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L. Md. Bhakshu & R. R. Venkata Raju

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RESEARCH ARTICLE

Chemical composition and *in vitro* antimicrobial activity of essential oil of *Rhynchosia heynei,* an endemic medicinal plant from Eastern Ghats of India

L. Md. Bhakshu[1], and R. R. Venkata Raju[2]

[1]*Department of Plant Sciences, University of Hyderabad, Gachibowli, Hyderabad, India, and* [2]*Department of Botany, Sri Krishnadevaraya University, Anantapur, Andhra Pradesh, India*

Abstract

Rhynchosia heynei Wt. & Arn. (Fabaceae) is an endemic medicinal plant found in the forests of Eastern Ghats of India. The essential oil obtained from the leaves by steam distillation was studied for antimicrobial activity and chemical characterization. The essential oil exhibited a broad spectrum of antimicrobial activity on eight bacterial and three fungal strains. The essential oil was characterized chemically using gas chromatography with flame ionization detector. The majority of the components of the essential oil of *R. heynei* are oxygenated terpenes, such as, terpineol, camphene hydrate, germacrene-D, humulene, and linalool. etc.

Keywords: Rhynchosia heynei*; Eastern Ghats; antimicrobial activity; essential oil components*

Introduction

Rhynchosia heynei Wt. & Arn. (Fabaceae), vernacular name Adavi vulava, is an endemic medicinal plant distributed in the Eastern Ghats of India (Pullaiah & Ramamurthy, 2001). The plant has been widely used for rheumatic pains, arthritis, and in skin diseases by the tribal people inhabiting the hill ranges of the Eastern Ghats. To confirm the medicinal properties in various human ailments, the present investigation was carried out to determine the antimicrobial activity and phytochemical evaluation of the essential oil obtained from the leaves of *R. heynei*, hitherto not reported. The results may have wide application in medicinal chemistry and pharmacological evaluation to enable the development of novel antimicrobial drugs.

Materials and methods

Plant material

Ethnomedicobotanical studies revealed that the plant was used by the Chenchu and Lambada tribes for rheumatic pains/arthritis (Bhakshu, 2002). The seeds were ground and boiled and the decoction given orally, or the paste of the fresh leaves applied externally. The paste of the leaves was used for cuts and wounds (Bhakshu, 2002). Leaf material was collected from Naramamidi Chervu of the Kurnool district of Nallamalais of the Eastern Ghats, India, in June 2001. A voucher specimen (24171) was deposited at the SKU Herbarium (Sri Krishnadevaraya University, Anantapur) and identified by the authors using authenticated flora (Gamble, 1935; Pullaiah & Ramamurthy, 2001) and by comparing authenticated specimens housed at the SKU Herbarium.

Extraction of essential oils

Five hundred grams of shade-dried leaves were pounded into a coarse powder and subjected to steam distillation, which was performed in a Clevenger type apparatus for 5 h (Anonymous, 1986; Mainsonneve & Sainte, 1975). The essential oil was obtained as an orange colored oil with a characteristic, pleasant odor (yield 0.35%, w/v), and was subjected to analysis of its chemical components and

Address for Correspondence: Professor R. R. Venkata Raju, Department of Botany, Sri Krishnadevaraya University, Anantapur – 515 003, Andhra Pradesh, India. Tel: 08554 225576. E-mail: rrvenkataraju@yahoo.com

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investigated for its *in vitro* antimicrobial activity. The moisture was removed by keeping the oil in a desiccator overnight, and then the oil was dissolved in *n*-hexane and stored in a cool place.

Gas chromatographic studies

Five microliters of essential oil were injected at 290°C into a column (stainless steel, 2 m × 3.21 mm) packed with OV_{17}, SS packed silica gel column (2M × 3.21 mm) mesh range 80–100, weighing 32% at a maximum temperature of 350°C. The flow gas was nitrogen with a split ratio of 1:30 and the septum sweep was held constant at 10 mL, using a Nucon gas chromatograph.

