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# In vitro antioxidant and antimicrobial effects of Streblus asper: An ethnomedicinal plant of Assam, India

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**Abstract:** *Streblus asper* Lour, a member of the Moraceae family, is a widely recognized ethnomedicinal plant in India, the Philippines, Malaysia, and Thailand. It is valued for its antimicrobial, antioxidant, anti-inflammatory, and antidiabetic properties. This study investigates the *in vitro* antioxidant and antimicrobial activities of *S. asper* hydroalcoholic extract, validating its traditional use among local communities in Assam. Plant samples, collected from Lakhimpur, Assam, and authenticated by the Botanical Survey of India, underwent extensive phytochemical screening, revealing the presence of cardiac glycosides, triterpenoids, phytosterols, coumarins, and anthocyanins. The antioxidant activity, assessed via the DPPH free radical scavenging method, yielded an IC50 value of 263.84±0.14 µg/ml, indicating significant radical scavenging potential. Antimicrobial efficacy was evaluated using the agar well diffusion method against *Escherichia coli* and *Staphylococcus aureus*, with a minimum inhibitory concentration of 60 µl and maximum activity at 120 µl, comparable to standard antibiotics such as azithromycin and penicillin G. Physicochemical standardization, including loss on drying, ash values, and extractive values, confirmed the extract's quality. These findings support the ethnomedicinal significance of *S. asper*, particularly as a dental stick in Assam for preventing oral ailments. The study emphasizes the plant's potential for developing novel therapeutic formulations and highlights the need for further research to explore its pharmacological applications, aiming to integrate it into modern medicine for societal health benefits.

***Keywords:*** *Antioxidant, Ethnomedicine, Moraceae, Antimicrobial, Streblus asper Lour.*

## 1. Introduction

The human race is mainly dependent upon the plants and herbs for food and medicine. As per reports a total of 7000 species of plants are available throughout the world which are edible. Plants can be a storehouse of energy and wellness in terms of heath benefits because of various phytochemicals and micro as well as macronutrients in them. The traditional use of plants is playing an important role acting as ethnomedicines amongst various tribes and communities. For example, medicinal plants are used as functional meals for medical and replacement needs as well as daily foods in China, Japan, Korea, and other nations [1]. One such plant is the *Streblus asper* Lour from the family Moraceae. The plant is indigenous to India, Philippines, Malaysia, Thailand and Sri Lanka. The plant is believed to have antimicrobial, anti-inflammatory, antioxidant, anti-pyretic, anti-plaque, activities and also used in diarrhoea and dysentery [2-4]. The plant is vastly used in India mostly in the North Eastern states of the country. The local people use the plant as dental sticks to brush the teeth and it helps to get rid of dental problems and periodontal problems. In Assam the plant is called as 'sora' and people believe that the use of this as a dental stick can prevent all the ailments related to teeth and from ancient time it has


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been a reason for the long-lasting teeth for the aged people in the state. The scientists have found that the plant is very rich in cardiac glycosides, triterpenoids as well as phytosterols [5]. The tree has a number of uses. In Thailand it is one of the potential sources for paper making. *Streblus asper* serves as a versatile resource, with sweet edible fruits, leaves for tea and cleaning, medicinal seeds, animal fodder, ornamental potential, valuable timber, and as a source of fuel in the form of firewood and charcoal, mainly in Vietnam.

## 2. Materials and Method

### *2.1. Collection and Authentication*

The plant samples were collected from the Lakhimpur district of Assam and authenticated by the Botanical Survey of India, Eastern Regional Centre, Shillong.

### *2.2. Chemicals*

Chloroform, ethanol, potassium hydroxide, phenolphthalein, sodium hydroxide, dinitrobenzene, glacial acetic acid, ninhydrin, sodium nitroprusside, isopropyl alcohol, ascorbic acid, nutrient agar, and nutrient broth were obtained from the Himedia Laboratories, Mumbai, India. HCl, diethyl ether, Wagner's reagent, resorcinol, barfoed's reagent, fehling's solution A and B, and ferric chloride were obtained from the Thermo Fisher Scientific India Pvt. Ltd. Dragendroff's reagent, sulphuric acid, acetone, millon's reagent, ammonia, iodine, and sodium bicarbonate were received from the Pallav Chemicals and Solvents Pvt. Ltd. Pyridine and DPPH was collected from the Sigma-Aldrich Chemical Co, Mumbai, India. Bromine water was collected from the Oriental Labs Retail Services. Methanol, Benedict's reagent was obtained from the Loba Chemie Pvt. Ltd.

### *2.3. Method*

#### *2.3.1. Extraction of Streblus Asper*

For decoction of the aerial parts of the *Streblus asper* an amount of 100 gm of the raw material was dissolved in 400 ml of distilled water and boiled for 15 minutes to get the aqueous concentrated extract of the plant [6]. Hydro-alcoholic maceration was carried out using a ratio of methanol and water.

