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# STUDIES ON *IN VITRO* EVALUATION OF ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF *STERCULIA FOETIDA* L. BARK

Akhtari Khatoon[1], Ashirbad Mohapatra[2] and Kunja Bihari Satapathy [*1]

P.G. Department of Botany [1], Utkal University, Vani Vihar, Bhubaneswar-751004, Odisha, India
Sri Jayadev College of Education and Technology [2], Naharkanta, Bhubaneswar-752101, Odisha, India

**Keywords:**

Agar well diffusion, Antibacterial activity, Antioxidant activities, *Sterculia foetida* L. bark, n-Hexane, Methanol

**Correspondence to Author:**
**Dr. Kunja Bihari Satapathy**

Plot No.-55/1733, Acharya Vihar, Bhubaneswar-751013, Odisha, India.

**E-mail:** kbs_bot@rediffmail.com

**ABSTRACT:** The objective of the present investigation was to evaluate the antibacterial and antioxidant potential of n-hexane and methanolic extracts of *Sterculia foeida* L. bark. The antibacterial potential of *Sterculia foetida* L. bark was tested against human pathogens causing diarrhoea and dysentery such as *Shigella flexneri* (MTCC-9543), *Salmonella enterica ser typhi* (MTCC-733), *Bacillus subtilis* (MTCC-1305), *Streptococcus mitis* (MTCC-2897), *Klebsiella pneumoniae* (MTCC-109) and *Staphylococcus aureus* (MTCC-1430) by using agar well diffusion method. The result of the study revealed that n-hexane extract was highly effective against *Shigella flexneri*, whereas *Klebsiella pneumoniae* showed no response and *Salmonella enterica ser typhi*, *Bacillus subtilis*, *Streptococcus mitis*, *Staphylococcus aureus* responded moderately. The methnolic extract had high inhibition against *Salmonella enterica ser typhi* (15.16 ± 0.20 mm) whereas *Shigella flexneri* showed no response and moderate effect against other test bacteria. The antioxidant activity by DPPH scavenging method resulted in significant antioxidant potential of n-hexane and methanolic extracts with $IC_{50}$ value of 51.26 and 66.84 respectively. Ciprofloxacin was taken as the reference antibiotic.

**INTRODUCTION:** With the advent of human civilization medicinal trees have been used both in the prevention and cure of various diseases of human and their pets. Primarily based on plant many system of therapy have been developed [1]. Plants have been a source of food, fiber and medicine since the beginning and the use of plants and its products has a long history that began with folk medicine and through the years has been incorporated into traditional and allopathic medicine system [2].

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Medicinal plants are natural sources of compounds that can be used against many diseases today [3]. The discovery of many natural and synthetic drugs is a remarkable progress in the field of medicine which has been made by the advancement in Science and Technology [4]. One of the most important therapeutic discoveries of the 20$^{th}$century is antibiotics that had high effectiveness against serious bacterial infections. However, only one third of the infectious diseases known have been treated from these synthetic products [5].

*Sterculia foetida* L. is a tall, deciduous tree belonging to the family Sterculiaceae. It produces more or less whorled, horizontal branches. The follicles are woody, boat shaped and bright red when ripe. The seeds are black, ellipsoid, 2 cm long, 10 - 15 in each follicle and not winged [6]. *S. foetida* has hidden potentiality for its medicinal and

economical importance [1]. Its gum could be used as controlled release matrix polymers [7]. The seed crude extract of *S. foetida* acted as insecticide to Asian army worm and as antifeedent to the semi-polar, *Achaea janata* [8]. *S. foetida* oil (3 %) in the diet definitely delayed sexual maturity of the female rat as determined by the criteria of age at the time vagina opened and the regularity and length of consecutive estrous cycles. Oil also used in the treatment of nausea and skin diseases [9].

The flavonoid content of *S. foetida* has anti-oxidant activity [10]. The seed extract of this plant used as aperient, diuretic and as insect repellant [11]. This plant have anti-fungal [12], laxative, astringent, carminative, anti-inflammatory and central nervous (CNS) depressant activity [13]. The roasted seeds of this plant can be eaten like chestnuts, and also the seed have potentiality for treating diseases like itch, skin diseases [14], and rheumatism [15].

