



## SHORT NOTE [NOTA CORTA]

### BIOLOGICAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF THE OLEORESIN OF *Shorea robusta* Gaertn. f.

### [ACTIVIDAD BIOLÓGICA Y EXPLORACIÓN FITOQUÍMICA DE LA OLEORESINA DE *Shorea robusta* Gaertn. f.]

K. Sri Rama Murthy<sup>1\*</sup>, N. Lakshmi<sup>1</sup> and D. Raghu Ramulu<sup>2</sup>

<sup>1</sup> School of Conservation Biology and Plant Biotechnology, Department of Biotechnology, Montessori Mahila Kalasala, Vijayawada - 520 010, Andhra Pradesh, India. E-mail: drksrmurthy@yahoo.com

<sup>2</sup> Department of Botany, Sri Krishnadevaraya University, Anantapur - 515 003, Andhra Pradesh, India

\*Corresponding author:

#### SUMMARY

*Shorea robusta* Gaertn.f. oleoresin (gum) extracts are used against the skin allergies, diarrhea, dysentery, astringency and is wide spread in different parts of Eastern Ghats of Southern Peninsular India. The objective of the present study was to evaluate the antimicrobial and phytochemical activity of resin extract against pathogenic microorganisms. Successive petroleum ether, methanol, benzene and aqueous extracts of *Shorea robusta* resin were tested for their phytochemical constituents, antibacterial and antifungal activity. The aqueous and methanolic extracts were found to be most effective against most of the tested organisms. The results confirmed the potential of this plant in the indigenous systems of medicine.

**Key words:** *Shorea robusta*; phytochemical screening; biological activity.

#### INTRODUCTION

*Shorea robusta* Gaertn.f. (Dipterocarpaceae) is widely distributed in India, Nepal and Bhutan. In India, the species is distributed from Himachal Pradesh to Assam, Tripura, West Bengal, Bihar and Orissa, Eastern districts of Madhya Pradesh extending further to the Eastern Ghats of Andhra Pradesh. The oleoresin (gum) of the aerial parts has been reported in indigenous system of medicine as it also used as an ingredient of ointments to heal wounds, burns, pains, skin diseases and to control diarrhea and dysentery (Saraswathy et al., 1992; Pullaiah and Rani 1999; Upadhyay et al., 1998; Misra and Ahmad 1997). The literature survey revealed that no biological activity and phytochemical works has been done so far with the oleoresin of this plant. The biological activity was

#### RESUMEN

Los extractos de oleoresina (goma) de *Shorea robusta* Gaertn.f. son empleados para el tratamiento de alergias cutáneas, diarrea, disentería y como astringente y se encuentra presente en diferentes áreas de los Ghats occidentales de la región sur de la India. El objetivo del presente estudio fue evaluar la actividad antimicrobial y actividad fitoquímica del extracto de la resina. Se realizaron extracciones de éter de petróleo, metanol, benceno y acuoso y se evaluaron sus constituyentes fitoquímicos y la actividad antibacterial y antifúngica. Los extractos acuosos y metanolicos fueron los más efectivos en los organismos estudiados. Los resultados confirman el potencial de esta planta en la medicina tradicional.

**Palabras clave:** *Shorea robusta*; exploración fitoquímica; actividad biológica.

screened against the micro organisms causing skin allergies, diarrhea and dysentery.

Medicinal Plants have been used for centuries as remedies for human diseases, because, they contain components of therapeutic value (Nostro et al., 2000). It is estimated that there are about 250,000 species of higher plants and the majority of them have not been examined for their pharmacological activities. The antimicrobial properties of certain Indian medicinal plants were reported based on folklore information (Ram et al., 2000; Nagalakshmi et al., 2001; Jayasinghea, 2002; Chowdhury et al., 2003; Mishra et al., 2005; Lavanya et al., 2006; Murthy and Kandimalla, 2008) and a few attempts were made on inhibitory activity against certain pathogenic bacteria and fungi (Taylor et al., 1995). The plant is well reputed in traditional system of medicine and used by tribal people to treat various diseases like skin

allergies, diarrhea, dysentery and also used as an astringent (Pullaiah and Rani, 1999). These are common in most of the tribal inhabitants due to lack of sanitation, potable water and awareness of hygienic food habits. Thus there is an increased need for the development of alternative antipathogenic substances. One possible approach is to screen local medicinal plants in search of suitable chemotherapeutic antibacterial and antifungal substances.

