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Antioxidant and Antimicrobial effects of the *Streblus asper* Lour

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ABSTRACT:

The *Streblus asper* Lour belonging to the family Moraceae is very popular in countries like India, Philippines, Malaysia, Thailand. It is a well-known ethnomedicine amongst the local tribes and communities of these countries. It is known for its promising antimicrobial, antiplaque, antihyperlipidemic, antioxidant, antidiabetic, anti-inflammatory, antipyretic properties. This study shows the evidence of promising actions based on the outcomes of the antimicrobial screening and antioxidant study performed using the extract. The physicochemical tests have been performed and the extensive phytochemical screening shows the presence of different metabolites in it. In the antimicrobial study the hydroalcoholic extract was found effective towards *E coli* and *Staphylococcus aureus.* The results of the study validate the traditional belief on the plant.

Keywords: *Streblus asper* Lour, Moraceae, Antimicrobial study, Ethnomedicine

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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 as daily foods in China, Japan, Korea, and other nations (Sivamaruthi et al., 2022).

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 diarrhea and dysentery (Ibrahim et al., 2013, Kumar et al., 2012, Sripanidkulchai et al. 2009). 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 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, triterpinoids as well as phytosterols (Zhu et al., 2019). 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.

1. Materials and Method

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, Shillonng.

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 were collected from the Oriental Labs Retail Services. Methanol, Benedict's reagent were obtained form the Loba Chemie Pvt. Ltd.

Methods

Extraction of *Streblus asper*

For maceration of the aerial parts of the *Streblus asper* an amount of 100 gm of the powdered raw material was dissolved in 400 ml of methanol and kept for 72 hours with. (Tandon et al., 2008).

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 (Shaikh, Patil, 2020).

(a) **Tests for alkaloids:** Dragendroff's reagent: To 3ml of extract few drops of Dragendroffs 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 killani test: To 1 ml filtrate 1.5 ml glacial 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 triterpinoids:** 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 terpinoids:** 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 fluroscence.

(s) Tests for gums and mucilages: 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.

Physical standardizations

The crude drug was grinded to powder and different physical standardization tests were performed (Madhavan et al., 2009).

Determination of loss on drying: About 2g of the air-dried crude drug was accurately weighed in a dried and tared petridish and kept in a hot air oven maintained at 110˚c for 4hours. After cooling in a desiccator, the loss in weight was recorded. This procedure was repeated until a constant weight was obtained.

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

Water soluble extractive: 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

Alcohol soluble extractive: 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

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 = Weight of the dish + Ash (after incineration)
Y = Weight of the empty dish
Z = Weight of the powdered drug taken

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.

$$\%\text{Acid insoluble ash} = \frac{X-Y}{Z} \times 100$$

Where, X = Weight of the dish + Ash (after complete incineration)
Y = Weight of the empty dish
Z = Total ash

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

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 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.

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

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

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.

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

Where, B = Blank

T = Test
W = Weight of the drug in gm

Detremination 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

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.

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.

Preparation of ascorbic acid standard curve: Ascorbic acid standard curve was prepared by following the methods of published reports with modifications (Stanvoic et al. 2014; Martinez et al. 2020; Nooman et al. 2008). 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.

Sample preparation: 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 made

upto 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)
IC50 values were estimated from the % inhibition versus concentration sigmoidal curve, using a non-linear regression analysis.
*Here the absorbance of the control was found to be 0.423 ± 0.016523.

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 evaluation (Nalawade, Bhat, Sogi, 2016).
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 was 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 petridish 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,

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

2. Results and Discussion

Phytochemical analysis: The phytochemical analysis of the plant extract it has been found that *Streblus asper* is a rich source of cardiac glycosides, triterpinoids, sterols and lignans.

Sl. No.	Metabolities	MECC (Methanolic Extract of *Curcuma caesia*)
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 Mucilages	Negative
16	Triterpenoides	Positive
17	Terpenoides	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 1: 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

Fig 1(a) Fig 1(b) Fig 1(c)

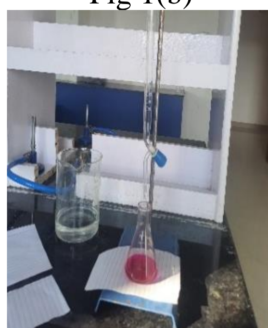


Fig 1(d)

Fig 1(a), (b), (c), (d): Physical standardizations

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 2: Preparation of ascorbic acid standard curve

