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Phytochemical Analysis and Evaluation of Antimicrobial Activity of *Senna alata* Linn. Flower Extract

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ABSTRACT

The aim of the present work is to evaluate the antimicrobial potential of *Senna alata (*Linn*).* flower extract. The flowers of *Cassia alata* (Linn) were subjected to phytochemical analysis, which identified several bioactive substances with potential antibacterial effects. Infections brought on by these bacteria can be treated using plant parts, as evidenced by the effects of water, acetone, and ethanol extracts on some pathogenic *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Bacillus subtilis*. Its usage in conventional medicine to treat skin, urinary tract, and gastrointestinal infections attests to the efficiency of the crude extracts at high temperatures and at pH levels close to neutrality. The appearance of resistance to the majority of the antimicrobial agents and the high costs of treatments as a result of this resistance, the pursuit of novel, risk-free, effective, and affordable strategies for the treatment of infectious diseases are some of its limitations. The various uses for this medicinal plant are skin problems brought on by germs, fungi, etc. The Ethanolic extract of *Senna alata* had outstanding effectiveness in antimicrobial sensitivity testing, and the majority of these organisms are part of the skin's normal flora. Eczema, pruritus, itching, as well as other skin problems are treated with decoctions of leaves, flowers, bark, and wood in the Indian medical systems of Ayurveda, Siddha, and Unani. Based on the statement, *S. alata* leaves contain a variety of phytochemicals that are essential for use in medicine, including alkaloids, saponins, flavonoids, terpenoids, tannins, anthraquinones, carbohydrates, and protein.

INTRODUCTION:

The botanical name of *Cassia alata is Cassia Senna alata*, and it is a member of the *Fabaceae family.* Takoma is its local Sindhi name, and *Cassia alata* is its Urdu name. This species of *Senna* is an annual shrub that can grow up to three feet tall. *Senna* has yellow flowers that develop into seedpods and fruits.

Geographical Distribution:

Senna alata is widely distributed in Ghana, Brazil, Australia, Egypt, India, Somalia, Sri Lanka, and all over Africa. It is an ornamental plant native to the Amazon Rainforest. Like other *Senna* species, it is cultivated in humid and tropic regions of Africa, Asia, West Indices, Mexico, Australia, South America, the Caribbean Islands, Polynesia, Hawaii, Melanesia, and different parts of India. In Philippines, Thailand, and Indonesia, this shrub is cultivated for medicinal shrub is widely dispersed and is cultivated for medicinal purposes. *Senna* contains many chemicals called Sennosides. Sennosides irritate the lining of the bowel, which causes a laxative effect.

The medicinal plants, a range of medications could be made and different plant parts have been employed for drug production since ancient times. For human primary health, developing nations rely on about 80% of individually used prepared plant-based medicine.

The ethanol extracts of leaves, flowers, stems and root barks of *Cassia alata* showed a broad spectrum of anti-microbial activity. The activity was increased on fractionation (petrol, dichloromethane, ethyl acetate), the dichloromethane fraction of the flower extract being the most effective.

They are effective in treating infectious infections while reducing many of the negative effects that are frequently associated with traditional antibiotics. *Senna* have been reported the chemicals being isolated on *Cassia alata* flowers hot ethanol solvent was used to extract the flowers of *Cassia alata.* The resulting extract was combined with various silica before being further dried.

The pharmacological effects of *Cassia alata* were listed as being antidiabetic, choleretic, analgesic, antibacterial, antiviral, antiulcer, hepatoprotective, depressive, antimalarial, and anthelmintic, as well as cardiovascular and anaesthetic. In terms of clinical medicine, the plant has not been known to have any negative effects. *Senna alata* contains major

pharmacologically important bioactive compounds including phenolics (chrysophenol, emodin, rhein, aloe-emodin, kaempferol and their glycosides), fatty acids (palmitic, oleic, linoleic acids), terpenoids (β-sitosterol, stigmasterol, campesterol) and anthraquinones (e.g. alatonal, alatinone).

