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**Research Article**

# Antimicrobial Activity Of Methanolic Extract Of *Spathodea Campanulata* Against Bacterial Pathogens

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**Abstract**

The antibacterial activity of methanol stem bark of *Spathodea campanulata* was investigated by testing the extracts against *Escherichia coli*, and *Staphylococcus aureus*. The minimum inhibitory concentration (MIC) of the methanol extract was determined against the two bacteria strains by using the disc diffusion method. Four different concentrations of *S. campanulata* such as (100 µl/ml, 200 µ/ml, 400 µl/ml) were prepared. After incubation for 24 hrs, the zone of inhibition was compared with standard antibiotics streptomycin (100 µg/ml).The present results revealed that the maximum zone of inhibition was observed in E.coli (mm) of 200 µg/ml concentration when compared to control. The result significantly paves the way for further application of *Spathodea campanulata* stem bark waste as antibacterial materials.

**Keywords**: *Spathodea campanulata,* anti-microbial activity, *Staphylococcus aureus, E.coli,*

**Introduction**

Phytochemistry is a branch of chemistry which embraces natural Product Organic Chemistry and Biochemistry. They are usually used to refer to compounds found in plants that are not required, for normal functioning of the body but that nonetheless have a beneficial effect on health or an active role in the amelioration of disease. In 1936, Szent-Gyorgy and Rusznyakprepared an extract from Paprika and subsequently from Citrus Juice which he claimed to have value beyond that of ascorbic acid in reducing capillary bleeding in man and in guinea pigs.

*Spathodea campanulata* Beauv (Bignoniaceae) is widely distributed through Africa and found in particular in Cameroon and Senegal. It is used in tradition herbal medicine for the treatment of ulcers, filaria, gonorrhea, diarrhoea, and fever. *S.campanulata* was also known in Cameroon traditional medicine to have a healing activity in burn wounds. The aim of the present study was to assess the burn healing effectiveness of the methanolic extract of the barks of *S.campanulata* Ointment (MEBSCO) in comparison to those of *centellaasiatica* and peru's balm in experimental burn model in rats. Experimental burn was made in rat under chloral anesthesia with electric iron (200 degrees) on the right and left side of the medianus line.

Topical applications of (MEBSCO) (2%, 10% and 49%) dose dependently decreased the score damage of the burn site. Treatment with 10% and 49% of MEBSCO varied the score damage from 5 to 1 +/- 0.4 and 5 to 0.2 +/-0.5 ($p<0.05$, $n=5$) respectively, at day 15 after experimental burn. As well as Casiatica (1%) and perus' balm (1%) ointments, MEBSCO (10% and 49%) induced a complete burn healing on the 19-20th post burn day. This study shows for the first time, the burn healing effectiveness of MEBSCO in experimental burn model. It also provides a rational use of the *S.campanulata* barks in traditional medicine to promote burn healing (Sy *et al*., 2005).

Determining appropriate treatment for an infectious disease requires both the isolation of an infectious agent from a patient with diseases and determination of susceptibility or resistance to anti-microbial agents used in the therapy. Even the Pneumococcuswas invariably susceptible to penicillin at levels of less that 0.03g ml is devel-

oping increasing resistance to this drug.

Clinical microbiologists can only recommend therapeutic agents based on their ***invitro*** activities and their background understanding of drug-bug interactions. At times, an antimicrobial agent that shows poor activity against an organism is used in a patient with good results, the opposite effect may also occur. This occurs because host immune factors play a significant role in the outcome of an infection.

## MATERIALS AND METHODS

### Microbial strains

Microbial strains Fresh clinical strains of *E.coli*, ATCC 6539, *S. aureus*ATCC 3450 were obtained from the IMTECH, Chandigarh, India. All the strains were stored at 4°C temperature until use.

### Preparation of plant extracts

Fresh plant material were washed with tap water, air dried at room temperature for 15-30 days, and then homogenized to fine powder. A sample (200 g) of each powdered plant material was soaked in ethanol (500 ml) for 48 h with constant stirring. The suspension was filtered through Whatman filter paper No. 1. The filtrate was concentrated under vacuum using a rota-vapor to obtain the dry ethanol extract and stored at 4°C until further use. These extracts were used for antibacterial activity.

