O$	208.3
Diisooctyl phthalate	22.247	849	$C2_4H_{38}O_4$	390.5
Di-n-octyl phthalate		844	$C_{24}H_{38}O_4$	390.6
Bis(2-ethylhexyl) phthalate		830	$C_{24}H_{38}O_4$	390.6
Squalene	24.277	952	$C_{30}H_{50}$	410.7
Supraene		930	$C_{30}H_{50}$	410.7
4H-1,3,2-Dioxaborin, 6-Ethenyl-2Ethyl-4-Methyl-4-(2-methylpropyl)-	25.800	758	$C_{12}H_{21}BO_2$	208.1
β-n-butyl ether of 11-Epi-Dihydroartemisin		693	$C_{19}H_{32}O_5$	340.5
1-Aminoinosine		680	$C_{10}H_{13}N_5O_5$	283.2
6,7-Epoxypregn-4-Ene-9,11,18-Triol-3,20-Dione, 11,18-Acetate		673	$C_{25}H_{32}O_8$	460.5
Cyclohexane, 1-(1,5-Dimethylhexyl)-4-(4-Methylpentyl)-	27.084	827	$C_{20}H_{40}$	280.5
19,19-Dimethyl_Eicosa-8,11-Dienoic acid		766	$C_{22}H_{40}O_2$	336.6
1-Octadecyne		763	$C_{18}H_{34}$	250.5
9-Eicosyne		753	$C_{20}H_{38}$	278.5
9, 19-Cyclolanostan-3-Ol, Acetate, (3. Beta)-	27.819	883	$C_{32}H_{54}O_2$	470.7
β-sitosterol acetate		851	$C_{31}H_{52}O_2$	456.7
Pseduosarsasapogenin-5, 20-Dien Methyl Ether		851	$C_{28}H_{44}O_3$	428.6
β-sitosterol		850	$C_{29}H_{50}O$	414.7

REV: Percentage of the compound concentration in the total extract

DISCUSSION

The antibacterial and antifungal study showcased a noble benefit of genus *Rhus* for application in pharma and phytotherapy. It was observed that the extract usage for primary screening exhibited all three extracts to be effective against selected bacteria but efficacy against fungi was effective at petroleum ether and methanol at different levels. Among the concentrations 100, 60, 30, 10 and 100 µL concentration (amount in the wells) showed the highest zone of inhibition *Klebsiella pneumoniae* (19 mm), *Staphylococcus aureus* (16 mm), *Escherichia coli* (10 mm), *Aspergillus niger* (08 mm), *Penicillium* sp. (06 mm) with petroleum ether extract, *Klebsiella pneumoniae* (18 mm), *Staphylococcus aureus* (25 mm), *Escherichia coli* (08 mm), *Aspergillus niger* (00 mm), *Penicillium* sp. (00 mm) with chloroform extract and *Klebsiella pneumoniae* (29 mm), *Staphylococcus aureus* (08 mm), *Escherichia coli* (15 mm), *Aspergillus niger* (00 mm), *Penicillium* sp. (07 mm) methanol extract, followed by 60 and 30 µL but at some observations 10 µL didn't shown any zone of inhibition. It was may be due to the presence of 31 compounds from chloroform extract of *R. semialata* may be few of them are very active in inhibiting the microorganisms growth in the findings. Several pharmacological studies conducted on *Dracontomelon dao* reported antimicrobial, antioxidant, anti-inflammatory, anti-diabetic and anti-trypanosomal activities[18-21] and the isolation of the chemical constituents of its several parts suggest the possession of medicinal and therapeutic properties[22,23]. It was evident from the study that different extracts used for primary screening to know the antimicrobial functions of the plant seed sample and found positive with few organisms in different concentrations. All the plant extracts showed prominent zone of inhibition against the bacteria and fungi at different concentration. Similar studies reported by Ragasa *et al.*[24] as antibacterial activity of essential oil composition of *Lasia spinosa* (L.) Thwarts may be associated with the contribution of the monoterpene, α-pinene, which was found to present as the predominant compound in essential oil comparing to other volatile compounds (camphene, δ-3-carene, caryophyllene) and is well known for antibacterial activity. The data of antibacterial efficacy of some other individual components e.g., camphene, limonene, caryophyllene has already been established[25]. The study of Magwa *et al.*[26] showed that the ethanolic extract from the sapwood of *Dracontomelon dao* exhibited antimicrobial activity against *S. typhimurium*, *K. pneumoniae*, *S. aureus*, *B. subtilis*, *C. albicans and A. niger* and also reported GC-MS analysis revealed that out of the 54 compounds found in the extract, 21 compounds have antimicrobial properties while 15 compounds have antioxidant properties. The antimicrobial activity of leaves oil of *Myristica fragrans* has been reported previously against fungi, such as *Candida tropicalis, Candida albicans* and *Candida glabrata*[27,28] and similar studies of Thileepan *et al.*[29] reported in addition to these three species, activity was demonstrated against *Candida krusei* and *Candida parapsilosis*. Inhibition of *Candida* spp., by both seed and leaf oils was noticed. GCMS chromatogram of the chloroform extract of *R. semialata* seeds showed the presence of more than 31 bioactive constituents with tridecane, decane, anethole, (Z) 6, (Z)9-pentadecadien-1-ol, squalene as the major constituents. Patil *et al.*[30] also reported similar compounds in GCMS studies of *Citrus medica* seeds and various biological activities. Out of the three solvent utilized, petroleum ether extract showed the best results for its efficacy against all the antimicrobial samples comprehended. Hence, implication of the findings can say that, the exhibited antibacterial activity of solvent extracts of *R. semialata* seeds is due to the presence of several types of compounds such as alkaloids, flavonoids, tannins and fatty acids in those extracts.

CONCLUSION

The study showed that the extracts from the seeds of *R. semialata* Indian traditional medicinal plant exhibited potent bioactive molecules and antimicrobial activity against 5 organisms, 3 bacterial strains (*Klebsiella pneumoniae, Escherichia coli* and *Staphylococcus aureus*) and 2 fungal (*Aspergillus niger* and *Penicillium* sp.) using four different concentrations. Phytochemical screening detected the presence of alkaloids, flavonoids, tannins, fixed oils and fats, terpenoids in petroleum ether extract, alkaloids, carbohydrates, flavonoids, tannins, phenols, fixed oils and fats in chloroform extract and alkaloids, flavonoids, tannins, phenols, fixed oils and fats in methanol extract, which were known to have potential antimicrobial properties with concentration dependent, active extract only studied for GCMS to know the compounds which are responsible for biological activities. These findings may add credence to the traditional medicinal claims on *Rhus semialata* seeds.

SIGNIFICANCE STATEMENT

This study enumerate the novel bioactive metabolites from the petroleum ether, chloroform and methanol extract of seed parts of *R. semialata* that can be useful as various therapeutic molecule for pharmaceutical industry. This study will help the researcher to uncover the critical areas of

pharmacology and pharmacognosy that many researchers were not able to explore. Thus a new protocol on identifying potential bioactive molecule from the diversity and especially non-edible seeds and wild plants and their products posses active principle may be arrived at for needful application as medicine or therapeutic drug.

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