The phytochemical study of *Senna alata* contains alkaloids, flavonoids, tannin, phenolic, saponin, steroidal, amino acid and proteins. These chemical constituents are studied by different types of extractions like maceration and percolation. In maceration two solvents are used that ethanol (99.9%) and acetone and percolation process also using ethanol solvent.

In extraction process of maceration and percolation, maceration in ethanol extraction using 15gms of fresh flowers in 50ml ethanol and rest it for 7 days without any disturbance, after collected the filtrate and acetone extraction process using as well as 15gms of fresh flowers in 50ml acetone and rest it for 7 days without any disturbance, after collected the filtrate. Percolation process using ethanol extraction by using 15gms of fresh flowers in 100ml of ethanol (99.9%) in conical flask for 4 hours and transfer it into percolator and added ethanol for covering the material, after 24 hours the filtrate was collected.

The percolation process of *Senna alata* was accurately weighed about 15gms and transferred to a beaker and added 100ml of ethanol and keep it aside for 24 hours. After 24 hours filtered and collected the filtrate.

Antibacterial activity was determined in *Senna alata* using 2 types of gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*) and 2 types of gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*) by Disc diffusion method. The test extractions used different concentrations of 500µg, 1000µg, and 2000µg. The activity finds in using of Mueller Hinton Agar (MHA) medium.

The bacterial activity in *Senna alata* extraction is compared to a standard drug (streptomycin 20µg) the activity is confirmed in clear zone around the disc was measured and expressed in millimetres.

PLANT PROFILE:

Botanical details:

Family	: *Fabaceae.*
Sub family	: *Caesalpinioideae*.
Binomial Name	: *Senna alata*.
Common Name	: candelabra brush, Christmas candle.
Genus	: *Senna*.
Foliage	: Yellow.
Origin	: Tropical South America (France, Columbia, Brazil).
Height	: 2-4 metres.
PH	: 5.5-6.5
Tribe	: Cassieae.
Growing condition	: Red loam soil, Alluvial loam soil, Rich clayed Rice fields.

Medicinal uses

- Senna Leaves - Helps to treat Rheumatoid arthritis, abdominal warms, and Gout.
- Senna Flower - Helps in the treatment of skin disease, Urinary Tract Disorder, Ulcer, and Liver disease.
- Senna Seed - Treats constipation and purpose for clean bowel syndrome.

MATERIALS AND METHODS:

Extraction:

Phytochemical Screening of Different extracts viz.., acetone, ethanol, and aqueous prepared after successive extraction methods were phytochemically screened by following standard

methods. All extracts were screened for different types of phytochemical groups such as alkaloids, flavonoids, phenols, tannins, proteins, saponins, steroids, amino acids and proteins.

Maceration:

The process was carried out by soaking in ethanol and kept for 24 hours and filtrated.

Ethanol extraction

Weigh about 15gms of fresh leaves was taken in beaker and added 50ml of ethanol and shaken it every 15min for 24hrs and kept it as side for 7 days. After 7 days the extraction is filtered in filter paper and separate the filtrate.

Aqueous extraction

Weigh about 15gms of fresh leaves was taken in beaker and added 50ml of water and shaken it every 15min for 24hrs and kept it aside for 7 days. Before 7 days the extraction was get contaminated.

Acetone extraction

Weigh about 15gms of fresh leaves was taken in beaker and added 50ml of acetone and shaken it every 15mins for 24hrs and kept it as side for 7 days. After 7 days, the extraction is filtered by using filter paper and separated the filtrate.