#### *2.3.2. Phytochemical Investigation*

Extensive phytochemical investigation was performed on the hydro-alcoholic extract of the plant to check the presence of the primary and the secondary metabolites [2]. The various tests performed are mentioned below:

(a) Tests for alkaloids: Dragendroff's reagent: To 3ml of extract few drops of Dragendroff's reagent was added and observed for the formation of a reddish precipitate. Wagner's reagent: Wagner's reagent (iodine in potassium iodide) of 2 drops was added to 3 ml of extract and observed for the formation of a red or brown precipitate.

(b) Tests for carbohydrate: Resorcinol test: To 2 ml of extract few crystals of resorcinol and equal volume of conc. HCl is added and heated and observed for the formation of rose colour. Barfoed's test: To 1 ml of extract 1ml of Barfoed's reagent is added and heated for 2 minutes and is observed for red precipitate.

(c) Tests for reducing sugar: Fehling's test: 1 ml each of Fehling's solution A and B are added to 1 ml of extract and boiled in water bath and observed for red precipitate. Benedict's test: 0.5 ml of Benedict's reagent is added to 0.5 ml of extract and observed for green or yellow or red colour.

(d) Tests for glycosides: Aqueous NaOH test: Alcoholic extract was dissolved in 1ml of water and to that few drops of Aq. NaOH solution was added, observed for yellow colour. Conc. $H_2SO_4$ test: To 5ml of the plant extract 2ml of glacial acetic acid, a drop of 5% $FeCl_3$ and conc. $H_2SO_4$ is added and observed for formation of a brown ring.

(e) Tests for cardiac glycosides: Bromine water test: To plant extract few ml of bromine water is added and observed for a yellow precipitate. Keller killiani test: To 1 ml filtrate 1.5 ml glacial

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acetic acid, a drop of 5% ferric chloride and conc. $H_2SO_4$ is added and observed for a bluish coloured solution.

(f) Tests for proteins and amino acids: Ninhydrin test: To 2 ml filtrate 2 drops of ninhydrin solution and 200 ml of acetone is added, observed for purple coloured solution. Millon's test: To 2 ml of filtrate few drops of Millon's reagent is added and observed for white precipitate.

(g) Tests for flavonoids: Ammonia test: To the filtrate 5ml of dilute ammonia solution, conc. $H_2SO_4$ are added and observed for yellow colour. Conc. $H_2SO_4$ test: To few ml of plant extract conc. $H_2SO_4$ and observed for orange colour.

(h) Test for Phenolic compounds: Iodine test: To 1 ml of extract few drops of dil. Iodine solution was added and observed for transient red colour. Ferric chloride test: To the extract solution few ml of 5% ferric chloride is added and observed for bluish black colour.

(i) Tests for tannins: Braymer's test: To 1 ml of filtrate 3ml distilled water, 3 drops of 10% ferric chloride solution are added and observed for blue green colour. Bromine water test: 10 ml of bromine water 0.5 gm of plant extract was added and observed for decolouration.

(j) Tests for saponins: Foam test: To 0.5 gm of plant extract 2ml water is added and vigorously shaken and observed for foam. $NaHCO_3$ test: To the plant extract few ml of sodium bicarbonate and distilled water is added and observed for formation of foam.

(k) Tests for phytosterols: Salkowski test: To filtrate few drops of conc. $H_2SO_4$ is added, shaken well and allowed to stand and observed for red colour in lower layer. Hesse test: To 5 ml of extract, 2 ml of chloroform, 2 ml of conc. $H_2SO_4$ are added and observed for the formation of a red layer.

(l) Tests for triterpenoids: Salkowski test: To the filtrate few drops of conc. $H_2SO_4$ is added, shaken well and allowed to stand and observed for appearance of golden yellow colour.

(m) Tests for terpenoids: Conc. $H_2SO_4$ test: To 2 ml of chloroform 5 ml of extract was added and evaporated on water bath, 3 ml of conc. $H_2SO_4$ is added and boiled in water bath, observed for a grey coloured solution.

(n) Tests for quinones: Alcoholic KOH test: To 1ml of extract few ml of alc. KOH is added and observed for red and blue colour. Conc. HCl test: To plant extract conc. HCl is added and observed for a green colour. $H_2SO_4$ test: 10 mg of extract was dissolved in isopropyl alcohol and to that a drop of conc. $H_2SO_4$ is added, observed for a red colour.

(o) Tests for anthraquinones: Borntrager test: 10 ml of 10 % ammonia solution and few ml of filtrate are shaken vigorously for 30 seconds and observed for pink, violet and red colour.

(p) Tests for anthocyanins: HCl test: To 2 ml extract 2 ml of 2N HCl is added and observed for a pink red solution.

