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Antibacterial activity of *Sauropus androgynus* (L.) Merr.

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SUMMARY

The leafy vegetable *Sauropus androgynus* (L.) Merr. which belongs to Family- Euphorbiaceae is commonly used as an effective medicinal herb in the treatment of diabetics, cancer, inflammation, microbial infection, cholesterol and allergy due to its antioxidant effect. In the present study an attempt was made to test the antibacterial effect against *Klebsiella pneumoniae* and *Staphylococcus aureus*. The response of antibacterial effect of the plant varies with the medium of extraction-aqueous or ethanol medium. The ethanol extract was having the higher inhibitory effect than the aqueous medium for both the type of bacteria. The inhibitory effect of plant extract was compared to the effect of antibiotic *Gentamicin* on bacterial culture.

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Key words : *Sauropus androgynus*, *Klebsiella pneumoniae, Staphylococcus aureus,* Antibacterial screening, Antibiotic Gentamicin

India is the store house of medicinal plants. About 70% of the rural folk depend on medicinal plants for their health care. Plants are known to contain innumerable biologically active compounds which possess antibacterial properties. Many life saving and essential drugs such as morphine, digoxin, aspirin, emetine, ephedrine etc. are extracted from medicinal plants and introduced into modern therapeutics. The phytochemicals can be extracted from natural source or can be synthesized artificially by drug industries.

Sauropus androgynus has been a popular leafy green perennial vegetable with high level of provitamin A, vitamin B, C, A and K, carotenoids, protein, fibre and minerals like potassium, calcium, phosphorus, magnesium and iron (Fletcher,1998). It is also known as Chekkurmanis or Sweet leaf bush or tropical asparagus. The dark green leaves provide a rich source of chlorophyll which is a valuable blood building element, cell rejuvenator, beneficial to blood circulation, and for regular bowel elimination. Mathew (2000) and Kumaran (2003) analyzed the nutritive value of leafy vegetables in Kerala and indicated that they are rich in various micro nutrients and phytochemicals having antioxidant properties which offer protection against heart disease and certain types of cancer (Saxena, 1999). *S. androgynus* is identified as potentially rich source of dietary flavonoids and antioxidants (Andarwulan *et al.*, 2010). According to Pullaiah (1999) *Sauropus* has a number of medicinal uses. Powdered roots and leaves are reported to be used as poultice for ulcers in the nose. The juice of the leaves mixed with the roots of *Punica granatum* and the leaves of *Jasminum sambac* is used in eye troubles. High blood pressure is lowered by eating raw leaves. Leaves are given as vegetable to nursing mothers to stimulate breast milk production. In lactating sheep also it induced milk production (Superayogy, 2000). It is also beneficial to cure anemia. It is suitable to prevent tiredness, to promote absorption from alimentary tract and to prevent chronic cardiovascular diseases.

The present study is concentrated to analyze the antibacterial activity of *Sauropus androgynus* taking *Klebsiella pneumoniae* and *Staphylococcus aureus* as experimental organisms.

MATERIALS AND METHODS

Glasswares:

Petri plates, conical flasks, test tubes, beakers, glass rods etc were used for the study. All these were washed thoroughly, dried and then sterilized in an autoclave.

Other requirements:

Autoclave, laminar airflow chamber, hot air oven, inoculation loop, Bunsen burner was unavoidable. In addition to these, filter paper discs, swabs, distilled water;

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ethanol and antibiotic disc (Gentamicin) were used.

Culture medium:

A nutrient medium prepared for growing microorganism in a laboratory is called culture medium. The composition of the media may vary according to the type of bacteria. In the present work, nutrient agar medium was used for the growth of the bacteria.