Percolation extraction

The percolation is the most common procedure for the preparation of tinctures and fluids extracts. The percolator is a conical vessel with a top opening in which is placed a circular drilled lid allowing the passage of liquid and subjecting the materials placed on it with slight pressure. The bottom has an adequate closure to allow passage of the fluid at a convenient rate. The *Senna* flowers weighed amount of 15gms is moistened prior to their placement in the percolator with a proper amount of 100ml of ethanol (99.9%), it's placed in a sealed container and left to stand for approximately four hours. After that time the *Senna* flowers were conveniently placed in the percolator so as to allow the even passage of fluid and complete contact with the plant material. The percolator was filled with solvent (99.9%) and covered up. The bottom outlet is opened until gets a regular dripping and then closes. More menstrum 50ml of ethanol 99.9% is added to cover all the materials and kept stand to soak in the percolator closed for 24 hours. After this time leave it to drip slowly and added enough

menstruum to a proportional volume of ¾ of the total volume required for the final product. The wet mass is pressed to extract the maximum residual fluid retained and supplemented with sufficient menstruum to get the proper proportion of chemical constituents in the filtrate.

Fig:1. Percolation of *Senna alata.*

PHYTOCHEMICAL TESTS

1. Alkaloid tests: -

Mayer's reagent test:

The filtrate after treating the extract with dilute hydrochloric acid was mixed with Mayer's reagent. Cream-colour precipitate formation shows that alkaloids are present in the test sample.

Dragondorff's test:

Dragondorff's reagent was added to filtrate of sample. Reddish brown coloured precipitate for formation indicates that alkaloid present in the test sample.

Wagner's reagent test:

Few drops of Wagner's reagent were added to the filtrate, formation of reddish brown precipitate formation indicates that alkaloids were present in the test sample.

Hager's reagent test:

Hanger's reagent was added to filtrate. Formation of yellow coloured precipitate shows that alkaloids are present in the test sample.

2. Flavonoid tests: -

Tollen's reagent test:

Test sample was treated with 2 ml of Tollen's reagent. Appearance of silver mirror shows that Flavonoids were present in the sample.

Ferric chloride test:

2-3 drops of ferric chloride solution was added to the test solution, appearance of dark green colour shows that flavonoid were present in the test drug.

3. Tannins and phenolic tests: -

Lead acetate test:

10% lead acetate solution was added to the test sample. Formation of white precipitate shows that phenolic compounds are present in the sample.

Ferric chloride test:

5% Fecl3 solution was added to the test solution. Formation of intense green colour shows that phenolic group is present in the test drug.

Gelatin test:

10% gelatin solution was added to the test solution, formation of white precipitates shows that phenolic compounds are present in the test sample.

4. Glycosides test (Test for cardiac glycosides):-

Keller-killani test:

Test solution was treated with 2ml of CH_3COOH containing in $FeCl_3$ in H_2SO_4, change of reddish-brown colour to blue colour shows that Cardiac Glycosides are present in the test sample.

Legal's test:

In this test pyridine (2 ml) and alkaline sodium nitroprusside solution (2 ml) was added to the solution of test drug. Formation of blood red colour indicates those cardiac glycosides are present in the test drug.

Baljet's test:

Test solution was treated with 2 ml of picric acid solution. Orange colour formation shows cardiac glycosides are present in the test sample.

5. Anthraquinone glycoside tests: -

Borntrager's test:

Test drug was boiled with H_2SO_4 solution (1 ml) for 5minutes in a test tube. Filter and filtrate were mixed with organic solvents like ether (or) chloroform. Organic layer was separated and shaken with dilute NH_3 solution. Formation of pink to red colour indicates that anthraquinone glycosides are present in the test drug.

Modified Borntrager's test:

Test solution was boiled with H_2SO4 solution (1 ml) for 5 minutes with the solution of 5% $Fecl_3$ followed by shaking with same volume of organic solvents like ether (or) chloroform. Organic layer was separated shaken with dilute NH_3 solution. Formation of pink to red colour indicates that anthraquinone glycosides are present in the test drug.

6. Saponin/steroidal glycosides tests: -

Foam test:

About 2 ml of test sample was shaken persistent by shows that saponins are present in the test drug sample.

Libermann's Burchard test:

Test sample was treated with 2 ml acetic anhydride and 2 ml concentration H2SO4, bluish green colour formation shows the presents of steroids in the sample.

