

## Antibacterial properties of an Indian traditional medicinal plant, *Rhynchosia scarabaeoides* (L.) DC (Fabaceae)

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### Abstract

*Rhynchosia scarabaeoides* (L.) DC plant parts are extensively used by traditional healers in India to treat a variety of bacterial diseases, such as dysentery, diarrhoea and skin disorders. This article reports the antibacterial activities of *n*-hexane, ethyl acetate and ethanol extracts belonging to the leaf, stem and root parts of *R. scarabaeoides* against five bacterial strains, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Staphylococcus aureus*, using an agar gel diffusion method. The range of inhibition zone (IZ) was found to be 15-24 mm and the minimum inhibitory activity (MIC) was found to be 1 µL/well. The IZ was found to be higher in ethyl acetate extracts while this was moderate in ethanol extracts, and no activity was seen with *n*-hexane extracts or root extracts. The MIC value of leaf ethyl acetate extract was found to be 1 µg against bacterial strains *P. vulgaris* and *S. aureus*, whereas 2 µg was found against *B. subtilis*, *K. pneumoniae* and *E. coli*. These results support the traditional usage of *R. scarabaeoides* plant parts in the treatment of bacterial infections. Interestingly, this plant was screened for antibacterial activity for the first time and was found to be active. Detailed chemical investigations are, therefore, warranted.

### Introduction

The genus, *Rhynchosia* belongs to the Fabaceae family, which includes more than 100 species; 22 species are to be found in India.<sup>1,2</sup> The species, *Rhynchosia scarabaeoides* (L.) DC (*Atylosia scarabaeoides* (L.) Benth.) is found in the tropics of both

hemispheres. It is widely available in the forests of Andhra Pradesh (Eastern Ghats of India) and in the native language, Telugu, is commonly called Gadi-chikkudu-kaya. It is of small to medium size and is found as twining or as erect herbs or shrubs. Its stem is very slender with a slight pubescence at the lower part. Leaves are sub-coriaceous or membranous, 0.75-2.5 cm wide, and are conspicuously gland-dotted with yellow flowers in short-peduncled racemes. Its pods are 1.25-1.5 cm long, and are glabrescent and mostly 2-seeded. The plant is characterized by its two ovules with a compressed central funiculus and often falcate pod, and by papilionaceous flowers which are beardless.<sup>1,2</sup>

All the parts of the *R. scarabaeoides* plant have been known for their medicinal uses since ancient times and are routinely used in Indian traditional medicine for the treatment of a variety of diseases.<sup>3-6</sup> Its leaf decoction is used for dysentery and, in particular, it is given with honey to women after childbirth.<sup>7</sup> The whole plant material is pounded in coconut oil and is applied on the scalp to control hair loss. It is also used to cure diarrhoea in cattle.<sup>7</sup>

The present study is aimed at investigating the antibacterial activity of *R. scarabaeoides* in order to validate its traditional use. All parts of *R. scarabaeoides* were, therefore, tested for antibacterial activity *in vitro*.

### Materials and Methods

#### Plant material

The plant materials of *Rhynchosia scarabaeoides* were collected from Rajahmundry (16° 59' N, 81° 47' E, Andhra Pradesh, India) in June 2010. The identity of this plant was confirmed by taxonomist, Prof. M Venkataratnam, (Department of Botany, Andhra University, Visakhapatnam, A.P., India) and the voucher specimens (100601) were deposited at the Department of Chemistry, GITAM University, India.

#### Soxhlet extraction

The leaves, stems and roots were collected and air dried. The dried and pulverized plant materials (10 gm) were packed in a Soxhlet and later extracted successively with hot hexane, ethyl acetate and ethanol for 12 h each. The resultant extracts were concentrated under vacuum to give hexane, ethyl acetate and ethanol concentrates.

#### Sample preparation

The crude extracts or control-drug stock solutions (1 mg/mL) were prepared by adding 50 µL of dimethyl sulphoxide (DMSO) to 1 mg of extract / drugs and made up to 1 mL with

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Key words: Indian System of Medicine, *Rhynchosia scarabaeoides*, Fabaceae, antibacterial activity, agar well diffusion, minimum inhibitory concentration, minimum inhibitory activity.

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Contributions: SC, VVSK, VSSK, study investigations; KKR, biological investigations; SKT, survey of ethnobotanical information, collection of plant parts, extraction and other phytochemical investigations.

Conflict of Interest: the authors have declared that there is no conflict of interest.

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double distilled water. These were used for antimicrobial tests. Later these solutions were gradually diluted as required.

#### Cultivation of microorganism

The five bacterial strains used in this study are: *Bacillus subtilis* (NCIM 2063), *Escherichia coli* (NCIM 2066), *Klebsiella pneumoniae* (NCIM 2957), *Proteus vulgaris* (NCIM 2027) and *Staphylococcus aureus* (NCIM 3021). All were obtained from the National Chemical Laboratory (NCL), Pune, India. The bacterial cultures were maintained on *Luria Bertani Agar* (LB) at 4° C temperature according to the methods of Sambrook *et al.*, 1999.<sup>8</sup> The subculture was carried out every 21 days using fresh medium.