### Antibacterial assay

### Determination of zone inhibition

The media (Muller-Hinton agar) were prepared according to the manufacturers' standard, 38 g/1000ml of distilled water. The methanolic extracts were dissolved to obtain different concentrations (50,100,200,400 µg/ml). Control used was methanol. Streptomycinwas used as positive reference standard having a concentration of 100 µl/ml for all bacterial strains. The organisms were maintained on nutrient agar plates and were revived for bioassay by subculturing in fresh nutrient agar for 24 h before being used. The agar wells diffusion method described by Kuete, (2010), was adopted. Briefly, nutrient agar was prepared by autoclaving and allowed to cool to 40-50°C before seeded with the test organism (in sterile petri-dishes of 90 mm diameter). The seeded plates were allowed to set and cylindrical plugs were removed from the agar plates by means of a cork borer to produce wells of approximately 6 mm diameter. The wells were equidistant from each other and the edge of the plate (Washington, 1995). The plates were incubated at 37°C for 24 h. antibacterial activity was determined by measuring the zone of inhibition surrounding the well. The zones of inhibition were then measured, recorded and compared with standard control, streptomycin (100 µg/ml). The assays were carried out under aseptic conditions. All tests were performed in triplicate.

### Statistical analysis

Data were expressed as mean ±standard deviation. The data obtained were subjected to ANOVA test to determine whether there was significant difference in zone of inhibition between extract concentrations and also antibiotic used.

### Results

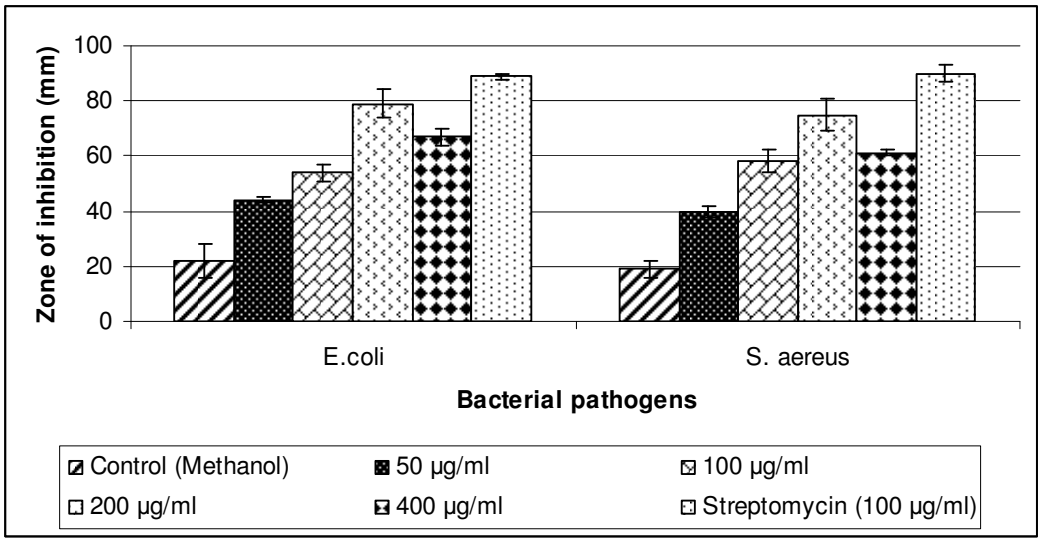
Fig 1: shows that the antibacterial activity of methanol extracts of leaf of *S. campanulata* like different concentration (50, 100, 200, 400 µg/ml) of were used in this study. The different two types of bacterial culture were used and analyzed for antibacterial activity and streptomycin was used in standard and control was used in methanol. The maximum of zone of inhibition (79mm) was observed in 200 µg/ml extract treated groups in *E. coli* and *S. aerues* (74 mm) when compared to control. The least zone of inhibition was observed in methanol extract (control groups) of both micro organisms. The streptomycin (100 g/ml) a standard antibiotic showed statistically ($p<0.05$) higher activity compared to methanol, and different concentration of leaf extracts.

### Discussion

Knowledge on plant uses is the result of many years of man's interaction and selection on the most desirable, the most vigorous and the most successful plant present in the immediate environment at a given time (Rindos, 1984). According to the World Health Organization, 80% of people in developing countries still depend on local medicinal plants to fulfill their primary health needs (WHO, 2002). Plants constitute an important source of active ingredients which differ widely in terms of structure and therapeutic properties. The continued investigation into the secondary plant metabolites for anti-infective properties has gained importance in recent years because of the alarming increase in resistance of pathogenic microorganisms to existing antibiotic. For instance, the emergence and spread of Salmonella resistance to many commonly used an-

tibiotics (Ciprofloxacin, Ampicillin, Chloromphenicol, Amoxicilllin) is now a subject of international concern. In the present study the effect of methanol extract of *S. campanulata*was used in antibacterial activity by using two different bacterial pathogens.