7. Test for amino acid / protein: -

Ninhydrin test:

Test sample was boiled with 5% Ninhydrin solution. Formation of violet colour shows that amino acids are present in the test sample drug.

Million's test:

Millions reagent was added to the test solution white precipitate is formed. Formation which turns to red after gentle heating shows that proteins are present in the test drug sample.

Biuret test:

Test drug sample was treated with 4% sodium hydroxide and 1% copper sulphate solution. Development of violet colour shows the presence of protein in the sample.

8. Carbohydrate tests: -

Molish test (General test):

1 ml of test sample was treated with alpha-napthol (alcoholic) along the sides of test tube, few drops of concentrated H_2SO_4 was added slowly, momentary purple to violet coloured ring formation at the junction shows that carbohydrates are present in the sample.

Fehling's test:

The test solution was boiled with few drops of equally mixed Fehling's A and Fehling's B solutions. Brick red coloured precipitate formation indicates that reducing sugars are present in the drug sample.

Barfoed's reagent test:

2 ml Barfoed's reagent was added to the test sample. Red cupric oxide formation indicates that carbohydrates are present in the test drug sample.

Benedict's reagent test:

2 ml Benedict's reagents were mixed with test sample and heated for 5 minutes Appearance of yellow green (or) red colour indicates present of various type of carbohydrates in sample.

RESULT:

Table:1. Phytochemical group tests of *Senna alata.*

Sl.No	Phytochemical groups	Ethanol	Acetone	Ethanol(percolation)
01	Alkaloids Test	+	+	+
02	Flavonoids Test	–	–	–
03	Tannins and Phenolic test	–	–	–
04	Cardiac/ Glycosides Test	–	+	–
05	Anthraquinone Glycosides Test	–	–	–
06	Saponin/ Steroidal Test	–	–	–
07	Amino acid & Protein Test	+	+	–
08	Carbohydrates Test	+	+	+

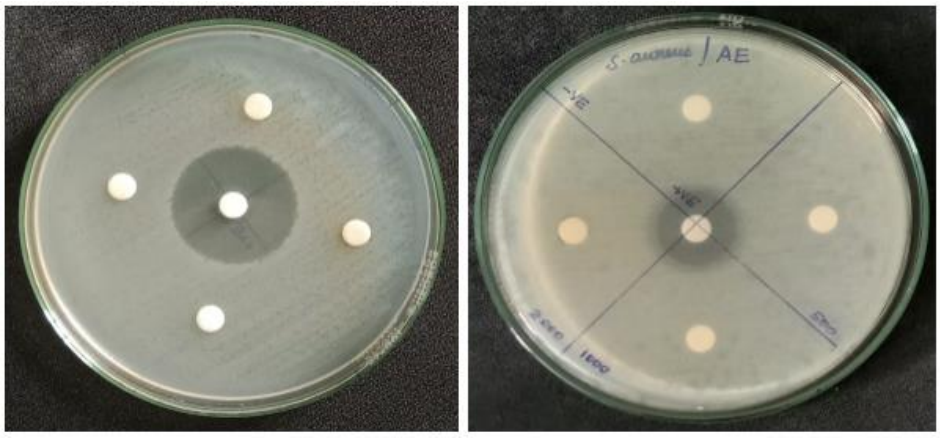

Fig:2. Antibacterial activity of Acetone extract of *Senna alata* on Staphylococcus aureus.