(q) Carboxylic acids: Effervescence test: To 1 ml plant extract 1ml of sodium bicarbonate is added and observed for effervescence.

(r) Tests for coumarins: NaOH test: To the plant extract, 10 % NaOH solution, Chloroform is added and observed for yellow fluorescence.

(s) Tests for gums and mucilage: Alcohol test: 100 mg of extract 10 ml of distilled water, 25 ml of absolute alcohol is added and observed for precipitate.

(t) Tests for resins: Turbidity test: To 10 ml of extract 20 ml of 4 % HCl is added and observed for turbidity.

(u) Tests for oils and fat: Saponification: The extract solution is applied on filter paper and checked for soap formation.

*2.4. Physical Standardization*

The crude drug was grinded to powder and different physical standardization tests were performed [7].

(i)Determination of loss on drying: About 2g of the air-dried crude drug was accurately weighed in a dried and tared Petri dish and kept in a hot air oven maintained at 110°c for 4hours. After cooling in a

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desiccator, the loss in weight was recorded. This procedure was repeated until a constant weight was obtained.

$$\%\text{LOD} = \frac{Loss\ in\ weight}{Weight\ of\ the\ drugs\ in\ grams} \times 100$$

(ii)Water soluble extract: About 5g of the coarsely powdered air-dried drug was macerated with 100ml of $CHC_{13}$-$H_2O$ in a closed flask for 24hrs, frequently shaken during the first 6hrs and was allowed to stand for 18hrs. Thereafter it was filtered rapidly and 25ml of the filtrate was evaporated to dryness in a tared flat-bottom shallow dish, dried at 105˚c, and weighed. The percentage of w/w of water-soluble extractive was calculated concerning the air-dried drug.

$$\%\text{Water soluble extractive} = \frac{X-Y}{Z} \times 100$$

Where, X = Final weight of the petri dish, Y = Initial weight of the petri dish & Z = Weight of the drug taken

(iii) Alcohol soluble extract: About 5g of the coarsely powdered air-dried drug was macerated with 100ml of $C_2H_5OH$ in a closed flask for 24hrs, frequently shaken during the first 6hrs and allowed to stand for 18hrs. Thereafter it was filtered rapidly and 25ml of the filtrate was evaporated to dryness in a tared flat-bottom shallow dish, dried at 105˚c, and weighed. The percentage of w/w of alcohol-soluble extractive was calculated.

$$\%\text{Alcohol soluble extractive} = \frac{X-Y}{Z} \times 100$$

Where, X = Final weight of the petri dish, Y = Initial weight of the petri dish & Z = Weight of the drug taken

(iv) Total ash: About 3g of crude powder was taken and weighed in a tared silica dish which was previously ignited and weighed. Scattered the powdered drug at the bottom of the dish and ignited it by gradually increasing the heat up to 550˚C until it is white indicating the absence of carbon. If the carbon-free ash cannot be obtained in this way, the charred mass was exhausted with hot water and residue was collected on an ashless filter paper, incinerated the residue, filter paper, and the filtrate, evaporated to dryness and ignited at a low temperature. The percentage of ash was calculated by incinerating completely the crude powdered drug and comparing its weight with the initial weight of the sample.

$$\%\text{Total Ash} = \frac{X-Y}{Z} \times 100$$

Where, X = Final weight of the petri dish, Y = Initial weight of the petri dish & Z = Weight of the drug taken

(v) Acid insoluble ash: The total ash was boiled with 25ml of 2M HCl for 5mins. The insoluble matter was collected on ashless filter paper, washed with hot water, ignited, cooled in a desiccator, and weighed. The percentage of acid-insoluble as concerning the air-dried was calculated as follows by incinerating the crude powdered drug completely and comparing its weight with the total ash of the sample.

Where, X = Final weight of the petri dish, Y = Initial weight of the petri dish & Z = Weight of the drug taken

(vi) Water soluble ash: The total ash was boiled with 25ml of water for 5mins and the insoluble matter was collected on an ashless filter paper, washed with hot water, and ignited for 15mins at a temperature not exceeding 450˚C. By subtracting the weight of the insoluble part from that of the total ash, weight of the soluble part of ash was obtained.

$$\%\text{Water soluble ash} = \frac{X-Y}{X} \times 100$$

Where, X= Total ash & Y = Water insoluble ash

(vii) Determination of acid value: About 10g of the drug was taken and added to 50ml of $C_2H_5OH$ and $(C_2H_5)2O$ (equal volumes) previously neutralized with 0.1M KOH to phenolphthalein solution. The flask containing the sample was connected with a reflux condenser, warmed slowly with frequent

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shaking until the sample dissolved. 1ml of phenolphthalein solution was added to it and titrated with 0.1M KOH until faint pink appeared. The acid value was determined.