**Fig:1. Effect of different concentration of*S. campanulata*extract on antibacterial activity of selected pathogens.**




Active compounds present in the crude ethanol extracts show the antibacterial activity with the dose dependant manner. If the active principle is present in high quantities, there could be other constituents exerting antagonistic effects of the bioactive compounds. So this may be happening with the ethanol flower extract where it shows more potency than the leaf extracts. Polyphenolic, flavonoids and tannins present in the ethanol extract may be responsible for the antibacterial activity. Tannin is known to show the antibacterial activity by precipitation the microbial proteins. Flavonoids are produced by the plants for the defense against the infection. So, use of the crude ethanol extract of this plant as an agent to control microbial pathogens needs further extensive research for their better economic and therapeutic utilization. The findings from this work may add to the overall value of the medicinal potential ethanol extract of leaf and flower extract of *S. campanulata*. Further phytochemical studies are required to determine the purified fractions/bioactive compounds responsible for the antibacterial activities of these species, which could serve as useful sources for new antimicrobial agents. From the above studies it can be concluded that the ethanol extracts of both Leaf and flower extract of S. campanulata exhibit significant antibacterial activity against pathogenic bacteria. The inhibited extracts showed high polyphenols, tannins and flavonoids content. Therefore this S. campanulata leaf and flower may be act as another source of natural antibiotic. This study reaffirms the ethanomedicinal property of *S. campanulata*.

The activity of plant extracts against bacteria have been studied for years, but in more intensified way during the last three decades. During this period, numerous antimicrobial screening evaluations have been published based on the traditional Chinese, African and Asian uses of plant-based drugs (Suffredim *et al*., 2004). In the present study, the results of antibacterial property of the 38 plant extracts against tested organisms varied depending on bacteria tested and concentration as previously reported by (Ravikumar*et al*., 2007; Rajesk *et al*., 2010). However, 38, 15 and 14 plants shows inhibition diameter against *Salmonella typhi*, *S. paratyphi B* and *S. paratyphi A* respectively. Nevertheless, negative results obtain with some of the plants extracts tested do not indicate the absence of bioactive constituents, nor that the plant is inactive. Active compound(s) may be present in insufficient quantities in the extracts to show activity with the dose levels employed (Taylor *et al*., 2001).

Lack of the activity can thus only be proven by using large dose (Farnsworth, 1993), if the active principle is present in high enough quantities, there could be other constituents exerting antagonistic effects or negating the positive effects of the bioactive agents (Jager*et al*., 1999). It was noticed that *Sennaalata*, showing the highest antibacterial activity on both pathogens with zone of inhibition of 24, 22.5, 20.5 mm on the *Salmonella paratyphi A*, *S. paratyphi B* and *S. typhi* respectively at 160 mg/ml. This could probably have been due to the fact that the rate of the active ingredients or constituents in the plant materials is higher compared to the other plants used in this research (Kunle&Egharevba, 2009). Preliminary phytochemical analysis of *S. alata* leaves showed that, they possess polyphenols, triterpenes and saponins. Phytoconstituents such as polyphenols, triterpenes and saponins have been reported to inhibit bacterial growth and to be protective to plants against bacterial and fungal infections (Mather &Gonzalel, 1982; Okwute, 1992). The presence of saponins in this plant must have exhibited direct antibacterial activity and suppression of bacterial virulence resulting to the antimicrobial activity seen in this study (Gills, 1992). This also corroborate with the report of Owoyale *et al*., (2005) who documented on the plant *Sennaalata* to be effective in treatment of fungal and bacterial diseases. The present study reveals that the maximum inhibition was observed in 200 µg/ml treated groups in both bacterial pathogens and when compared to control.