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

Table 3: Sample preparation

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

Table 4: IC50 values of the tested sample

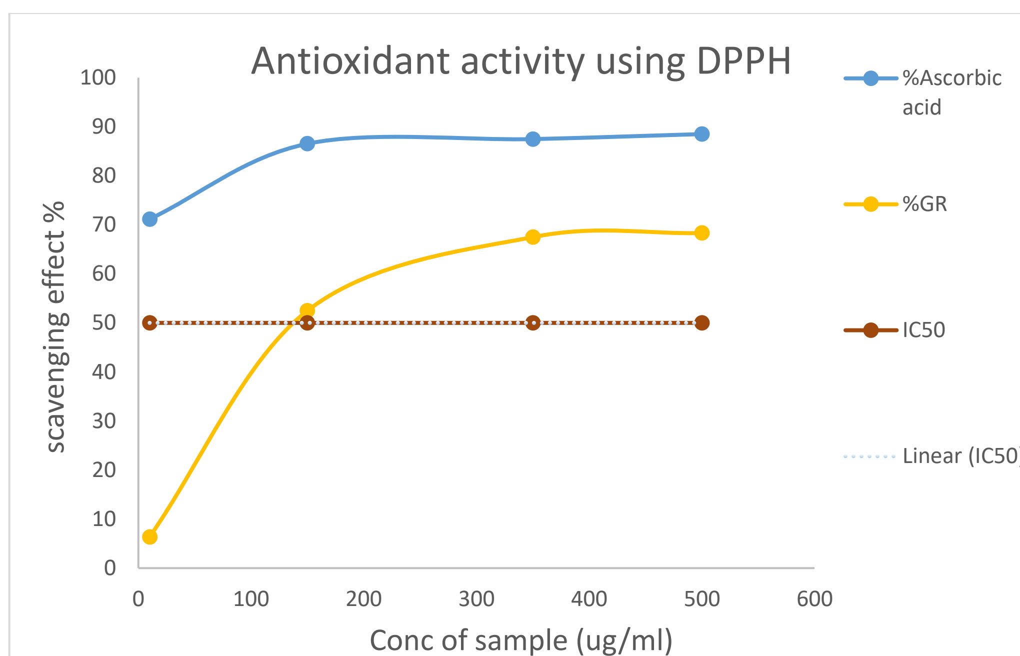
Sl No	Sample Id	Equation	IC_{50}(μg/ml)
1.	GR	y=0.1182x+18.814	263.84±0.14

Table 5: Preparation of standard curve of ascorbic acid

Sl No	Ascorbic acid (ug/ml)	Absorbance				
		R1	R2	R3	Mean	SD
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 6: % DPPH scavenging activity of ascorbic acid and the test samples at different concentrations

Conc of sample (ug/ml)	%Ascorbic acid	%GR
10	71.15839243	6.382978723
150	86.5248227	52.4822695
350	87.47044917	67.49408983
500	88.49487786	68.321513



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.

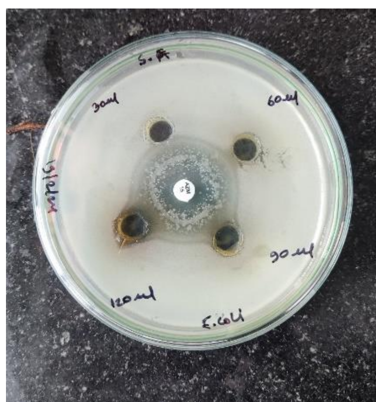


Fig 2(a)

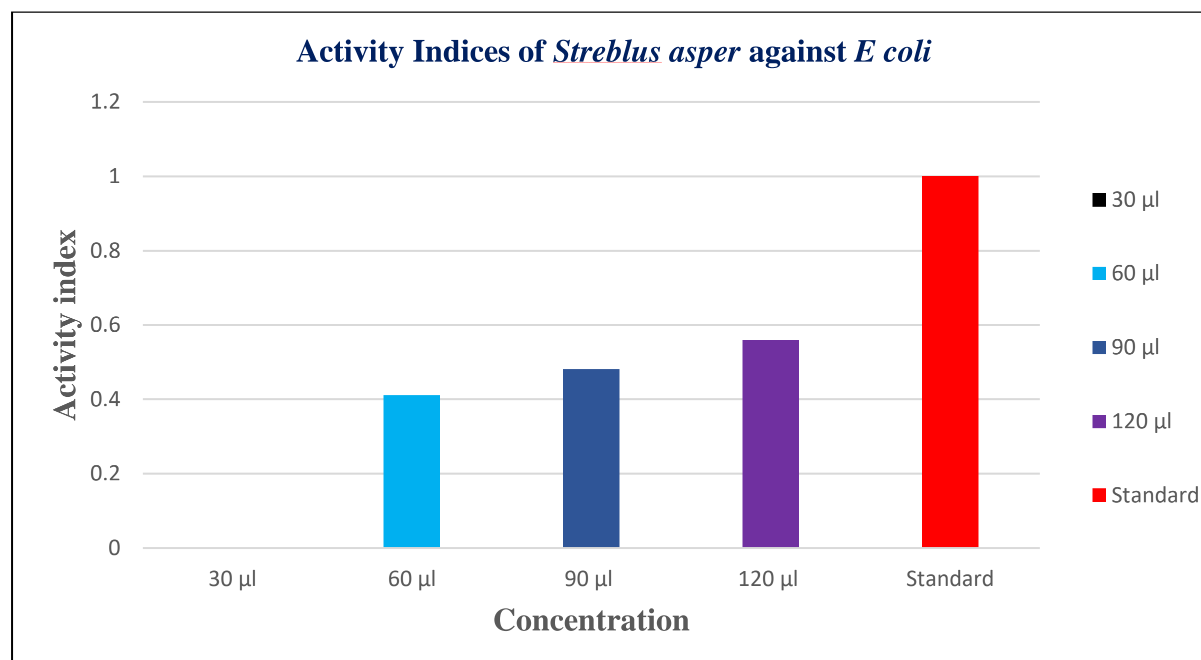
Fig 2(b)