## MATERIALS AND METHODS

### Plant material

Oleoresin of *Shorea robusta* (gum) collected from the evergreen forest of Donubai hills ranges of Srikakulam district of Eastern Ghats, in February 2006. The ethanobotanical information regarding the drug-yielding plant was recorded using the standard methods (Croom, 1983, Hamilton, 1995). The information on name, part used, purpose, mode of administrations were recorded in the field notebooks as well as on the audiotapes. The sample specimens were collected in bulk quantities for analysis. Based on the folk evidences regarding the effective utilization for different skin allergies, diarrhea, dysentery and astringent, the samples were collected and screened for antimicrobial properties. The voucher specimens (No.2095) were identified with the help of regional floras (Pullaiah and Rani, 1999) and deposited in MMK Herbarium, Vijayawada.

### Preparation of extracts

The plant oleoresin (gum) was shade dried (about 60g) and successively extracted with different solvents (250 ml) using a soxhlet apparatus for 6 h. The extracts were filtered and concentrated under reduced pressure, below 40°C to dryness. Phytochemical analysis for major phytoconstituents of the plant extracts were undertaken using standard qualitative methods as described by various authors (Rizk and Bashir 1980, Fadeyi *et al.*, 1989, Odebiyi and Sofowora, 1990). The plant extracts were screened for the presence of biologically active compounds like alkaloids, flavonoids, glycosides, phenolics, saponins, steroids, tannins, Triterpenoids.

### Microorganisms and culture media

*Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus coagulans*, *Bacillus cereus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus griseus*, (gram positive) *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas fluorescence*, (gram negative) *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans*, *Penicillium chrysogenum* (fungi) were

obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India. The tested organisms were sub-cultured at 37°C and maintained on nutrient agar media for bacteria and sabouraud agar medium for *Candida albicans*.

### Antimicrobial assay

The antimicrobial activity of the ethanol extracts of each sample was evaluated by using disc diffusion method (Bauer *et al.*, 1966). Petriplates containing the aliquotes 20 ml of respective medium was seeded with selected microbial strains. Five millimeters of nutrient broth was inoculated with a loop (6 mm) of bacteria / yeast and incubated at 35°C for 6 h. One millimeter of broth was taken at 0.6 optical density (at log phase, ca.108 cells/ml) and inoculated on the nutrient agar and transferred to 180 x 20 mm petri dishes. The sterile Whatmann No. 1 filter paper discs of 6 mm diameter were impregnated with 1000-5000 µg of concentrated plant extracts and placed on the surface of the freshly inoculated medium. Standard antibiotic discs viz. Ampicillin, Tetracycline, Gentamycine, and Clotrimazole (Hi-Media, Mumbai) were used as positive controls. Ethanol and water alone served as negative controls. The assessment of antimicrobial activity was based on measurement of inhibition zones formed around the discs. The media were incubated for 24 hrs at 37°C and the diameters of the inhibition zones were recorded. Three independent trials were conducted for each concentration.

## RESULTS AND DISCUSSION

The aqueous, methanol, petroleum and benzene extract of oleoresin of *Shorea robusta* were tested (Table 1). Different extracts inhibited the growth of 12, 11, 9 and 6 of the 15 used microorganisms, respectively. Aqueous extracts of *Shorea robusta* exhibits significant activity against *Bacillus coagulans*, *Escherichia coli*, *Bacillus cereus* and moderate inhibition on *Salmonella typhi* and *Bacillus subtilis* and less activity against *Proteus vulgaris* and *Pseudomonas fluorescence*. However, ethanolic extracts also exhibited significant activity against *Staphylococcus aureus*, *S. epidermidis* and *Escherichia coli*, moderate inhibition on *Candida albicans* and *Bacillus coagulans*. In a nutshell, methanol extract showed more significant activity. The petroleum ether and benzene extracts showed less inhibitory activity when compare with the above two extracts. The Petroleum ether showed activity against *Escherichia coli*, *Aspergillus flavus* and *Candida albicans* and whereas benzene extracts worked against *Bacillus licheniformis*, *Bacillus cereus* and *Aspergillus flavus*.