Identification of components

The oil was spiked with a standard mixture of *n*-alkane series (C_8–C_{23}) and analyzed under the abovementioned conditions. The retention indices were calculated and chemical components were identified by application of a modified Kovats procedure (Kovats, 1965).

Antimicrobial assay

Bacterial and fungal strains used for the study (Table 1) were obtained from the Microbial Type Collection Center (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. Microorganisms used in the present investigation were maintained on the appropriate media such as nutrient agar, nutrient broth, Mueller–Hinton agar, and Czapek–Dox agar. Standard antibiotics, namely, ampicillin, tetracycline, and vancomycin (30 µg/ 6 mm disk), were used as positive controls to compare the inhibition activity of the essential oil of *R. heynei*. The antibiotics were obtained from Hi-Media Laboratories, Mumbai, India. The media and the glassware were properly sterilized in an autoclave and all other manipulations were conducted under aseptic conditions.

Test organisms

Standardized cultures of microbial strains, namely, *Bacillus ccereus* (MTCC 1429), *B. subtilis* (MTCC 121), *Escherichia coli* (MTCC 1687), *Micrococcus luteus* (MTCC 1541), Proteus vulgaris (MTCC 1771), *Pseudomonas aeruginosa* (MTCC 1688), *Staphylococcus aureus* (MTCC 737), *Candida albicans* (MTCC 183), *Candida tropicalis* (MTCC 187), and *Aspergillus niger* (MTCC 1377) used in the investigation were purchased from MTCC, IMTECH, Chandigarh, India.

Preparation of samples for antimicrobial assay

Antimicrobial susceptibility tests were determined by employing standard disk diffusion and dilution techniques (Bauer et al., 1966; Russel & Furr, 1977). Whatman No.1 filter paper disks of 6 mm diameter were placed in dry Petri plates and sterilized in an autoclave under stipulated conditions. The sterile disks were dipped in the test oil samples and shaken thoroughly. These filter paper disks were allowed to dry and then carefully placed over the spread cultures and incubated at 35 ± 2°C, 24 h for bacterial strains, or 28 ± 2°C, 48 h for fungal strains. Paper disks dipped in Tween 80 alone served as negative controls. Disks impregnated with antibiotics served as positive controls and were used for comparison with the antimicrobial activity of the test oil.

The zone of inhibition surrounding the paper disk indicated antimicrobial activity, which was measured accurately to the nearest millimeter by means of a metric ruler. In all cases where the zone of inhibition was found to be more than 10 mm, it was necessary to distinguish between microbistatic or microbicidal activity. Microbicidal activity was confirmed by transferring a loop of culture from the inhibition zone to fresh sterilized nutrient broth and incubating under standardized conditions. Various standard antibiotics were tested simultaneously for all microbes under similar conditions

Table 1. Antimicrobial activity of essential oil of *Rhynchosia heynei* (leaves).

Test microorganism	Diameter of zone of inhibition (mm)		Minimum inhibitory concentration (MIC, µg/mL)
	Essential oil (pure)	Standard antibiotic (30 µg/disk)	
Gram-positive bacteria			
Bacillus cereus MTCC 1429	20 ± 0.5	22[a]	10
Bacillus subtilis MTCC 121	12 ± 0.3	22[a]	50
Micrococcus luteus MTCC 1522	22 ± 0.5	26[b]	10
Staphylococcus aureus MTCC 737	21 ± 0.5	24[b]	10
Gram-negative bacteria			
Escherichia coli MTCC 1687	16 ± 0.5	22[b]	12
Pseudomonas aeruginosa MTCC 1688	22 ± 0.3	28[b]	10
Klebsiella pneumoniae MTCC 109	—	22[b]	—
Proteus vulgaris MTCC 1771	—	24[b]	—
Fungi			
Candida albicans MTCC 183	20 ± 0.5	24[b]	10
Candida tropicalis MTCC 187	22 ± 0.5	22[c]	7.5
Aspergillus niger MTCC 1377	10 ± 1	20[c]	1000

[a]Ampicillin; [b]tetracycline; [c]vancomycin; —, resistant.

so as to compare the inhibition activity exhibited by the essential oil. The experiments were conducted in triplicate and the average inhibition zone was taken for each microorganism and the results tabulated.