Acid value = $\frac{n}{w} \times 5.61$

Where, n = No. of ml of 0.1M KOH & w = Weight in grams of the substance

(viii) Determination of saponification value: The saponification value was determined by taking 2g of the drug in a tared 250ml of a conical flask, weighed accurately, and added to it 25ml of 0.5N alcoholic KOH. The flask was heated on a steam bath under a suitable condenser to maintain reflux for 30min, frequently rotating the contents. 1ml phenolphthalein was added and titrated the excess KOH with 0.5N HCl. A blank determination was performed under the same conditions, The difference between the blank and the test reading gives the number of ml of KOH required to saponify 1g fat.

Saponification value= $\frac{(B-T)\times 28.05}{W}$ in mg $\frac{KOH}{1g}$

Where, B = Blank, T = Test & W = Weight of the drug in gm

(ix) Determination of ester value: The ester value is the number of mg of KOH required to saponify the esters in 1g of a sample.

Ester value = Saponification value – Acid value

(x) Determination of foaming index: About 1g of the drug was taken in a 500ml conical flask containing 100ml of boiling water. This was allowed to moderately boil for 30min, cooled, filtered into a 100ml volumetric flask., and added a sufficient amount of water through the filter to dilute to the volume. The decoction was poured into 10 stoppered test tubes of height 16cm and diameter 16mm in successive portions of 1ml, 2ml, 3ml, 4ml, 5ml, 6ml, 7ml, 8ml, 9ml, 10ml. Stoppered test tubes were shaken through in a lengthwise motion for 15sec, 2shakes per second, and allowed to stand for 15min. The height of the foam was measured. The height of the foam was measured. The volume of the plant material decoction was determined.

$$\text{Foaming index} = \frac{1000}{a}$$

- If the height of the foam in every tube is less than 1cm, the foaming index is less than 100.
- If height of foam of 1cm is measured in any tube, the volume of the plant material decoction in this tube(a) is used to determine the index. If this tube is the 1st or 2nd tube in a series, then we need to prepare an intermediate dilution in a similar manner to obtain more precise result.
- If the height of the foam is more than 1cm in every tube, the foaming index is over 1000 which is not possible and the determination should be repeated using a new series of dilution of the decoction to obtain a result.

*2.5. Antioxidant Activity Using DPPH Method*

Chemicals preparation: 4 mg of 2, 2-diphenylpicrylhydrazyl (DPPH) was dissolved in 100 ml of methanol to make the concentration 40 µg /ml. The working solution was obtained by diluting DPPH solution with methanol to attain an absorbance of about 0.98±0.02 at 517 nm using the spectrophotometer. 18 mg of ascorbic acid (used as standard) was dissolved in 2 ml of methanol to make the concentration 9 mg/ml.

Ascorbic acid standard curve was prepared by following the methods of published reports with modifications [6-8]. From the stock solution (1 mg/ml), different working solutions were prepared as: 10, 150, 350 and 500 µg/ml concentrations by using methanol for dilution. It was followed by adding DPPH (as given in the following Table 1) and then incubation in dark condition for 5 minutes. The coloured solution was then measured for absorbance using UV-VIS spectrophotometer at 517 nm.

The samples provided were dried to powdered form using a hot air oven. Then weighed 1 mg of the powdered sample and dissolved in 1 ml of solvent (methanol/chloroform). From the stock solution (1 mg/ml), different working solutions will be prepared as: 10, 150, 350 and 500 µg/ml concentrations by using methanol for dilution. 1.5 ml of each diluted sample was taken in different test tubes followed by addition of 1.5 ml DPPH (as given in the following Table 2). The final volume of all the test tubes was

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made up to 3 ml with methanol. After that all the test tubes were incubated for 5 minutes in dark condition at room temperature. Finally, absorbance for all the tubes was measured at 517 nm.
The percentage inhibition was calculated using equation-
% DPPH scavenging effect or % inhibition = 100 x (A control – A sample)/A control)

$IC_{50}$ values were estimated from the % inhibition versus concentration sigmoidal curve, using a non-linear regression analysis.