The phytoconstituents of seeds like sterculic acid triglycerides [16], cyclopropenoid fatty acids contains anti-fungal compounds [12], insecticidal, anti-viral, hormonal, antibiotic, carcinogenic or antitumour activities [17]. Extracts, fractions and pure compounds of *S. foetida* seeds have insecticidal activities against *Sitophilus oryzae* L., *Callosobruchus chinensis* L. and *Tribolium castaneum* [18]. Fruit bodies (epicarp and endocarp) of *S. foetida* were used to explore novel approaches for biosynthesis of silver nanoparticles and then antibacterial property of synthesized nanoprticles was observed against some gram positive and gram negative bacteria [19].

In the present investigation, n-hexane and methanolic extracts of *Sterculia foetida* L. bark have been evaluated for their antimicrobial and antioxidant activities.

## MATERIALS AND METHODS:

### Collection and identification of plant material:

The plant *Sterculia foetida* L. was collected from the "Chandaka reserve forest" area near Bhubaneswar, Odisha in the month of March, 2014. Identification of the voucher specimen was done by available literature [20]. The voucher specimens were deposited in the herbarium of Post Graduate Department of Botany, Utkal University, Vani Vihar, and Bhubaneswar. The bark was collected in bulk amount, washed in running tap water, dried under shade and made to coarse powder form.

### Processing of plant material and preparation of extract:

The collected bark was shade dried and ground to form coarse powder and had been successively extracted with the solvent n-hexane and methanol by Soxhlet apparatus [21] and the extract was recovered under reduced pressure in a rotatory evaporator. The extracts were kept in desiccators for further use.

### Evaluation of the extracts for antibacterial activity:

The *in-vitro* antibacterial screening was carried out against selected bacterial pathogens causing diarrhoea and dysentery in human. The bacterial pathogens were *Shigella flexneri* (MTCC-9543), *Salmonella enterica ser typhi* (MTCC733), *Bacillus subtilis* (MTCC1305), *Streptococcus mitis* (MTCC2897), *Klebsiella pneumoniae* (MTCC-109) and *Staphylococcus aureus* (MTCC-1430). These species were procured from Microbial Type Culture Collection Centre (MTCC) and Gene Bank, Chandigarh, India. The remaining bacterial species were provided by Post Graduate Department of Microbiology, OUAT, Bhubaneswar and Odisha. These organisms were identified by standard microbial methods [22]. The antibacterial screening of the extracts was carried out by determining the zone of inhibition using agar well diffusion method [21] for bacteria. Ciprofloxacin was taken as reference antibiotic.

### Standard drugs used and preparation of doses for antibacterial assay:

Ciprofloxacin was used as Reference Antibiotics (RA). The stock solutions of RA were prepared in 10 % dimethylsulphoxide (DMSO) to give a concentration of 0.5 mg/ml for RA.

### Agar well diffusion assay:

Agar well diffusion method [21] was followed to determine the zone of inhibition of microbes in Nutrient Agar (NA, Hi Media Laboratories Ltd., Mumbai). Plates were swabbed (sterile cotton swabs) with 8 hr old broth culture of bacteria. Wells (8 mm diameter and about 2 cm apart) were

made in each of these plates using sterile cork borer. Stock solution of plant extracts were prepared at a concentration of 3 mg/ml and about 50 µl of the solvent extracts were added aseptically into the wells and allowed to diffuse at room temperature for 2 hrs. Control experiments comprising inoculums without plant extract were set up. The plates were incubated at 37 °C for 24 hrs for bacterial pathogens. Triplicates were maintained and the diameter of the zone of inhibition (mm) was measured and statistical analysis was carried out.

**Antioxidant Test:**

Antioxidant potential of the extract was estimated on the basis of the extract's scavenging activity of stable DPPH radical. First 25 mg of extract was dissolved to 50 ml of methanol to prepare stock solution. Then 250, 200, 150, 100 and 50 µg / ml solution was prepared by diluting the stock solution. Then 1.5 ml of solution from the above was added to 1.5 ml of DPPH. This was kept in dark room for 20 min for allowing reaction. After that, absorbance or OD was measured by using UV Spectrophotometer at 517 nm against blank prepared. A blank was prepared without adding the extract. 10 mg of dry ascorbic acid was dissolved in 10 ml of methanol to prepare 5 different concentrations viz. 10, 20, 30, 40 and 50 µg / ml of ascorbic acid. That was used as standard. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

DPPH scavengered (5) = A Control – A Text × 100

(Absorbance of the central reaction – Absorbance in the present of sample of the extracts × 100)

The antioxidant activities of the extracts were expressed in $IC_{50}$.