**Table 1. Antibacterial activities of leaf and stem extracts of *Rhynchosia scarabaeoides*.**

Micro-organism	Control*	Zone of growth inhibition in mm					
		Hexane Ext.		EtOAc Ext.		Ethanol Ext.	
		Leaf*	Stem*	Leaf*	Stem*	Leaf*	Stem*
<i>Bacillus subtilis</i>	15±1	ND	ND	16±1	14±2	16±1	15±1
<i>Escherichia coli</i>	14±1	ND	ND	16±1	15±2	15±1	13±1
<i>Klebseilla pneumonia</i>	11±1	ND	ND	16±1	14±2	15±1	12±1
<i>Proteus vulgaris</i>	15±1	ND	ND	22±2	18±1	18±2	15±1
<i>Staphylococcus aureus</i>	15±1	ND	ND	24±1	18±2	24±1	15±1

ND, not detected; Ext, extract; EtOAc, ethyl acetate; \*10 µL/well; Control: ampicillin.

### Antimicrobial assay

A pour plate method was adopted for antibacterial screening.<sup>8,9</sup> Inoculum of test organisms was prepared by growing pure isolate in nutrient broth at 37° C overnight. These cultures were sub-cultured in fresh nutrient broth and were incubated at 37° C for 3 h to obtain log phase culture. The agar plates were prepared by a pour plate method using 20 mL nutrient agar medium per bacteria. The molten sterile medium was cooled to 45° C and was mixed thoroughly with 10 µL of growth culture of the test organism under study (1×10<sup>8</sup> cells). It was then poured into sterile petriplates and allowed to solidify. Wells (6 mm) were made with sterile gel puncture and 10 µL of plant extract was aseptically added to each well. A control drug, ampicillin (10 µg/µL), was used as standard positive antibacterial agent along with plant extract samples. These nutrient agar plates were incubated at 37° C for 24 h. The diameter of zone inhibition was measured in mm using a Himedia zone reader. The minimum inhibitor concentration (MIC) was determined by an agar diffusion method. The extracts were incorporated into nutrient agar at concentrations from 1 µL to 10 µL. A control plate without the extract was also set up. The lowest concentration of extract that inhibited the growth of microorganisms was considered the MIC.

### Results and Discussion

The antibacterial activities of *Rhynchosia scarabaeoides* extracts were tested against five bacterial strains: *Bacillus subtilis*, *Escherichia coli*, *Klebseilla pneumonia*, *Proteus vulgaris* and *Staphylococcus aureus* (Table 1). Data were expressed as the mean±standard deviation of the inhibition zone (IZ) of three independent experiments on different days. The IZ (10 µL/well) values of extracts ranged from 12-24 mm whereas that of the control drug, ampicillin, ranged from 11-15 mm. It was found that the hexane extracts of leaf and stem parts of *R. scarabaeoides* did not demonstrate any activity against the microorganisms tested, whereas ethyl acetate and ethanol extracts demonstrat-

ed different levels of antibacterial activity against different bacteria. No activity was seen with root extracts.

The ethyl acetate extracts of *R. scarabaeoides* demonstrated a broad spectrum of antibacterial activity ranging from gram positive to gram negative bacteria. Interestingly, leaf ethyl acetate extracts were found to demonstrate significant activity against all tested microorganisms, with an IZ ranging from 15-24 mm. MIC values were 1 µg against *P. vulgaris* and *S. aureus* whereas these were 2 µg against *B. subtilis*, *K. pneumoniae* and *E. coli* (Table 2). Ethanol extracts also demonstrated antibacterial activity but with moderate inhibitory activity. Also, it was found that *R. scarabaeoides* plant extracts showed higher activity against two bacterial strains, *S. aureus* and *P. vulgaris*, among the screened bacteria. Based on these results, it is evident that the selected plant extracts demonstrated potential antibacterial activities. This supports the use of the plant as an antibacterial agent as part of the Indian system of medicine.<sup>7</sup>

An intensive literature search found that similar antibacterial activities were reported in some previous studies of other species of the genus, *Rhynchosia* viz. *R. beddomei* and *R. suaveolens*.<sup>10,11</sup> It was also seen that a few *Rhynchosia* species had been investigated previously for phytochemicals<sup>12-14</sup> but no phytochemical studies have so far been reported on *R. scarabaeoides*, in spite of general knowledge of its traditional use. Chemotaxonomic studies indicate that *Rhynchosia* species normally contain flavonoids and a phenolic type of constituents. For example, gallic acid and its analogs were reported from species such as *R. volubilis* and *R. minima*, isovitexin (flavonoid), and 8-C-prenylquercetin 7,4'-dimethyl ether (flavanol) were reported from the leaves of *R. cyanosperma* along with gallic acid derivatives.<sup>15-17</sup> These compounds are biologically important and proven to be active in a considerable number of pharmacological studies.<sup>16-19</sup> For example, antibacterial biphenyl constituents 4-(3-methyl-but-2-enyl)-5-methoxy-[1,1'-biphenyl]-3-ol and 2-carboxy-4-(3-methyl-but-2-enyl)-5-methoxy-[1,1'-biphenyl]-3-ol were reported from *R. suaveolens*.<sup>11</sup> Therefore, it would be worth while carrying out a phytochem-

**Table 2. Minimum inhibitory activity values of leaf ethyl acetate extracts.**

Micro-organism	MIC (µg/well)
<i>Bacillus subtilis</i>	2
<i>Escherichia coli</i>	2
<i>Klebseilla pneumonia</i>	2
<i>Proteus vulgaris</i>	1
<i>Staphylococcus aureus</i>	1

ical investigation of *R. scarabaeoides* in order to isolate the active constituents responsible for the antibacterial activity found in the present study.

### Conclusions

Our findings support the traditional usage of *Rhynchosia scarabaeoides* plant parts in the treatment of bacterial infections because of their significant antibacterial activity. These plant parts merit further investigation.

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