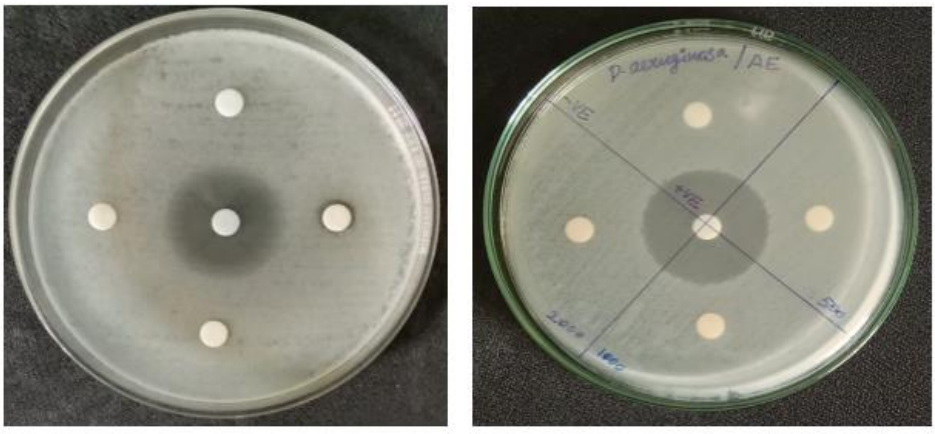

Fig:3. Antibacterial activity of Acetone extract of *Senna alata* on Pseudomonas aeruginosa.

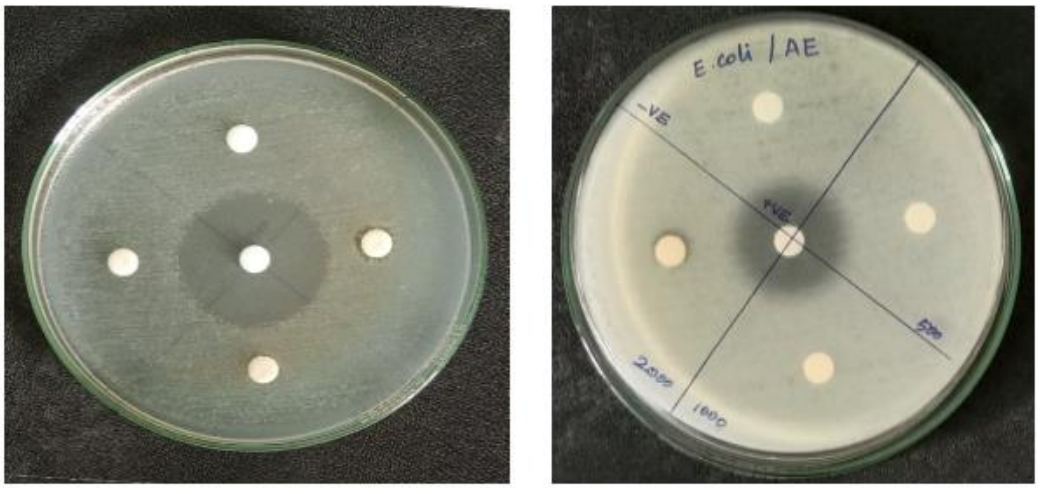

Fig:4.Antibacterial activity of Acetone extract of *Senna alata* on Escherichia coli.