Composition of the nutrient agar medium

Peptone	-10 g
Meat extract	- 10 g
Sodium chloride	- 5g
Agar	- 10 g
Distilled water	- 1000 ml

Human pathogen under study:

The pure culture of human pathogen was isolated from the clinical samples of Polyclinic Laboratory, Thrissur. *Klebsiella pneumoniae* from urine and *Staphylococcus aureus* from pus sample. A sterile inoculation loop was dipped into the culture and streaked over the nutrient medium. These colonies were picked up with an inoculating loop and transferred to a test tube containing about 10 ml of nutrient broth to form a pure culture. Incubate at 25^0 C for 2-8 hours till moderate turbidity appears.

Composition of nutrient broth:

Bacto peptone - 5g
Beef extract -1g
Distilled water - 1000ml

Special features of selected microorganisms:

Klebsiella pneumoniae

Section : Facultative Gram negative rods
Family : Enterobacteriaceae
Genus : *Klebsiella*
Species : *pneumoniae*

Disease caused – severe enteritis in children, pneumonia, urinary tract infection etc.

Staphylococcus aureus

Section : Faculative Gram positive rods
Family : Micrococcaceae
Genus : *Staphylococcus*
Species : *aureus*

Disease caused – Skin abscesses, impetigo, wound infection, pneumonia, and other systematic infection.

Method:

Preparation of plant extract:

Two types of plant extract were made.

Ethanolic extract:

The leaves of *Sauropus androgynus* were shade dried and powdered. 2g of which was taken and 45 ml of ethyl alcohol and 5 ml distilled water were added. It was shaken continuously and kept for overnight. The extract was taken and residue discarded. The extract which was taken in a clean beaker was allowed to evaporate so that plant extract became more concentrated.

Aqueous extract:

Fresh leaves of plant were collected, 2g leaves were washed thoroughly under running tap water, chopped and ground well. The homogenate obtained was filtered using a sterile cloth and poured into a test tube.

Preparation of filter paper disc:

Filter paper discs (6mm diameter) were punched from Whattmann's No.1 filter paper. The filter paper disc was autoclaved by placing in a sterile Petri dish.

Screening of anti- bacterial activity:

The nutrient agar was prepared, sterilized and poured in sterile Petri plates and allowed to solidify. The culture suspensions were swabbed on the nutrient agar medium. Three filter paper discs were placed in a single Petri dish. Two of the filter paper discs namely A and B were loaded with 0.03µ ml plant extract using micropipette. Disc A was saturated with ethanol extract and disc B was saturated with aqueous extract. Readymade antibiotic disc of Gentamicin (10µg) were brought for comparison.

All were incubated in an oven at 37^0C for 24 hours. After 24 hours of incubation, the plates were observed for the formation of zone of inhibition and the diameter of inhibition zone was measured using a measuring scale. The antibacterial activity of the plant extracts were determined by measuring the diameter of inhibition zone of plant extracts and the inhibition zone of the antibiotic solution.

Statistical analysis:

Standard deviation of the data was found out and significance levels were compared with one way ANOVA test and p value < 0.05 using graph pad instat software.

RESULTS AND DISCUSSION

The dried ethanolic extract of *Sauropus* showed average inhibition zone of 13.66mm diameter against *K.pneumoniae* and aqueous extract showed average inhibition zone of 8.66mm diameter. The antibiotic gentamicin had inhibition zone of 20mm diameter (Table 1 and Fig. 1)

Table 1 : Sensitivity pattern of *Sauropus androgynus* against *Klebsiella pneumoniae*

Sample	Diameter of inhibition zone (mm)			Average zone diameter (mm)	P value
	Expt. 1	Expt. 2	Expt. 3		
A	14	15	12	13.66 ± 0.577	P < 0.001
B	9	10	7	8.66 ± 0.577	P <0.001
G	18	20	22	20 ± 0.30	

A - Dried ethanol extract
B - Aqueous extract
G - Antibiotic – Gentamicin

Table 2 : Sensitivity pattern of *Sauropus androgynus* against *Staphylococcus aureus*

