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# Phytochemical and Antimicrobial Potency of the Aqueous and Methanol Leaf Extracts of *Icacina senegalensis*

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## ABSTRACT

**Background:** An assessment of phytochemical composition and antimicrobial activity of the aqueous and methanol leaf extracts of *Icacina senegalensis* was carried out. **Methodology:** Phytochemical screening and antibacterial properties of both the aqueous and methanol leaf extracts of *Icacina senegalensis* were evaluated against clinical isolates of *Staphlococcus aureus*, *Shigella* spp., *Salmonella typhi*, *Pseudomonas aeruginosa*, *Serratia marcescens* and *Escherichia coli* using agar diffusion method. **Results:** Phytochemical screening revealed the presence of saponins, tannins, alkaloids, flavonoids, terpenoids, steroids and phenols. The aqueous and methanol leaf extracts exhibited good activity against the test organisms. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the leaf extract ranged from 2-60 $\mu$g mL$^{-1}$. **Conclusion:** The findings indicate that both the aqueous and methanol leaf extracts of *Icacina senegalensis* contain bioactive components that have broad spectrum antibacterial properties.

**Key words:** *Icacina senegalensis*, leaf, phytochemical composition, antibacterial agent, aqueous, methanol

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## INTRODUCTION

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Mahesh and Satish, 2008). Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health (Parekh and Chanda, 2006). There has been an increasing incidence of multiple resistance in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed for the treatment of infectious diseases. This has necessitated a search for new antimicrobial substances from various sources including medicinal plants. Researchers are increasingly becoming involved in the screening of such plants with the aim of establishing their potential antibacterial effects and identifying the compounds responsible for their antibacterial properties (Aibinu *et al*., 2007; Ndukwe *et al*., 2007; Akuodor *et al*., 2011).

*Icacina senegalensis* A. Juss (Family: Icacinaceae) is a savannah suffurex with glabrous or pubescent leafy shoots of about 2-3 feet high and a large fleshy tuber with creeping roots. The plant is indigenous to west and central Africa (Sarr *et al*., 2011; Mbatchou and Dawada, 2012). It grows wild on light sandy soils in the savannah areas of Senegal, Gambia, Ghana, Nigeria, Guinea, Central African Republic, Congo and parts of Sudan. Different parts of the plant, especially the leaves, root and stem are widely employed in traditional medicine. The leaves are taken as a decoction for feverish condition. Extract of the leaves in water or diluted alcohol has been used as antihyperglycemic agent (N'diaye *et al*., 2008).

The aim of the present study was to investigate the phytochemical composition and antibacterial activity of the leaf extracts of *Icacina senegalensis* which have been claimed to possess some ethnomedicinal properties.

## MATERIALS AND METHODS

**Collection and preparation of plant materials:** Fresh leaves of *Icacina senegalensis* were collected in July, 2012 from a farm land in Orlu, Imo State, Nigeria. The plant material was identified and authenticated by Mr. Frank I. Apejoye of Department of Botany, University of Calabar, Nigeria. A voucher specimen (No. 620) has been deposited in the University of Calabar Herbarium. The leaves were cleaned, cut into smaller pieces, air-dried at room temperature for 7 days and pulverized to dry powder using a mortar and pestle.

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**Extraction of plant material:** Eighty grams of the leaf powder was extracted in water and methanol by maceration for 24 h with constant shaking. The mixture was concentrated to dryness in a water bath to obtain 5 g (6% w/w) for water and 4 g (5% w/w) for methanol of methanol extract. The leaf extract was subsequently reconstituted in an appropriate volume of distilled water for the study.

**Phytochemical analysis:** The phytochemical screening of the aqueous and methanol leaf extract of *Icacina senegalensis* was carried out to determine the presence of the following compounds; alkaloids, tannins, saponins, terpenoides, phlobataninns, flavonoids, steroids cardiac glycosides, phenols and anthraquinones using standard procedures (Inyang-Agha, 2006; Kasolo *et al*., 2010; Ajayi, 2008).

**Test organisms:** Clinical isolates of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli* and *Shigella* spp. obtained from Departmen of Microbiology, University of Calabar Teaching Hospital, Calabar, Nigeria, were used for the experiment. Purity plates of each bacterial isolates were obtained by culturing on their respective selective media. Biochemical tests were performed to re-identify and confirm the identity of the isolates. Fres plates of the test organisms were made from the isolate cultures obtained o agar slants. Discrete colonies of fresh cultures of different bacterial isolates were then picked and suspended in 5 mL nutrient broth in Bijou bottles an incubated for 24 h at 37°C prior to antimicrobial susceptibility testing.