**RESULT AND DISCUSSION:**

**Antibacterial Screening:**

The bark extract of this plant subjected to antibacterial screening against three gram-positive (*Bacillus subtilis*, *Streptococcus mitis* and *Staphylococcus aureus*) and three gram-negative (*Shigella flexneri*, *Salmonella typhi* and *Klebsiella pneumoniae*) bacteria causing diarrhoea and dysentery. The results indicated that n-hexane extract of *Sterculia foetida* L. exhibited highest zone of inhibition against *Shigella flexneri* (13.3 ± 0.24), least against *Salmonella typhi* (10.06 ± 0.12) and resistant to *Klebsiella pneumoniae*. This extract exhibited moderate activity against *Streptococcus mitis* (11.26 ± 0.24), *Bacillus subtilis* (10.50 ± 0.28) and *Streptococcus aureus* (10.33 ± 0.24). While methanolic extract exhibited highest zone of inhibition on *Salmonella typhi* (15.16 ± 0.20), least against *Staphylococcus aureus* (10.13 ± 0.12) and moderate against *Klebsiella pneumoniae* (12.13 ± 0.12) followed by *Streptococcus mitis* (11.5 ± 0.08) and *Bacillus subtilis* (10.93 ± 0.12). **(Table 1** and **Fig. 1)**

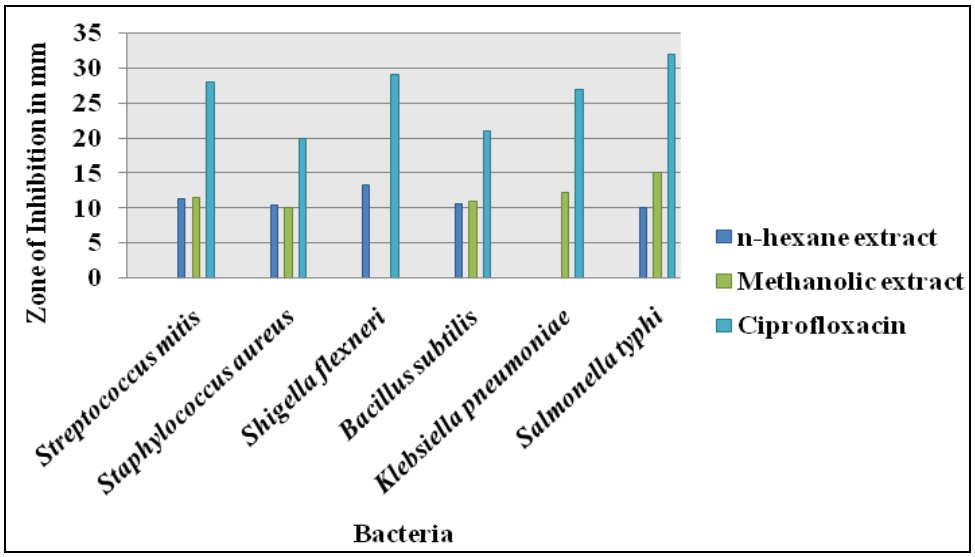


**FIG.1: *IN VITRO* ANTIBACTERIAL ACTIVITY (ZONE OF INHIBITION IN MM) OF DIFFERENT PLANT EXTRACTS OF *STERCULIA FOETIDA* L.**

**TABLE 1: *IN VITRO* ANTIBACTERIAL ACTIVITY (ZONE OF INHIBITION IN MM) OF DIFFERENT PLANT EXTRACTS OF *STERCULIA FOETIDA* L.**

| Test organisms | Zone of Inhibition in mm | | |
|---|---|---|---|
| **Bacteria** | **n-hexane extract (3 mg/ml)** | **Methanol extract (3 mg/ml)** | **Ciprofloxacin (RA) (0.5 mg/ml)** |
| *Streptococcus mitis* | 11.26 ± 0.24 | 11.5 ± 0.08 | 28±0.816 |
| *Staphylococcus aureus* | 10.33 ± 0.24 | 10.13 ± 0.12 | 20±0.816 |
| *Shigella flexneri* | 13.3 ± 0.24 | ---- | 29±1.69 |
| *Bacillus subtilis* | 10.50 ± 0.28 | 10.93 ± 0.12 | 21±0.816 |
| *Klebsiella pneumoniae* | ---- | 12.13 ± 0.12 | 27±1.69 |
| *Salmonella enteric ser typhi* | 10.06 ± 0.12 | 15.16 ± 0.20 | 32±1.69 |

Results expressed as mean ± S.D. of three determinations, (--) denotes no zone of inhibition, (RA) denotes reference antibiotics