*2.6. Antimicrobial Activity Study*

There are different techniques for evaluating the in-vitro antimicrobial activity, like Agar disk diffusion method, Antimicrobial gradient method, Agar well diffusion method, Agar plug diffusion method, Cross steak method etc. Here we have used the agar well diffusion method for the antimicrobial activity [9]. All the apparatus used in the experiment were sterilized first in the autoclave. Along with the apparatus the nutrient agar solution was also placed in the autoclave for sterilization. All the apparatus and the agar solution were then transferred into the laminar air flow. Inside the laminar airflow the nutrient agar solution was poured into the petri-dish and the UV is turned on placing all the apparatus along with the agar plates. The hardened agar plates are transferred into the incubator at 37° C for 24 hours to check if there is any bacterial growth to confirm sterility. Again, in the laminar air flow the bacterial inoculum is spread over the agar plate. With a sterile cork borer 4 holes were made to apply different concentrations of the sample, 30µl, 60 µl, 90 µl and 120 µl. With the help of a micropipette the different concentrations were poured into the wells. Two microbial strains *E coli* and *Staphylococcus aureus* are used to check the activity. At the centre the standard antibiotic i.e. azithromycin for *E coli* and penicillin G for *Staphylococcus aureus* are used. The Petri dish is then covered with the cover and sealed with the parafilm to prevent any contamination. The dish is transferred into the incubator to incubate at 37° C for 24 hours. After 24 hours the plate is taken out and the zone of inhibition or diameter is measured for different concentrations. Activity indices for different concentrations are found out and the MIC (Minimum inhibitory Concentration) is found out.
Activity index is measured using the following formula-

$$\text{Activity index} = \frac{\text{Zone of inhibition by sample}}{\text{Zone of inhibition by standard}}$$

## 3. Results and Discussion

*3.1. Phytochemical Analysis*

From the phytochemical analysis of the plant extract it has been found that *Streblus asper* is a rich source of cardiac glycosides, triterpenoids, sterols, coumarins and anthocyanins.

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**Table 1.**
The phytochemical analysis results of *Streblus asper*.

| Sl. No. | Metabolites | ME (Methanolic Extract of Streblus asper) |
|---|---|---|
| 1 | Alkaloids | Negative |
| 2 | Carbohydrate | Negative |
| 3 | Reducing sugar | Negative |
| 4 | Phlobatannins | Negative |
| 5 | Glycosides | Negative |
| 6 | Cardiac Glycosides | Positive |
| 7 | Oils and Fats | Negative |
| 8 | Proteins and Amino acids | Negative |
| 9 | Flavonoids | Negative |
| 10 | Phenolic Compounds | Negative |
| 11 | Resins | Negative |
| 12 | Tannins | Negative |
| 13 | Saponins | Negative |
| 14 | Phytosterols | Positive |
| 15 | Gums and Mucilage | Negative |
| 16 | Triterpenoids | Positive |
| 17 | Terpenoids | Negative |
| 18 | Quinones | Negative |
| 19 | Anthraquinones | Negative |
| 20 | Carboxylic Acids | Negative |
| 21 | Coumarins | Positive |
| 22 | Anthocyanins | Positive |

Physical standardizations: The results of different physical standardisation tests are given in the table below-

**Table 2.**
Results of physical standardization.

| Sr. no | Parameters | Mean values |
|---|---|---|
| 1 | Total ash | 16.67% |
| 2 | Acid insoluble ash | 4% |
| 3 | Water soluble ash | 16% |
| 4 | Water soluble extractive value | 2.20% |
| 5 | Alcohol soluble extractive value | 14.06% |
| 6 | Loss on drying | 10% |
| 7 | Foaming index | Less than 100 |
| 8 | Saponification value | 53.3 |
| 9 | Acid value | 0.28 |
| 10 | Ester value | 53.02 |


**Figure 1.**
(a), (b), (c) & (d) are the Physical standardizations.

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*3.2. Antioxidant Activity*

The antioxidant activity is tested for the sample using the DPPH free radical scavenging activity and the percentage inhibition values are listed in the table 7 and the $IC_{50}$ value of *Streblus asper* (GR) is found to be 263.84±0.14.

**Table 3.**
Preparation of ascorbic acid standard.

| Sl No. | Sample(mg/ml) | | Methanol(ml) | Add 1.5ml of DPPH | Incubation in dark for 5mins at RT and the absorbance taken at 517nm |
|---|---|---|---|---|---|
| | µg/ml | µl | | | |
| 1 | 0 | 0 | 1.5 | | |
| 2 | 10 | 3 | 1.497 | | |
| 3 | 150 | 50 | 1.45 | | |
| 4 | 350 | 116 | 1.384 | | |
| 5 | 500 | 166 | 1.334 | | |

**Table 4.**
Sample preparation.

| Sl No. | Sample(mg/ml) | | Methanol (ml) | Add 1.5ml of DPPH | Incubation in dark for 5mins at RT and the absorbance taken at 517nm |
|---|---|---|---|---|---|
| | µg/ml | µl | | | |
| 1 | 10 | 30 | 1.47 | | |
| 2 | 150 | 450 | 1.05 | | |
| 3 | 350 | 1.05 | 0.45 | | |
| 4 | 500 | 1.5 | - | | |

**Table 5.**
$IC_{50}$ values of the tested sample.

| Sl. No | Sample Id | $IC_{50}$(µg/ml) |
|---|---|---|
| 1. | GR | 263.84±0.14 |