**Determination of antibacterial activity:** The antibacterial activities of the aqueous and methanol leaf extracts of *Icacina senegalensis* was determined using agar diffusion method of (Osadebe and Ukwueke, 2004; Adebayo and Ishola, 2009; Akuodor *et al*., 2011). Broth cultures of the test isolate (0.5 mL) containing $1\times10^5$ CFU $mL^{-1}$ of organism were introduced into sterile petri-dish and 15 mL of Muller Hinton agar was added. The content was properly mixed and allowed to solidify. Holes were bored in the plates using a standard sterile cork borer of 6 mm in diameter and the leaf extract reconstituted in distilled water at varying concentrations of 6.25, 12.5, 25, 50 and100 $\mu g\ mL^{-1}$ were applied in each of the wells in the culture plates. The experiments were carried out in duplicate. The plates were allowed to stand for 1 h for pre-diffusion of the extract to occur and incubated at 37°C for 24 h. At the end of the incubation, the diameter of the zones of inhibition were measured and recorded in mm.

**Determination of Minimum Inhibitory Concentration (MIC):** The minimum inhibitory concentration was determined by the method as described by Andrews (2001). Six sterile tubes were arranged in a test tube rack and 0.5 mL of sterile nutrient broth was transferred into each test tube. Extracts concentrations of (3.125, 6.25, 12.5, 25, 50 and 100 $\mu g\ mL^{-1}$) were prepared by serial dilution. The test tube organism (0.5 mL) was taken and transferred into each of the test tube containing the mixture of the broth and the extract and then incubated at 37°C for 24 h. The MIC was recorded as the least concentration of plant extract that completely inhibited the growth of the organism.

**Determination of Minimum Bactericidal Concentration (MBC):** The minimum bactericidal concentration of the plant extract on the clinical bacterial isolates was carried out according to National Committee for Clinical Laboratory Standard (NCCLS, 1993). A loopful of broth was collected from the determination of MIC tubes which did not show any growth and streaked on a sterile nutrient agar. All the plates were then incubated at 37°C for 24 h. The least concentration of the leaf extracts with no visible growth after incubation was taken as the minimum bactericidal concentration.

## RESULTS

**Phytochemical studies:** Phytochemical screening of the extracts revealed the presence of saponins, flavonoids, steroids, terpenoids, alkaloids, cardiac glycosides and phenols while phlobatannins and anthraquinones were not detected. The presence of saponins, flavonoids, alkaloids and steroids in the plant's part is an indication that the plant is of pharmacological importance. These classes of compounds are reported to show important biological activities (Ghoghari and Rajani, 2006; (Hadacek, 2002; Panda and Kar, 2007) (Table 1).

Table 1: Phytochemical constituents of the aqueous and methanol leaf extract of *Icacina senegalensis*

| Components | Aqueous extract | Methanol extract |
|---|---|---|
| Alkaloids | ++ | +++ |
| Tannins | + | +++ |
| Saponins | ++ | +++ |
| Terpenoids | - | + |
| Flavonoids | - | +++ |
| Phenol | +++ | +++ |
| Steroid | - | ++ |
| Cardiac glycosides | - | +++ |
| Phlobatannins | - | - |
| Anthraquinones | - | - |

+: Slight presence, ++: Medium presence, +++: Heavy presence, -: Absence

Table 2: Antimicrobial activities of the aqueous and methanol leaf extracts of *Icacina senegalensis*

| | Extract ($\mu$g mL$^{-1}$) zone of inhibition (mm) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 6.25 | | 12.5 | | 25 | | 50 | | 100 | |
| Organisms | AE | ME | AE | ME | AE | ME | AE | ME | AE | ME |
| *S. aureus* | 12 | 6 | 12 | 6 | 11 | 6 | 11 | 6 | 6 | 6 |
| *Shigella* spp. | 12 | 8 | 15 | 8 | 17 | 6 | 14 | 9 | 11 | 7 |
| *S. typhi* | 8 | 6 | 12 | 8 | 17 | 10 | 14 | 9 | 6 | 9 |
| *P. aeruginosa* | 14 | 13 | 14 | 15 | 15 | 6 | 12 | 9 | 10 | 6 |
| *S. marcescens* | 22 | 19 | 22 | 20 | 25 | 16 | 28 | 19 | 29 | 16 |
| *E. coli* | 10 | 8 | 18 | 8 | 15 | 12 | 6 | 6 | 6 | 7 |

AE: Aqueous extract, ME: Methanol extract

Table 3: Antimicrobial effects of MIC and MBC ($\mu$g mL$^{-1}$) of the aqueous and methanol leaf extracts of *Icacina senegalensis*

| | Aqueous extract | | Methanol extract | |
|---|---|---|---|---|
| Test organisms | MIC | MBC | MIC | MBC |
| *Salmonella typhi* | 5 | 10 | 15 | 30 |
| *Staphlococcus aureus* | 2 | 4 | 30 | 60 |
| *Shigella spp.* | 2 | 4 | 15 | 30 |
| *Sarratia marcescens* | 2 | 4 | 15 | 30 |
| *Pseudomonas aeruginosa* | 2 | 4 | 15 | 30 |
| *Escherichia coli* | 4 | 8 | 10 | 20 |

MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration

**Antibacterial activities of the aqueous and methanol leaf extracts of *Icacina senegalensis*:** The results of antimicrobial activities of the aqueous and methanol leaf extracts against the test organisms are shown in Table 2. The zones of inhibition of the isolates are a function of the antimicrobial activities of the extracts. The activities of the leaf extracts were shown to be concentration dependent. The extracts showed significant inhibition against all the test organisms. However, the aqueous extract showed more activity than the methanol extract. Distilled water used as respective controls were inactive against the bacteria.

**Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC):** Table 3 shows the results of MIC and MBC determination on the test organisms. The lowest MIC and MBC of 2 and 8 $\mu$g mL$^{-1}$ were demonstrated against *S. aureus*, *Shigella* spp., *S. marcescens* and *P. aeruginosa* while the MIC and MBC values ranging between 4-60 $\mu$g mL$^{-1}$ were demonstrated against *S. typhi* and *E. coli*, respectively.

## DISCUSSION

Medicinal plants are important source of potentially useful structures for the development of new chemotherapeutic agents. Many reports are available on the antibacterial, antifungal, antiviral, anthelmintic and anti-inflammatory properties of plants (Bylka *et al*, 2004; Behera and Misra, 2005; Govindarajan *et al*., 2006; Mahesh and Satish, 2008). Several studies have been conducted in the past that focus on the antimicrobial properties of herbs, spices and their derivatives such as extracts and decoctions (Hsieh *et al*., 2001; Alma *et al*., 2003). Some of these observations have helped in identifying the active constituent responsible for such activities and in developing drugs for therapeutic use in human. The phytochemical screening of the aqueous and methanol leaf extract of *Icacina senegalensis* revealed the presence of alkaloids, tannins, saponins, terpenoids, flavonoids, phenols, steroids and cardiac glycosides. These secondary metabolites have been established to be frequently responsible for the antimicrobial properties of most medicinal plants (Esimone *et al*., 2003; Adejumobi *et al*., 2008; Dewanjee *et al*., 2008). Thus, the presence of the above bioactive components may account for the high antimicrobial activity of the aqueous and methanol leaf extract of *Icacina senegalensis* against some selected microorganisms. The extracts showed good activity against pathogenic bacterial strains such as *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Shigella* spp., *Eschrichia coli* and *Serratia marcescens* with the aqueous extract having the highest zones of inhibition. Plant based products have been effectively proven for their utilization as source for antimicrobial compound (Mahesh and Satish, 2008). The demonstration of activity against the test bacteria provides scientific bases for the local application of this plant in the treatment of various ailments. However, the activities of the aqueous and methanol leaf extracts of *Icacina senegalensis* against both Gram-negative and Gram-positive bacteria tested may indicate a broad spectrum of activity. This observation is very significant because of the possibility of developing therapeutic substances that will be active against multidrug-resistant organisms.

Aqueous extract of *Icacina senegalensis* exhibited strong antibacterial potential compared to methanol extract. The activity of both extracts showed a concentration dependent inhibitory effect on all the organisms tested. More so, the very high significant antibacterial activity shown by the extracts is beneficial as it indicates probably the emergency of a new antibiotic with such a wide spectrum of activity. In addition, the fact that treatment of infection caused by bacteria such as *S. aureus*, *S. marcescens*, *P. aeruginosa*, *S. typhi*, *Shigella* spp. and *E. coli*, are increasingly becoming difficult further strengthens the importance of these findings and the need for a continuous search for chemotherapeutic agents.

Apart from antimicrobial activities, the leave extract are also exploited for therapeutic purpose to cure several disorders. The leaf extracts of *Icacina senegalenses* was found to possess antimalarial and anthyperglycemic activities (Sarr *et al*., 2011; N'diaye *et al*., 2008).

Recently, attention has been directed towards extracts and biologically active compounds isolated from medicinal plants. More so, the use of medicinal plants plays a vital role in covering the basic health needs in developing countries and these plants may offer a new source of antibacterial, antifungal and antiviral agents with significant activity against infective microorganisms (Kumar *et al*., 2012).

The results of the present investigation clearly reveal the antibacterial importance of *Icacina senegalensis* leaf extracts and suggests that this plant could be exploited in the management of diseases caused by the bacteria in human. The bioassay-guided fractionation and further characterization of the active principles responsible for the antibacterial potential of the plant is underway in our laboratory.

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