In-vitro Antioxidant and Antibacterial Activity of Methanolic Extract of *Shorea robusta* Gaertn. F. Resin

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ABSTRACT

The methanolic extract of the resin of *Shorea robusta* was subjected to investigate its antioxidant and antibacterial properties its utility in free radical mediated diseases including diabetic, cardiovascular, cancer etc. The methanol extract of the resin was tested for antioxidant activity using scavenging activity of DPPH (1,1-diphenyl-2-picrylhydrazil) radical method, reducing power by $FeCl_3$ and antibacterial activity against gram positive and gram negative bacteria using disc diffusion method. The phytochemical screening considered the presence of triterpenoids, tannins and flavoniods. Overall, the plant extract is a source of natural antioxidants which might be helpful in preventing the progress of various oxidative stress mediated diseases including aging. The half inhibition concentration (IC_{50}) of resin extract of *Shorea robusta* and ascorbic acid were 35.60 µg/ml and 31.91 µg/ml respectively. The resin extract exhibit a significant dose dependent inhibition of DPPH activity. Antibacterial activity was observed against gram positive and gram negative bacteria in dose dependent manner.

Key Words: *Shorea robusta*, antioxidant, antibacterial, Disc-diffusion, DPPH.

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INTRODUCTION

Oxidative stress on human health has become a serious issue. Under stress, our bodies produce more reactive oxygen species (ROS) than enzymatic antioxidants and non-enzymatic antioxidants. This imbalance leads to cell damage and health problems. A lack of antioxidants, which can quench the reactive free radicals, facilitates the development of degenerative diseases, including cardiovascular diseases, cancers, neurodegenerative diseases, alzheimer's disease and inflammatory diseases [1, 2].

Many of the previous literature show large number of plants that can be used against diseases, in which reactive oxygen species and free radical play important role [3]. Aromatic and medicinal plants are known to produce certain bioactive molecules which react with other organisms in the environment, inhibiting bacterial growth. Many studies reported the activities of medicinal plants on pathogenic microorganisms [4].

The chosen medicinal plant namely *Shorea robusta* resin belongs to the Dipterocarpaceae family. *Shorea robusra* Gaertn.f. is widely distributed in India, Nepal and Bhutan. In India, the species are 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 [5]. Different parts of the plant are traditionally used for the treatment of diverse purposes. The leaves are used to treat wounds, ulcers, itching, leprosy, gonorrhea, cough, earache and headache [6, 7].

The oleoresin exuded from the cut bark has astringent and detergent properties [8]. The bark is also used to treat diarrhea [9], dysentery [10], wounds [11], ulcers and itching [12]. In Unani system of medicine, the resin is used for treating menorrhagia, enlargement of spleen and for relieving eye irritations [13].

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In ayurveda the leaves are used as anthelmintic and alexiteric. *Shorea robusta* leaf extract has been found to possess significant anti-inflammatory activity [14].
Therefore, the present study were to investigate the *Shorea robusta* resin *in-vitro* antioxidant activity by reducing power assay and DPPH assay and *in-vitro* antibacterial activity by disc diffusion method.

MATERIALS AND METHODS

Plant material

Natural *Shorea robusta* resin was purchased from local market of Delhi, India. *Shorea robusta* resin was identified by department of Raw Materials Herbaruim and Museum (RHMD), National Institute of Science Communication and Information Resources (NISCAIR), Delhi, India. The powdered material stored in air tight polythene bags until use.

Preparation of extract

The above powdered material was successively extracted with methanol in Soxhlet apparatus for 48 hrs. The extracts was concentrated by evaporation and subjected to freeze drying till dry powder was obtained [15].

Phytochemical screening

The phytochemical screening of methanol extract was done to identify the main groups of chemical constituents present in methanol extract of *Shorea robusta* resin [16].

In vitro antioxidant activity

DPPH- Assay

The scavenging ability of the natural antioxidants of the plant extract towards the stable free radical DPPH was measured by the method of Shimada *et al*. [17]. Briefly, a 2 ml aliquot of DPPH methanol solution (25µg/ml) was added to 0.5 ml sample solution at different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance was measured at 517 nm in a spectrophotometer. L-Ascorbic acid was used as the standard. Radical scavenging activity (%) = 100-[(Ac-As) / AcX100] Where AC = control is the absorbance of the control and AS = sample is the absorbance of reaction mixture (in the presence of sample). All tests were run in triplicates (n = 3), and the average values were calculated.