**Table 6.**
Preparation of standard curve of ascorbic acid.

| Sl No | Ascorbic acid (µg /ml) | Absorbance | | | | |
|---|---|---|---|---|---|---|
| | | R1 | R2 | R3 | Mean | Standard Deviation |
| 1 | 0 | 0.442 | 0.412 | 0.415 | 0.423 | 0.016523 |
| 2 | 10 | 0.136 | 0.114 | 0.116 | 0.122 | 0.012166 |
| 3 | 150 | 0.068 | 0.051 | 0.052 | 0.057 | 0.009539 |
| 4 | 350 | 0.058 | 0.049 | 0.052 | 0.053 | 0.004583 |
| 5 | 500 | 0.049 | 0.048 | 0.049 | 0.048667 | 0.000577 |

**Table 7.**
Calculation for $IC_{50}$ Value of the sample.

| Samples | Equation | $IC_{50}$ concentration | Standard Deviation (SD) | $IC_{50}$±SD |
|---|---|---|---|---|
| GR | y=0.1182x+18.814 | 263.84 | 0.14 | 263.84±0.14 |

**Table 8.**
% DPPH scavenging activity of ascorbic acid and the test samples at different concentrations

| Concentration of sample (ug/ml) | %Ascorbic acid | %GR | $IC_{50}$ value |
|---|---|---|---|
| 10 | 71.15839243 | 6.382978723 | 50 |
| 150 | 86.5248227 | 52.4822695 | 50 |
| 350 | 87.47044917 | 67.49408983 | 50 |
| 500 | 88.49487786 | 68.321513 | 50 |

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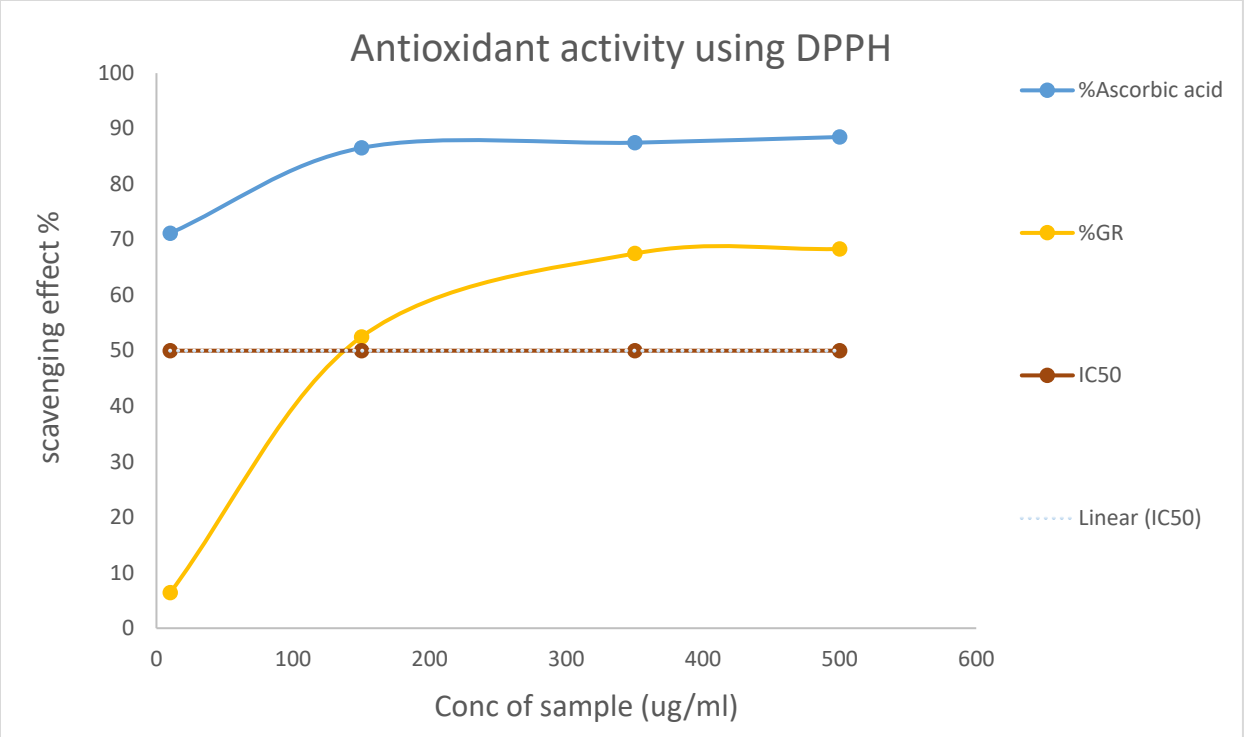
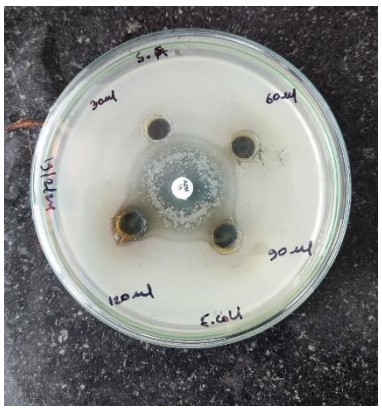



**Figure 2.**
Graphical representation of the DPPH free radical scavenging activity of Streblus asper hydroalcoholic extract compared to ascorbic acid standard, showing percentage inhibition at various concentrations (10, 150, 350, and 500 µg/ml) with an IC50 value of 263.84±0.14 µg/ml.