The fact that *Salmonella typhi* was more susceptible to the extract of *Pseudarthriaconfertiflora*, *Terminaliaglaucescens*, *Sennaalata, Dacryodesedulis* and *Stereospermumacuminatissimum* indicated the potency of these plants against typhoid fever. *Sennaalata* and *Rauvolfiavomitoria* were found to be the most sensitive against *Salmonella paratyphi A*. However, *Salmonella paratyphi B* was sensitive to the extract of *Sennaalata, Pseudarthriaconfertiflora* and *Rauvolfiavomitoria*. The antibacterial activity of *Terminaliaglaucescens*, *Sennaalata, Dacryodesedulis* and *Rauvolfiavomitoria* have been reported in the literature (Owoyale *et al*., 2005; Obame *et al*., 2008; Adebayo and Ishola, 2009; Ayepola, 2009; Ogundiya *et al*., 2009; Ajibesin *et al*., 2011; Bolou *et al*., 2011; Omogbai&Eneh, 2011). In local area, these plants are being used in the treatment of typhoid fever, diarrhea, dysentery etc. So, the uses of these plants as antibacterial agent are justified; since they showed good inhibition zones. Demonstration of low MIC (128 µg/ml) especially by *Vitexdoniana* is an indication that the phytoconstituents of the plant have therapeutic potential. Similar report revealed the antimicrobial potency of *Vitexdoniana* with MIC range from 0.4-128 µg/ml (Iroha *et al*., 2010). It is important to highlight that Vitexdoniana has been thoroughly studied phytochemically and its antimicrobial activity has been evaluated (Kubmarawa *et al*., 2007; Iroha *et al*., 2010). In this study, its antibacterial activity was demonstrated. It is no surprising the activity shown by this plant, since other species of the same genus (*V*. *negundo*) have shown to have antibacterial, anti-inflammatory and anti-fungal activity (Dharmasiri *et al*., 2003; Panda *et al*., 2009). In the present study the methanol extract 200 *µg/ml* of *S. campanulata* maximum highest inhibition was observed.

**Conclusion**

The study has demonstrated the antimicrobial activity of the stem bark extracts of *S. campanulata* against two strains of bacteria. The methanol extract of *S. campanulata* showed the best antibacterial activity. The broad antimicrobial activity of *S. campanulata* is supportive of its folklore use in the treatment of wounds and other topical infections.

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**References**

Szent-Gyorgy and Rusznyak; 1936. Vitamin P: Flavonols as Vitamins,*Nature*, , 27

Sy GY, Nongonierma RB, Ngewou PW, Mengata DE, Dieye AM, Cisse A, Faye B. 2005. Healing activity of methanolic extract of the barks of Spathodea campanulata Beauv (Bignoniaceae) in rat experimental burn model. *Dakar Med*. **50**(2):77-81.

Kuete V. 2010 Potential of cameroonian plants and derived products against microbial infections: Planta Medica, **76**:1-13.

Washington J. 1995. Manual of clinical microbiology. 4th Edn., Lennette, E., A. Balows, W.J. Haausler and H.J. Shadomy (Eds.), *American Society of Microbiology*, Washington, pp: 967-970

Rindos D.1984. Origins of Agriculture: Anevolutionary perspective. Cornell University.Orlando: Academic Press, INC.

World Health Organisation. WHO traditional medicine strategy 2002-2005.World Health Organization, 2002.

Suffredim IB, Sarder HS, Goncalves AG. 2004. Screening ofantibacterial extracts from plants native to Brazillian amazon Rain Forestand Atlantic Forest. *Brazillian J. Med. Biol. Res*,**37**:379-384.

Ravikumar PHS, Makari HK, Gurumurthy H. 2007. In vitroantimicrobial activity of ethanol extract of *Thevetiaperuviana*. *EJEAFChe*,**6**(9):2318-2322.

Rajesh K, Harsha R, Mohammed GA, Hareesh AR,Thammanna GSS, Dinesha R, Satish KBP, Irfan AM. 2010. Antimicrobial activity of ethanol extract of leaf and flower of *Spathodeacampanulata*P.Beauv.*Research Journal of Pharmaceutical, Biological and Chemical Sciences*,**1**(3):691-698.

Taylor JLS, Rabe T, McGraw LJ.2001. Towards the scientific validation of traditional medicinal plants. *Plant Growth Regul*,**34**:23-37.

Farnsworth NR. 1993. Biological approaches to the screening and evaluation of natural products. In: Rasoanaivo P, Ratsimamanga- Urverg S (Eds) Biological evaluation of plants with reference to the Malagasy Flora, Madagascar, pp. 35-43.