Reducing power assay

The Fe^{3+} reducing power of the extract was determined by the method of Oyaizu [18] with slight modifications. The extract (0.75 ml) at various concentrations was mixed with 0.75 ml of phosphate buffer (0.2 M, pH 6.6) and 0.75 ml of potassium hexacyanoferrate [$K_3Fe(CN)_6$] (1%, w/v), followed by incubating at 50°C in a water bath for 20 min. The reaction was stopped by adding 0.75 ml of trichloroacetic acid (TCA) solution (10%) and then centrifuged at 3000 r/min for 10 min. 1.5 ml of the supernatant was mixed with 1.5 ml of distilled water and 0.1 ml of ferric chloride ($FeCl_3$) solution (0.1%, w/v) for 10 min. The absorbance at 700 nm was measured as the reducing power. Higher absorbance of the reaction mixture indicated greater reducing power.

In vitro antibacterial activity

The antibacterial activity of methanol extract of *Shorea robusta* resin evaluated by paper disc diffusion method using nutrient agar medium against following microorganism *S. aureus, B. subtilis*, (Gram positive) and *E. coli, P. aeruginosa* (gram negative). Petri plates containing the aliquots 20 ml of respective medium was seeded with selected bacterial strains. Five millimeters of nutrient broth was inoculated with a loop (6 mm) of bacteria and incubated at 37 ^{0}C for 6 h. One millimeter of broth was taken at 0.6 optical density 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 impegranated with 30 and 50 µg of concentrated plant extract and placed on the surface of the freshly inoculated medium. Standard antibiotic discs ciprofloxacin was used as standard. Water alone served as control. The assessment of antibacterial activity was based on the measurement of inhibition zones formed around the discs. The media were incubated for 24 hrs at 37 ^{0}C and the diameters of the inhibition zones were recorded [19].

Statistical analysis

Tests were carried out in triplicate for 3–5 separate experiments. The amount of extract needed to inhibit free radicals concentration by 50%, IC_{50}, was graphically estimated using a nonlinear regression algorithm.

RESULTS AND DISCUSSION

In vitro Antioxidant activity

DPPH- Assay

DPPH radical scavenging activity of resin extract of *Shorea robusta* and standard as ascorbic acid are presented in (Table 1 and figure 1). In the DPPH assay, the antioxidant was able reduce the stable radical DPPH to the yellow colored 1, 1-diphenyl-1, 2-picryl hydrazine. The molecule of 2, 2-diphenyl-1-picryl hydrazine is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole. The proton transfer reaction of the DPPH radical by a scavenger causes a decrease in absorbance at 517 nm, which can be followed by a common spectrophotometer set in the visible region. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability. The half inhibition concentration (IC_{50}) of resin extract of *Shorea robusta* and ascorbic acid were 35.60 µg/ml and 31.91 µg/ml respectively. The resin extract exhibit a significant dose dependent inhibition of DPPH activity. The potential of L-ascorbic acid to scavenge DPPH radical is directly proportional to the concentration. The DPPH assay activity is near to standard as ascorbic acid.

Table 1. DPPH radical scavenging activity of *Shorea robusta*

Concentration (µg/ml)	% inhibition	
	Ascorbic acid	*Shorea robusta*
20	20.34±0.340	25.40±0.010
40	59.78±0.060	50.70±0.021
60	78.90±0.090	71.10±0.043
80	86.60±0.088	80.42±0.060
100	100.00±0.090	95.61±0.080
IC_{50}	31.91µg/ml	35.60µg/ml

Table 2. Reducing power activity of *Shorea robusta* extract

Concentration (µg/ml)	Absorbance	
	Ascorbic acid	*Shorea robusta*
100	0.52±0.04	0.70±0.01
200	0.82±0.05	0.60±0.02
400	1.54±0.06	0.81±0.04
600	1.70±0.03	1.09±0.04
800	3.44±0.03	2.11±0.01
1000	3.61±0.03	2.27±0.08

Table 3. Antibacterial activity of *Shorea robusta* resin extract

Name of bacteria	Zone of inhibition (mm)		
	Ciprofloxacin	*Shorea robusta* (30µg/disc)	*Shorea robusta* (50µg/disc)
Staphylococcus aureus	18	12	16
Bacillus subtilis	14	10	12
Pseudomonas aeruginosa	15	10	13
E. coli	17	12	15

Reducing power assay

For the measurements of the reducing ability, the Fe^{3+}-Fe^{2+} transformation was investigated in the presence of *Shorea robusta.* The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. (Table 2 and figure 2) depict the reductive effect of *Shorea robusta.* The reducing power of *Shorea robusta* increased with increasing dosage. All the doses showed significantly higher activities than the control exhibited greater reducing power, indicating that *Shorea robusta* consist of hydrophilic polyphenolic compounds that cause the greater reducing power.