### *3.3. Antimicrobial Study*

The antimicrobial study was performed using well diffusion method with two bacterial strains that are *E coli* and *Staphylococcus aureus* and the activity index was found out. The MIC (Minimum inhibitory concentration) in both the bacterial strains are found to be 60 µL. The inhibition zone of standard antibiotic azithromycin was 2.9 cm and was compared with the different concentrations of the sample and the MIC is found to be 60 µl and the maximum activity was shown at 120 µl.




(a)

(b)

**Figure 3.**
(a) & (b) are the Zone of inhibition of standard and the sample in *E coli*.

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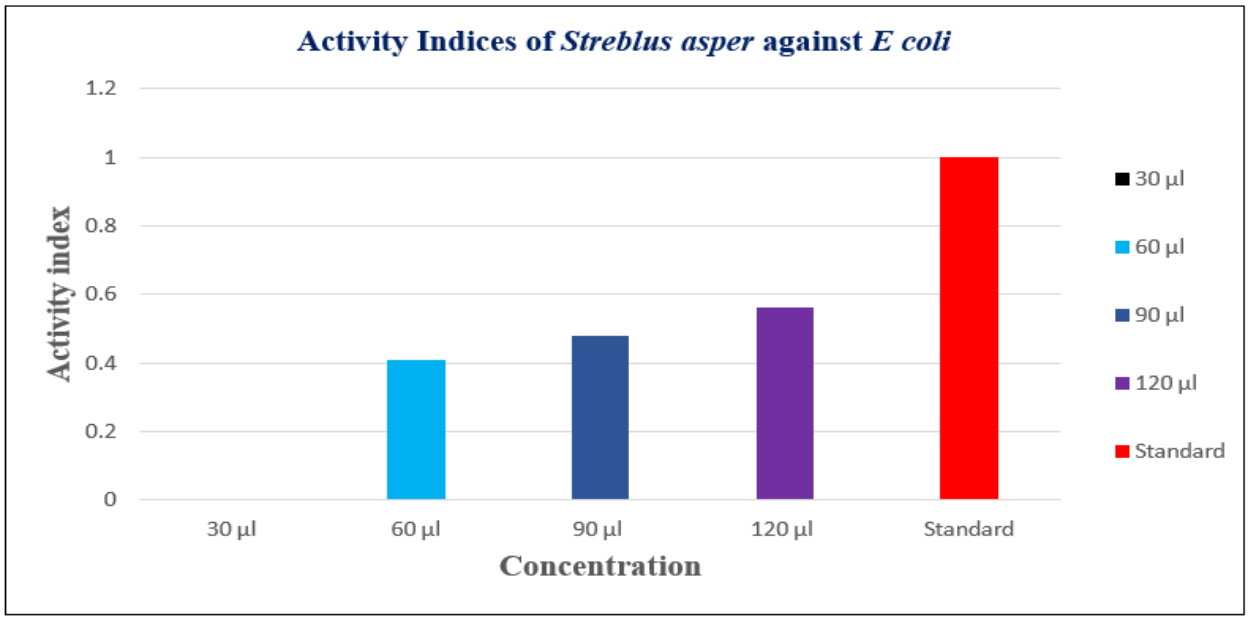
**Table 9.**
Activity indices of different concentrations against *E coli.*

| Sl. No. | Sample | Concentration | Diameter | Activity Index |
|---|---|---|---|---|
| 1 | Hydro-Alcoholic Extract of *S. asper* | 30 µl | 0 cm | 0 |
| 2 | | 60 µl | 1.2 cm | 0.41 |
| 3 | | 90 µl | 1.4 cm | 0.48 |
| 4 | | 120 µl | 1.5 cm | 0.56 |