Jager AK, Hutchings A, Van Staden J. 1999. Screening of plants used by southern African traditional healers in the treatment of dysmenorrhoea for prostaglandin-synthesis inhibitors and uterine relaxing activity. *J. Ethnopharmacol*,; 64:9-14.

Kunle OF, Egharevba HO. 2009. Preliminary studies on Vernonia ambigua: Phytochemistry and Antimicrobial Screening of the Whole Plant.*Ethnobotanical Leaflets*.**13**: 1216-21.

Mather SB, Gonzalel L. 1982. Identification of terpenoids from leaves of*Piptocarphaperitora*and their biological activities. *J. Nat. Prod*,**45**:495-496.

Okwute SK. 1992. Plant derived pesticidal and antimicrobial agents for use in agriculture. A review of phytochemical and biological studies on some Nigerian plants. *J. Agric. Sci. Technol*,; **2**:62-70.

Gills LS. 1992. Ethnomedical uses of plants in Nigeria. University of Benin press, Nigeria.P. 276.

Owoyale JA, Olatunji GA, Oguntoye SO. 2005. Antifungal and antibacterial activities of an alcoholic extract of *Sennaalata*leaves. *J.Appl. Sci. Environ, Mgt*, **9**(3):105-107.

Obame LC, Edou P, Bassole IHN, Koudou J, Agnaniet H, Eba F, Traore AS. 2008. Chemical composition, antioxidant and antimicrobial properties of the essential oil of *Dacryodes edulis*(G.Don)H.J. Lam from Gabon. *African Journal of Microbiology Research*,**2**:148-152.

Adebayo EA, Ishola OR. 2009. Phytochemical and antimicrobial screening of crude extracts from the root, stem bark, and leaves of *Terminaliaglaucescens. African Journal of Pharmacy and Pharmacology*, **3**(5):217-221.

Ayepola OO. 2009. Evaluation of the antimicrobial activity of root and leaf extracts of *Terminaliaglaucescens. Advances in Natural and Applied Sciences*, **3**(2):188-191.

Ogundiya MO, Kolapo AL, Okunade MB, Adejumobi JA. 2009. Assessment of phytochemical composition and antimicrobial activity of *Terminaliaglaucescens*against some oral pathogens.*EJEAFChe*,**8**(7):466-471

Ajibesin KK, Ekpo BA, Bala DN, Essien EE, Adesanya SA. 2008. Ethnobotanical survey of AkwaIbom State of Nigeria.*Journal of Ethnopharmacology*,**115**: 387-408.

Ajibesin KK, Essien EE, Adesanya SA. 2011. Antibacterial constituents of the leaves of *Dacryodesedulis. African Journal of Pharmacy and Pharmacology*,**5**(15):1782-1786.

Bolou GEK, Attioua B, N'Guessan AC, Coulibaly A, N'Guessan JD, Djaman AJ. 2011. Évaluation*in vitro* de l'activité antibactérienne des extraits de *Terminaliaglaucescens*planch. Sur *Salmonella typhi* et *Salmonella typhimurium*. *Bulletin de la Société Royale des Sciences de Liège*, **80** : 772 – 790.

Omogbai BA, Eneh TO. 2011. Antibacterial activity of *Dacryodesedulis*seed extracts on food-borne pathogens. *Bayero Journal of Pure and Applied Sciences*, **4**(1):17-21.

Onocha PA, Ekundayo O, Oyelola O, Laakso Kubmarawa D, Ajoku GA, Enwerem NM, Okorie DA. 2007. Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. *African Journal of Biotechnology*, **6**(14):1690-1696.

Iroha IR, Ilang DC, Ayogu TE, Oji AE, Ugbo EC.

2010. Screening for anti-typhoid activity of some medicinal plants used in traditional medicine inEbonyi State Nigeria. *African Journal of Pharmacy and Pharmacology*, **4**(12):860-864.

Dharmasiri MG, Jayakody JRAC, Galhena G, Liyanage SSP, Ratnasooriya WD. 2003. Anti-inflammatory and analgesic activities of mature fresh leaves of *Vitex negundo*. *J. Ethnopharmacol*, **87**: 199-206

Panda SK, Thatoi HN, Dutta SK. 2009. Antibacterial activity and phytochemical screening of leaf and bark extracts of *Vitexnegundo*I. From simimipal biosphere reserve, Orissa. *Journal of Medicinal Plants Research*,**3**(4):294-300.