## Antioxidant Study:

Methanolic extract of *Sterculia foetida* L. showed comparatively high antioxidant activity than n-hexane extract and ascorbic acid. The $IC_{50}$ value of methanol extracts, n-hexane extract and ascorbic acid was found 66.84, 51.26 and 25.63 respectively. **Table 2** and **Fig. 2 (i) & 2 (ii)**

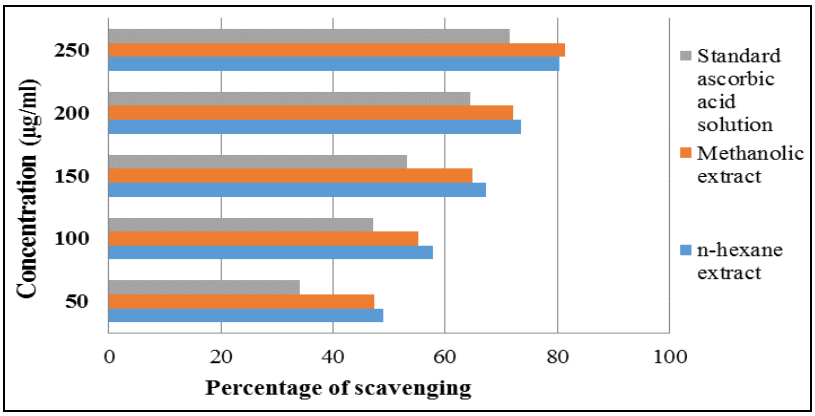


**FIG.2 (i): *IN VITRO* ANTIOXIDANT ACTIVITY OF METHANOLIC AND n-HEXANE EXTRACTS OF *STERCULIA FOETIDA* L. BARK SHOWS PERCENTAGE OF SCAVENGING.**

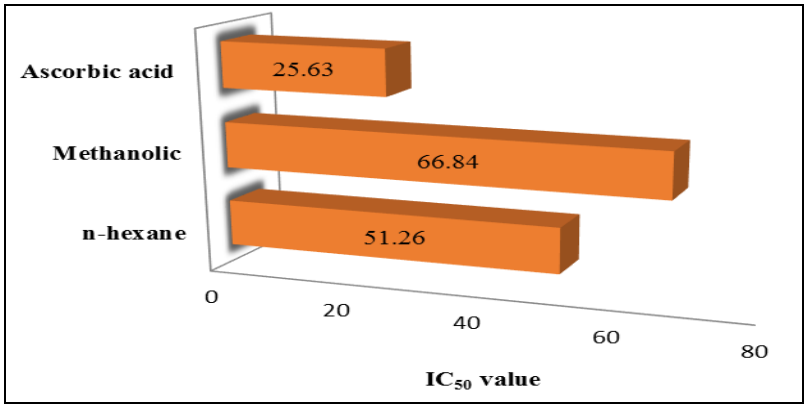


**FIG.2 (ii): $IC_{50}$ VALUE OF METHANOLIC AND N-HEXANE EXTRACTS OF *STERCULIA FOETIDA* L. BARK.**

**TABLE 2: ANTIOXIDANT ACTIVITY OF *STERCULIA FOETIDA* L.**

| | % of scavenging | | | $IC_{50}$ value | | |
|---|---|---|---|---|---|---|
| **Conc. (µg/ml)** | **n-hexane extract.** | **Methanolic extract.** | **Standard Ascorbic acid solution** | **n-hexane extract** | **Methanolic extract** | **Standard ascorbic acid solution** |
| 50 | 48.87 | 47.25 | 34.02 | | | |
| 100 | 57.76 | 55.24 | 47.13 | 51.26 | 66.84 | 25.63 |
| 150 | 67.25 | 64.85 | 53.12 | | | |
| 200 | 73.56 | 72.14 | 64.36 | | | |
| 250 | 80.36 | 81.45 | 71.45 | | | |

**CONCLUSION:** It was found that *Sterculia foetida* L. had a significant inhibitory effect against bacterial strains. The methanolic extracts of this plant showed more antimicrobial activity than n-hexane extract. The inhibitory effect of the extract of *Sterculia foetida* L. against pathogenic bacteria used in the present study indicates that the plant can be a potential candidate for various drug developments for the treatment of aliments caused by most of the pathogens. The n-hexane extract have more activity on *Shigella flexneri* where as its methanolic extract resulted in a better activity on *Salmonella typhi*. Since the n-hexane extract of *Sterculia foetida* registered lowest $IC_{50}$ value, it showed highest antioxidant potential. Further Studies are recommended for the isolation of important chemical constituents which may be specific for the antibacterial and antioxidant activity.

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**CONFLICTS OF INTREST:** All the authors have none to declare.

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