Table 1. Antimicrobial activity of *Shorea robusta* resin extracts <sup>a</sup>

Microorganism	P	M	B	A	Standards <sup>b</sup>
<b>Bacteria</b>					
<i>Bacillus subtilis</i> (G +) NCIM 2063	12 (21)	-	6(14)	20(8)	NT
<i>Bacillus licheniformis</i> (G +) MTCC 1483	9 (7)	13(5)	8(15)	12(8)	16
<i>Bacillus coagulans</i> (G +) NCIM 2030	-	9(13)	-	26 (9)	14
<i>Bacillus cereus</i> (G +) MTCC 1305	-	-	10(8)	27(5)	14
<i>Salmonella typhi</i> (G+) MTCC 537	12 (7)	-	7(14)	25(11)	12
<i>Staphylococcus aureus</i> (G +) NCIM 2079	6(13)	14(31)	-	-	16
<i>Staphylococcus epidermidis</i> (G +) NCIM 2493	-	12(20)	7(12)	13(23)	13
<i>Staphylococcus griseus</i> (G +) MTCC 1540	-	9 (14)	-	10(19)	14
<i>Escherichia coli</i> (G -) NCIM 2065	13 (26)	11(12)	-	26(8)	14
<i>Proteus vulgaris</i> (G -) NCIM 2857	7(14)	9 (12)	-	11(14)	13
<i>Pseudomonas fluorescense</i> (G -) MTCC 748	7(17)	8 (12)	-	11(3)	10
<b>Fungi</b>					
<i>Aspergillus flavus</i> MTCC 1884	17 (10)	5 (18)	9(13)	14(5)	7
<i>Aspergillus niger</i> MTCC 1785	-	-	-	-	NT
<i>Candida albicans</i> MTCC 1637	10(6)	10(31)	-	14 (3)	9
<i>Penicillium chrysogenum</i> MTCC 1996	-	11(18)	-	-	8

<sup>a</sup> values (zone of inhibition in mm) are the mean of three replicates; minimum inhibitory concentration (mg / ml) are reported in parentheses, when determined. P, petroleum ether extract; M, methanol extract B, benzene extract; A, Aqueous extract; (all tested at 25mg/ml); - no inhibition; NT-not tested.

<sup>b</sup> Ampicillin (10 mg / ml) for *Bacillus*, *Staphylococcus* species, Tetracycline (30 mg / ml) for *E. coli*, Gentamycine (10 mg / ml) for *Proteus* and *Pseudomonas* species and Clotrimazole (25 mg / ml) for fungal species.

Phytochemical screening of these extracts showed the presence of alkaloids, carboxylic acids, fatty acids, phenols, saponins and steroids (Table 2.) Catechols, coumarins, proteins, tannins, volatile oils were observed in low concentrations. We suspect that the tannins found in the phytochemical analysis of the extracts could be responsible for the antibacterial activity. Triterpenoids have astringent actions, which form the basis for their therapeutic applications (Edward *et al.*, 1970). It was found that the plants which contain triterpenoids had antimicrobial activity (Trease and Evans, 1989).

The results of antimicrobial activity are comparable with the results of the previous researches using extracts of other species like *Azadirachta indica* (Ram *et al.*, 2000), *Chukrasia tabularis* (Nagalakshmi *et al.*, 2001), *Toona ciliata* and *Amoora rohituka* (Chowdhury *et al.*, 2003), *Aglaia spectabilis* (Lavanya *et al.*, 2006), *Walsura trifoliata* (Murthy and Kandimalla 2008). The effectiveness may be due to the cumulative action of different compounds present in the plant parts (Bai 1990). They include alkaloids, Flavonoids, triterpenoids and other compounds of phenolic nature and are classified as active antimicrobial compounds (Rojas *et al.*, 1992). This study revealed that the oleoresin extracts of *Shorea robusta* had antimicrobial activity on certain pathogens with a broad spectrum result to standard antibiotics.

Table 2. Phytochemical screening of petroleum ether (P), methanol (M), benzene (B); aqueous (A) extracts of *Shorea robusta* resin extracts <sup>a</sup>

Constituents	P	M	B	A
Alkaloids	+	+++	-	+++
Anthocyanidins	-	-	-	-
Carboxylic acids	++	+	+	-
Catechols	-	+	++	+
Coumarins	-	++	-	-
Fatty acids	+	+++	+	-
Fixed oils	+	+	-	+
Flavonoids	++	+++	++	+
Glycosides	++	+++	+	+
Phenols	+	++	+++	++
Proteins	-	-	-	+
Quinines	-	-	-	-
Resins	+++	++	+++	++
Saponins	+	++	+	+
Steroids	-	++	+	-
Tannins	+	-	+++	+
Triterpenoids	+++	+++	++	++
Volatile oils	+	-	-	+

- not detected; + low concentration; ++ high concentration; +++ very high concentration.

## CONCLUSION

Based on the results obtained in the current study, it may be concluded that *Shorea robusta* resin have a stronger and broader spectrum of antimicrobial

activity against a number of pathogenic microorganisms and the extracts may be used to discover bioactive natural products that may serve as basic source for the development of new drugs for therapy of skin allergies, diarrhea, dysentery and astringency. The results obtained also provide support to the uses of the plants in traditional medicine. However, the toxicological analysis of the active compounds is necessary in order to assess its tolerance in the human body when administered.

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