Fig 2(a),(b): Zone of inhibition of standard and the sample in *E coli*

Table 7: Activity indices of different concentrations of sample 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

The inhibition zone of standard antibiotic azithromycin (25 mcg) 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.



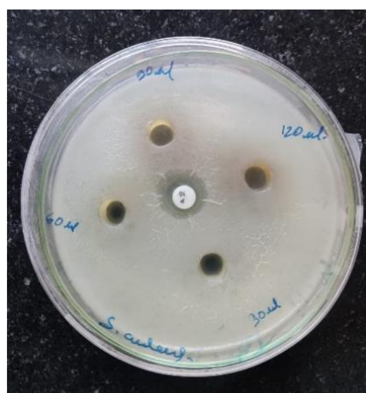


Fig 3(a)

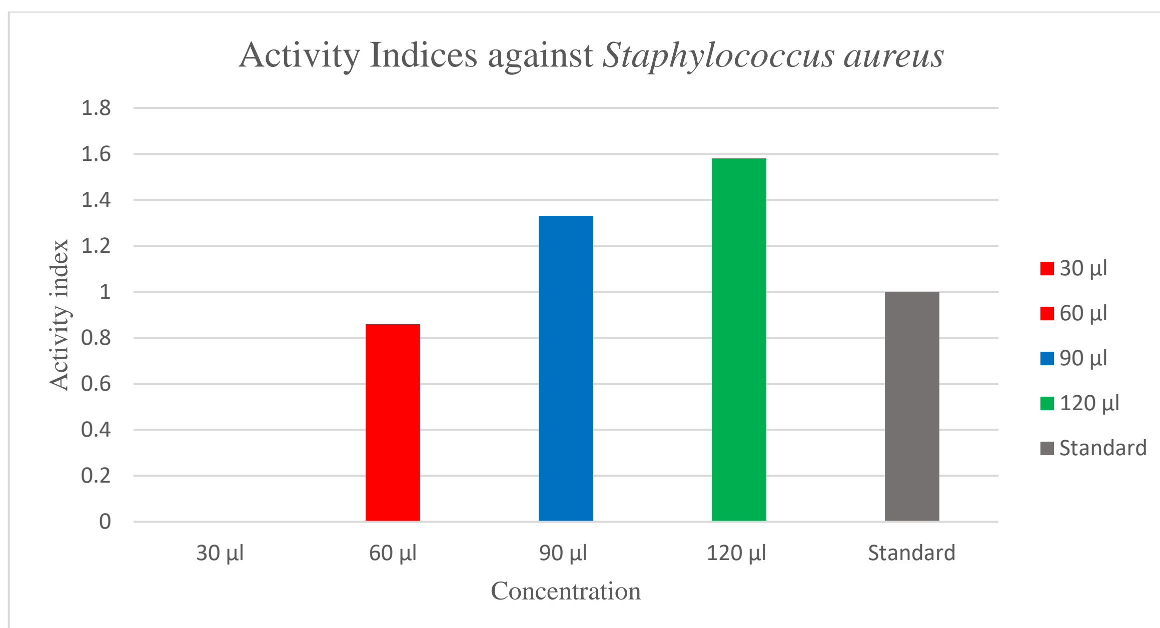
Fig 3(b)

Fig 3(a), (b): Zone of inhibition of the standard and the sample in *Staphylococcus aureus*

Table 8: Activity indices of different concentrations of sample 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

The inhibition zone of the standard antibiotic penicillin G (10 mcg) was found to be 1.2 cm and was compared with the different concentrations of the sample and the MIC was found to be 60 µl and the highest activity was at 120 µl.



3. Conclusion

It can be concluded from the study that the *Streblus aspr* 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 curing various ailments to serve the society.

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