Sample	Diameter of inhibition zone (mm)			Average zone diameter (mm)	P value
	Expt.1	Expt.2	Expt.3		
A	11	10	13	11.33 ±0.5774	P < 0.001
B	7	8	10	8.333 ±0.5777	P < 0.001
G	15	14	15	14.66 ±0.00	

A - Dried ethanol extract
B - Aqueous extract
G - Antibiotic – Gentamicin

On comparison, the activity of ethanolic and aqueous extract against *K.pneumoniae* showed that the dried ethanolic extract had more inhibitory activity. The ethanol extract showed inhibition zone of 13.66mm diameter while aqueous extract had only 8.66mm inhibition zone. Compared to gentamicin (20mm), ethanolic extract had less activity.

The dried ethanol extract of *Sauropus* showed an average inhibition zone of 11.33mm diameter against *S.aureus*. The aqueous extract showed inhibition zone of 8.333mm diameter and the antibiotic disc of gentamicin showed an inhibition zone of 14.66mm. (Table 2 and Fig. 2).

Here also when the activity of both extracts was compared against *S.aureus*, the dried ethanolic extract showed high inhibition zone (11.33mm) than aqueous extract (8.333mm). Compared to gentamicin (14.66 mm), both extract showed less activity.

Among the two extracts studied the best activity was noticed by ethanolic extract against both *Klebsiella pneumoniae* and *Staphylococcus aureus*. Ethanolic extract showed more inhibition zone against *K. pneumoniae* (13.66 mm) than *S. aureus* (11.33mm). The aqueous extracts showed less activity towards both

Fig. 1 : Activity of ethnolic and aqueous extract against *Klebsiella pneumoniae*
(a) Dried ethanol extract
(b) Aqueous extract
(G) Antibiotic-Genamicin

Fig. 2 : Activity of ethnolic and aqueous extract against *Staphylococcus aureus*
(a) Dried ethanol extract
(b) Aqueous extract
(G) Antibiotic-Genamicin

bacteria, against *Klebsiella* aqueous extract had a inhibition zone of 8.66mm and against *Staphylococcus* 8.333mm diameter. Compared with control (gentamicin) aqueous extract and ethanolic extract showed less activity. The control gentamicin showed 20mm inhibition zone against *Klebsiella pneumoniae* and 14.66mm against *Staphylococcus aureus*.

Screenings of antibacterial activity of various extracts of *S. androgynus* have shown that they can be used in the treatment of diabetes (Usha and Palaniswamy, 2008). An essential oil from *Sauropus* is strongly antibacterial and may be used to treat *Candidiasis* and other fungal conditions (Prajapathi, 2003). As it possesses many medicinal properties, this leafy vegetable can be considered as a medicinal plant.

Different types of phytochemicals like riboflavin, niacin, ascorbic acid, carotenoids, and lycopene were extracted from *S. androgynus* including the most important alkaloid papaverin. These phytochemicals have been reported to provide medical or health benefits. For example, b carotene to prevent lung and skin cancer, niacin to prevent recurrent heart attacks, ascorbic acid in improving immune system, riboflavin for lesion treatment and lycopene in the prevention of cancer (Mahanum *et al.*, 1999). The papaverin content of *Sauropus androgynus* was about 580mg/100g fresh leaf (Padmavathi and Prabhakaran Rao, 1990).

Plant based remedies have always been an integral part of traditional medicine throughout the world. *S.androgynus* is a multivitamin, multinutrient containing green leafy vegetable and as a medicinal leafy vegetable its importance is much higher. The present study of the antibacterial activity played an important role in extracting the effective compound for the treatment of bacterial infection. Natural products and their derivatives represent more than 50% of the drugs in clinical use in the world (Cowan, 1999). Enormous health problems are arising today, due to drug resistant micro organism and the emergence of unknown disease causing microbes. In this context, this identification of natural antibacterial product in common medicinal plant help us to extract the chemotherapeutic agent for drug designing with utmost efficacy and availability at affordable rate to common people.

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