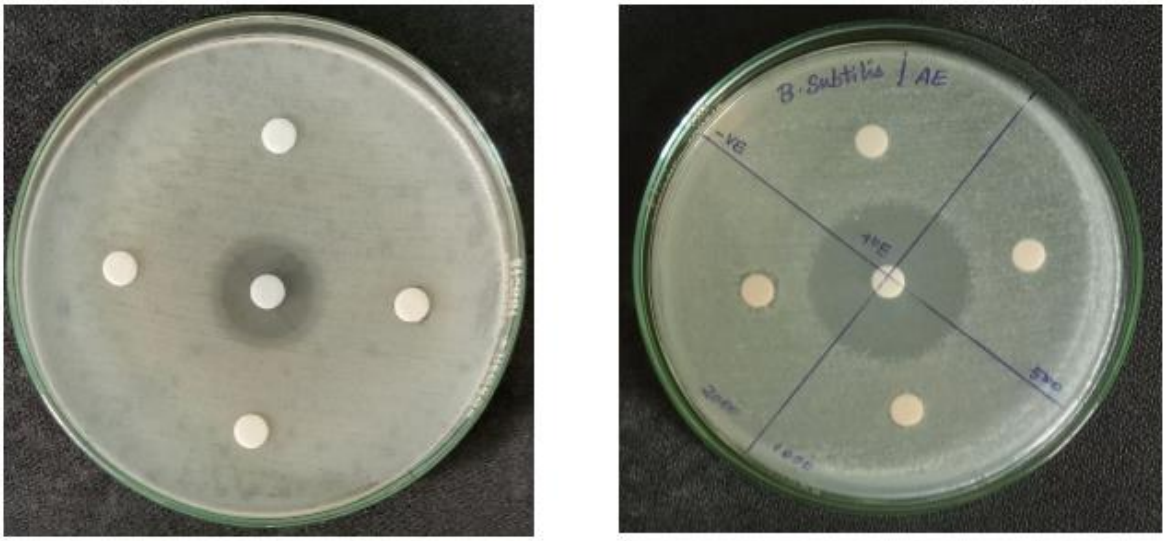

Fig:5. Antibacterial activity of Acetone extract of *Senna alata* on Bacillus subtilis.

ETHANOL EXTRACTION OF ANTIBACTERIAL ACTIVITY:

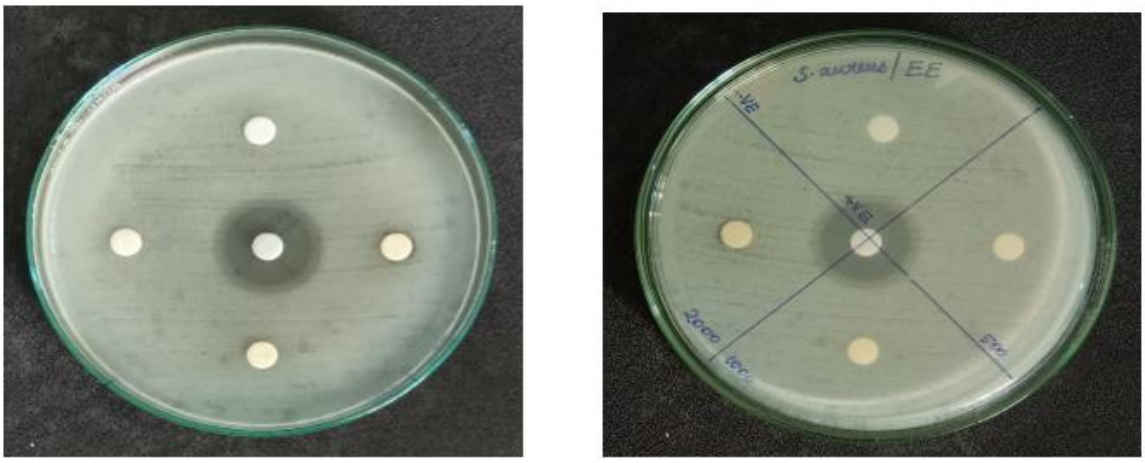

Fig:6. Antibacterial activity of thanol extract of *Senna alata* on Staphylococcus aureus.

Fig:7. Antibacterial activity of Ethanol extract of *Senna alata* on Pseudomonas aeruginosa.