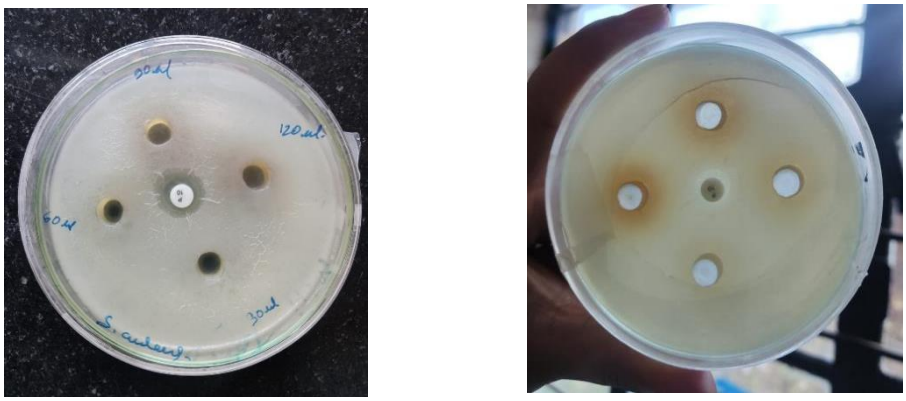



**Figure 4.**
(a) & (b) are Zone of inhibition of standard and the sample in *Staphylococcus aureus.*

**Table 10**.
Activity indices of different concentrations against *Staphylococcus aureus.*

| Sl. No. | Sample | Concentration | Diameter | Activity Index |
|---|---|---|---|---|
| 1 | Hydro-Alcoholic Extract of *S. asper* | 30 µl | 0 cm | 0 |
| 2 | | 60 µl | 1.2 cm | 0.86 |
| 3 | | 90 µl | 1.6 cm | 1.33 |
| 4 | | 120 µl | 1.9 cm | 1.58 |

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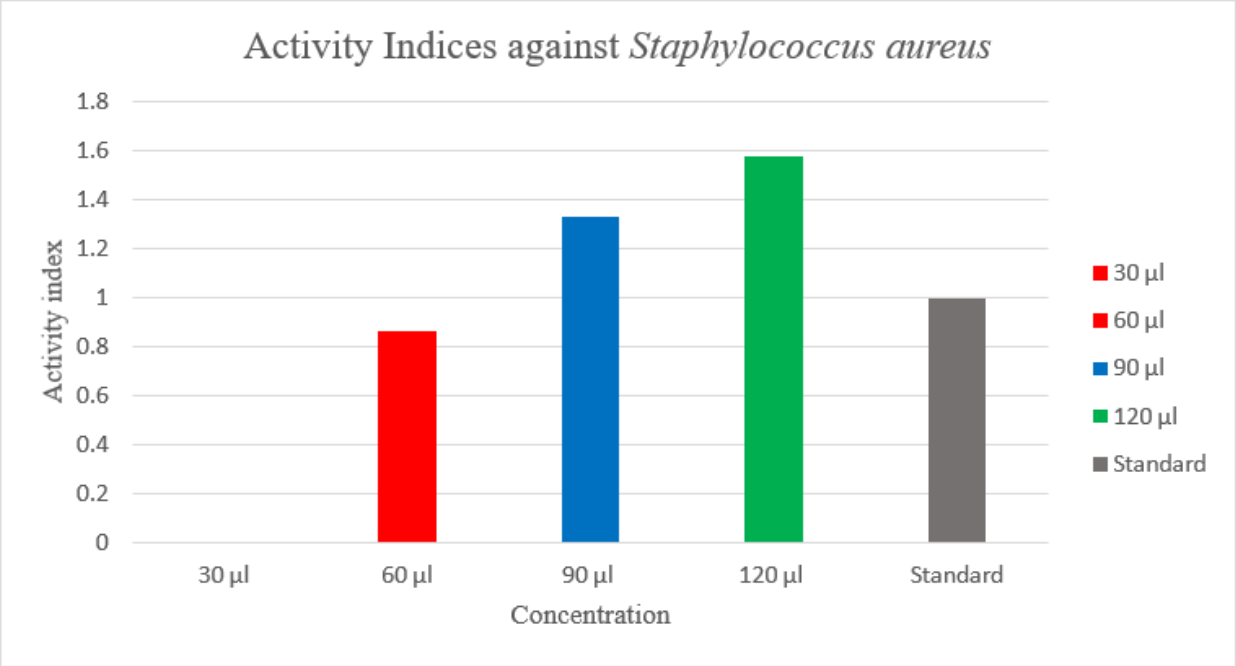


**Figure 5.**
Illustration of the antimicrobial activity of *Streblus asper* hydroalcoholic extract, depicting zones of inhibition for different concentrations (30 µl, 60 µl, 90 µl, and 120 µl) against *Escherichia coli* and *Staphylococcus aureus*, alongside standard antibiotics (azithromycin for E. coli and penicillin G for S. aureus), measured via the agar well diffusion method.

## 4. Conclusion

It can be concluded from the study that the *Streblus asper* Lour is a pharmacologically active plant and the extract is having promising antioxidant and antimicrobial properties as the results suggest. Further study on the plant is important to reveal its medicinal and therapeutic properties so that it can be used for formulations for curing various ailments to serve the society.

## Transparency:

The authors confirm that the manuscript is an honest, accurate, and transparent account of the study; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained. This study followed all ethical practices during writing.

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