Calculation of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) was calculated by using a microdilution method (Kiehlbauch, 2000; National Committee for Clinical Laboratory Standards, 2000) for the susceptible microorganisms. The essential oil was serially diluted and different concentrations were examined separately in broth cultures against the sensitive bacterial and fungal species. The essential oil of different concentrations was added to sterile broth medium, inoculated with microorganism, and incubated at 35°C. The broth culture was incubated overnight and observed for turbidity. The MIC was determined as the lowest concentration of essential oil that completely inhibited the growth of microorganism overnight (Table 1). The MIC for *Aspergillus niger* was determined using Czapek–Dox agar plates pretreated with different concentrations of essential oil and observing visible colonies. The least concentration of oil that inhibited growth of the fungus was noted as the MIC and recorded (Table 1).

Results and discussion

The essential oil of *R. heynei* leaves exhibited a broad spectrum of antimicrobial activity. The screening revealed that the inhibitory activity was highest against *Micrococcus luteus*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, followed by *M. roseus*, *Bacillus cereus*, *Candida tropicalis*, *C. albicans* and *Aspergillus niger*, whereas *Klebsiella pneumoniae* and *Proteus vulgaris* were resistant to the tested oil. The oil exhibited very significant MICs on some tested microorganisms, namely *Bacillus cereus*, *Micrococcus luteus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*, at 10 μg/mL. Based on the MIC of the essential oil it was observed that the tested oil was significantly effective on *Pseudomonas aeruginosa* and *Micrococcus luteus*, which needs further studies to characterize the active principle, as the microbicidal activity was similar to that of the standard antibiotics used (Table 1).

This is the first report of the phytochemical components of the essential oil, in which about 98% of the components were identified, as depicted in Table 2, using gas chromatography. The majority of the components of the essential oil of *R. heynei* were oxygenated terpenes, which are reported as highly lipophilic. The high concentration of oxygenated monoterpenes constituting the oil might be responsible for its antimicrobial properties (Saller et al., 1998). Important constituents in the essential oil of *R. heynei* such as terpineol, camphene hydrate, germacrene-D, humulene, linalool, etc., might be responsible for the medicinal properties of the plant. Further investigation including pharmacological studies should be carried out to characterize the active principle.

Table 2. Chemical components of essential oil of *Rhynchosia heynei* (leaves).

Name of compound	Retention index	%
1-Pentanol	744	71.98
Terpineol	1169	0.192
Camphene hydrate	1150	16.373
Germacrene-D	1484	4.071
Menthe-1,8-dien-4-ol	1700	0.479
Unknown	1872	0.569
Humulene	1451	0.304
Stearic acid	2193	0.407
Tetradecane	1405	0.942
Unknown	2953	1.325
Linalool	1552	1.698
Tetracosanoic acid	2685	1.66

Conclusions

The antimicrobial activity of *Rhynchosia heynei* has been demonstrated, justifying its local usage for the treatment of various diseases by the Adivasi tribes. Its activity against microorganisms, especially Gram-negative bacteria such as *E. coli* and *P. aeruginosa*, means that the plant can be used in the treatment of wound infections, otitis media, and gastroenteritis, and its activity against *S. aureus*, *B. subtilis*, *Micrococcus luteus*, *Candia albicans*, *C. tropicalis*, and *Aspergillus niger* can be employed in developing novel chemotherapeutic agents against urinary tract infections and other related infections, food-borne diseases, and skin infections. Further research against a wide range of bacteria and fungi as well as toxicological investigations and further purification needs to be carried out, with a view to developing novel antibiotic substances.

***Declaration of interest*:** The authors report no conflicts of interest.

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