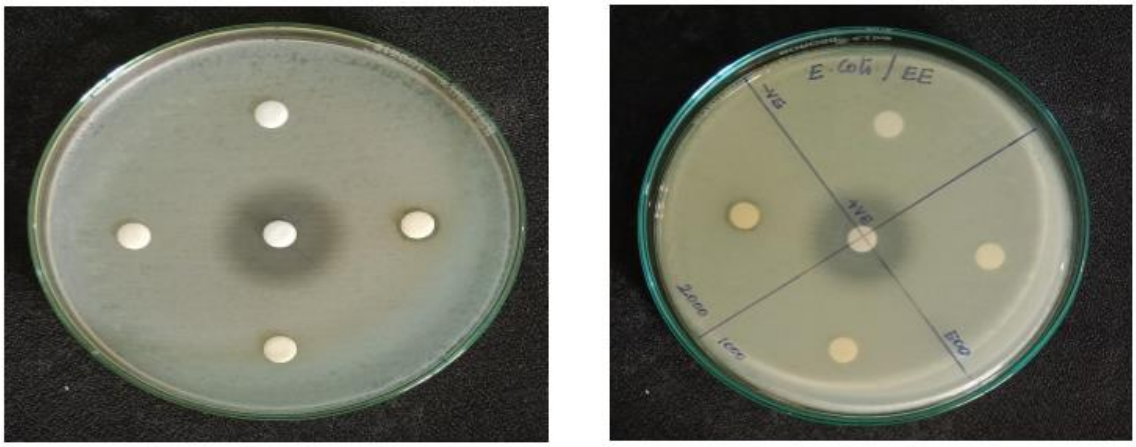

Fig:8 Antibacterial activity of Ethanol extract of *Senna alata* on Escherichia coli.

Fig:9. Antibacterial activity of Ethanol extract of *Senna alata* on Bacillus subtilis.

Table:2: Types of organisms used to study antimicrobial activity of *Senna alata.*

Staphylococcus aureus	Gram positive
Bacillus subtilis	Gram positive
Pseudomonas aeruginosa	Gram negative
Escherichia coli	Gram negative

Disc-diffusion method:

The antibacterial activity of the test samples was carried out by disc diffusion method. The targeted microorganisms were cultured in Mueller-Hinton broth and incubated for 24 hrs. The petri dishes containing Mueller Hinton agar (MHA) medium were cultured with diluted bacterial strain. The prepared discs were placed on the culture medium. Test samples (500, 1000 and 2000µg) were injected to the sterile disc. Standard drug Streptomycin (20µg) was used as a positive reference standard to determine the sensitivity of microbial species tested. Then the inoculated plates were incubated at 37 °C for 24 h. The diameter of the clear zone around the disc was measured and expressed in millimetres as its antibacterial activity.

Table:3. Zone of inhibitions of *Senna alata* vs Streptomycin standard on different microorganisms by Disk Diffusion method.

Samples	**Zone of Inhibition (mm)**											
	Microorganisms											
	S.aureus			***B.subtilis***			***P.aeruginosa***			***E. coli***		
Concentration	**500**	**1000**	**2000**	**500**	**1000**	**2000**	**500**	**1000**	**2000**	**500**	**1000**	**2000**
AE	**-**	**-**	**-**	**-**	**-**	**10**	**-**	**-**	**-**	**-**	**-**	**8**
EE	**-**	**-**	**7**	**-**	**-**	**10**	**-**	**-**	**-**	**-**	**-**	**8**
Streptomycin (20µg)	**19**			**29**			**28**			**21**		

DISCUSSION

- *Senna alata* flowers extract has significant antimicrobial activity against Broad spectrum of microorganisms, the antibacterial activity of extracts against *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa and Escherichia coli* reported for the first time. The microbial studies indicate the potential for the discovery and more drugs from plants.

- The Ethanolic extract was shown to be as potent as anthraquinones increase in extract concentration has increased the zone of inhibition (antibacterial) activity.

- The results clearly showed that terpenoids, flavonoids and anthraquinones were abundantly found in ethanolic extracts of *Senna alata* flowers.

CONCLUSION:

- The present study throw light on the anti-bacterial efficacy of *Senna alata* flowers this study offers a valuable source for the discovery of alternatives to the present anti-bacterial drugs. The study also concludes that *Senna alata* flowers this study offers valuable source for the discovery of alternatives to the present anti-bacterial drugs. This study also concludes that *Senna alata* flowers contain a number of pharmaceutically important phytochemicals such as alkaloids, saponins flavonoids, terpenoids, tannins, anthraquinones, carbohydrates and proteins.

- A further study of the extracts in progress to isolate, characterize and elucidate the structure of the bioactive compounds present which were responsible for the potent antimicrobial activity.

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