In vitro antibacterial activity

The antibacterial activity of plants is related to their zone of inhibition against the some of the pathogenic organisms. The result of this study showed that methanolic extract of *Shorea robusta* resin inhibited the growth of various species of gram positive and gram negative bacteria (Table 3 and figure 3, 4). The inhibition zone of gram positive bacteria was found to be *Staphylococcus aureus* (12-16 mm), *Bacillus subtilis* (10-12 mm) and gram negative bacteria *Pseudomonas aeruginosa* (10-13 mm), *E.coli* (12-15 mm). In the present study, the growth of all pathogenic bacteria was remarkably inhibited by methanolic extract of *Shorea robusta* resin which was significantly similar to ciprofloxacin.

CONCLUSION

Shorea robusta is an important ayurvedic drug which has also been studied extensively by different investigators. It stimulates the antioxidant, antibacterial, wound healing and anti-inflammatory activity due the presence of polyhenols, falavonoids and triterpenoids. It could be beneficial in prevention of oxidative stress and various types of bacterial infections. It was promoted by reported of antioxidant activity of *Shorea robusta* leaves[11].

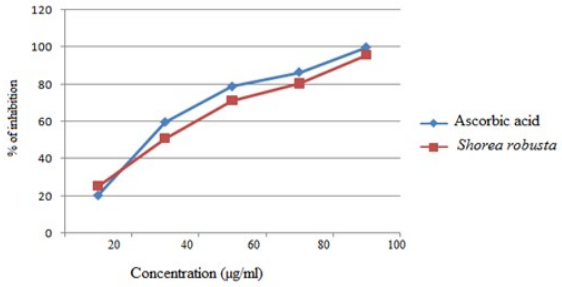


Figure 1. DPPH radical scavenging activity of *Shorea robusta* resin

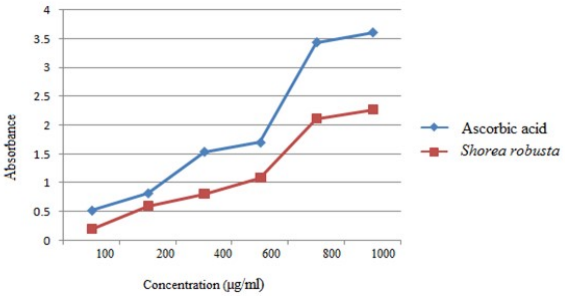
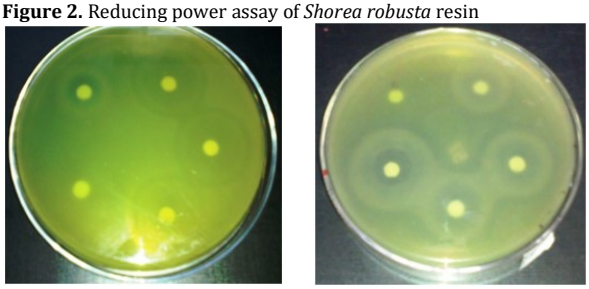


Figure 2. Reducing power assay of *Shorea robusta* resin



(A) **(B)**

Figure 3. Antibacterial activity against (A) Gram negative bacteria and (B) against gram positive bacteria

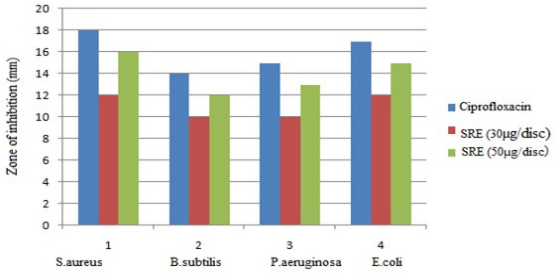


Figure 4. Antibacterial activity of methanolic extract of *Shorea robusta* resin at different concentrations

This work has gathered experimental evidence in the *Shorea robusta* resin extract as natural antioxidant and antibacterial agent for its capacity to scavenge reactive oxygen and protect cell from oxidative damage and thus could effective against oxidative stress and inhibited the growth of gram positive and gram negative bacteria.

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