RESEARCH ARTICLE

Comparative phytochemical and antimicrobial activities of polar solvents tuber extracts of Icacina senegalensis A. Juss *(Icacinaceae)*

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Abstract

Aim: This study was designed to assess phytochemical and antimicrobial activities of methanol (Met), aqueous (Aq), and methanol-aqueous (Met:Aq) extracts of *Icacina senegalensis* tuber.

Methodology: Phytochemical screening of the various tuber extracts was performed using standard methods. Antimicrobial testing was performed on clinical isolates of *Staphylococcus aureus, Shigella dysenteriae, Salmonella typhi, Peudomonas aeroginosa, Serratia marcescens,* and *Escherichia coli* using the well diffusion method. The minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of the extracts were determined by the tube dilution method.

Results: The following phytochemicals were detected: alkaloids, phenols (all extracts), phytate, oxalate (Met), saponin (Aq), steroids, and cardiac glycosides (Met:Aq). All three extracts displayed good antibacterial activities against the test organisms.

Aq had the least MIC (1.41-11.25 µg/mL) and MBC (5.63-22.5 µg/mL) against the test organisms. Similar values for Met and Met:Aq were (3.125-6.25; 12.5-50 µg/mL) and (3.125-25; 25-50 µg/mL) respectively. Therefore the antibacterial potencies of the extracts seemed to be in the order of Aq > Met> Met:Aq.

Conclusion: The bioactive (phytochemical) components of the different polar solvents extracts of *Icacina senegalensis* tuber possess wide spectrum antibacterial potency. Thus, a combination of these extracts in antibacterial drug formulation is an attractive option for enhanced efficacy.

Keywords: *Icacina senegalensis*, tuber, phytochemical composition, antibacterial agent, methanol, aqueous

Running title: *I. senegalensis* tuber: antimicrobial potency

1.0. Introduction

Medicinal plants have been and will continue to be the mainstay of health systems in most underdeveloped and developing economies of the world [1]. This is because they double as good nutritional sources and super drug stores in some instances. A great majority of orthodox medicines owe their sources either directly or indirectly to plants. Ethnopharmacological information on them is most often gleaned from traditional knowledge base and remains a spring board for drug discovery. However, the hydra-headed problem of high cost of synthetic drugs, drug abuse, drug resistance, and toxicity has compelled the WHO, the scientific world, and pharmaceutical industries to fall back to medicinal plants for succor [2,3]. In recent times, much effort has been put into the production, evaluation and standardization of herbal medicines [1,4,5]. A guideline on "Specifications, test procedures and acceptance criteria for herbal substances, herbal preparations and herbal medicinal products / traditional herbal medicinal products" [6] is in the public domain. This is quite encouraging.

Icacina senegalensis A. Juss (*Icacinaceae*) is a perennial shrubby plant with starch-rich underground tuber. It is native to the sandy arid regions of Asia and sub-Saharan Africa[7]. In most parts of West Africa where it is found, all the parts are put to use as food and medicine. Ethnopharmacological uses of the leaves include the treatment of malaria, internal haemorrhages, cough, chest infections and feverish conditions [8], while the seed and tuber flours are eaten [9]. Scientific studies have confirmed antimalarial [8,10], and antibacterial [7] potencies of the leaves. Ethanol root extract of this plant has been found to possess antidiabetic activities [11]. Until recently, when antimalarial activity was discovered in methanolic tuber extract of *Icacina senegalensis* [12], not much was known of medicinal attributes of the tuber of this plant. In this study, we sought to assess the phytochemical and antimicrobial activities of methanol, aqueous, and a 50:50 methanol-aqueous extracts of *Icacina senegalensis* tuber.

2.0. Materials and Methods

2.1. Plant sample collection

Whole plant samples of *I. senegalensis* collected in July 2012 from a farmland situated less than 5 Km from the Orlu Local Government Area secretariat, Imo State, Nigeria, was identified and authenticated by Mr. Frank Apejoye of the Department of Botany, University of Calabar. A specimen (voucher No. 0620) was deposited at the department's herbarium for future reference.

The tubers were detached from the plant stems, washed to remove dirt, and further cut into tiny pieces. The cut tuber samples were oven-dried at 40^{o}C for 10 days and ground to powdery form prior to extraction.

2.1.1. Preparation of plant tuber extracts

From the ground tuber powder, 400g was extracted in analytical grade methanol (BDH, England) in a soxhlet apparatus for 24 h to obtain the methanol (Met) extract. It was subsequently concentrated to dryness in a water bath (Memmert, Germany). For methanol-aqueous (Met:Aq) extract, 20g of tuber powder was steeped in 80ml 1:1 methanol-distilled water mixture; while for aqueous extract (Aq), 20g of ground tuber was steeped in 80ml distilled water. Both were preserved under sterile condition for 72 h, after which the respective concoctions were filtered using muslin cloth, and the filtrates evaporated to dryness. The extracts so obtained were refrigerated at 4^{o}C until needed.

2.2. Phytochemical screening

The constituents of the extracts were screened for the presence of alkaloids, tannins, saponins, terpenoids, phlobatannins, flavonoids, phenols, steroids, phytates, oxalates, glycosides and anthraquinones using standard procedures [7,13].

2.3. Antibacterial screening

2.3.1. Test organisms

Clinical isolates of *Staphilococcus aureus*, *Shigella dysenteriae, Salmonella typhi, Peudomonas aeroginosa, Serratia marcescens,* and *Escherichia coli* were obtained from Microbiology Department, University of Calabar Teaching Hospital, Calabar. Re-identification and culturing of pure isolates of the test organisms was performed as earlier reported [7].

2.3.2. Antibacterial Susceptibility Test

The agar diffusion technique as earlier described [14] was used to determine the antimicrobial activity of the plant extracts. Into sterile petri-dishes, broth culture of the respective test isolates (0.2ml) were introduced and 10ml of Muller-Hinton agar added. They were properly mixed and allowed to solidify. The extracts were reconstituted to obtain serial concentrations of 100, 50, 25, 12.5, 6.25 and 3.125 µg/mL. Using a standard sterile cork borer, holes (6mm diameter) were then bored in the plates into which 0.4 ml of the various concentrations of the plant extracts were transferred. The plates were made in triplicates with one for the standard drug (tetracycline) and another for the negative control (water-methanol). All plates were incubated at room temperature for 1 h and thereafter incubated at 37^0C for 24 h. At the end of the incubation, the inhibition zones formed on the medium were evaluated.

2.3.3. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Extracts

The MICs and MBCs of the extracts were determined by the tube dilution method as previously described [15]. The tuber extracts were diluted into different concentrations of 100, 50, 25, 12.5, 6.25 and 3.125 µg/mL (Met and Met:Aq), and 45.0, 22.5, 11.25, 5.62, 2.81 and 1.41 µg/mL (Aq). Methanol-water mixture was used as the control. To each of the dilutions was added 0.2ml broth culture of the test organisms. The tubes were incubated at 37^0C for 24h after which they were observed for turbidity. The lowest concentrations that exhibited least turbidity were taken as the MBCs while the highest concentrations that showed no turbidity were taken as the MICs of the respective extracts.

3.0. Results

3.1. Phytochemical composition

Phytochemical screening results (Table 1) show that alkaloids and phenols were present in all extracts; phytates and oxalates, in Met only; saponins, in Aq only; and steroids and cardiac glycosides, in Met:Aq only.

3.2. Antimicrobial susceptibility

As shown in Table 2, all the extracts displayed some antibacterial sensitivity at all doses tested most of which were of greater potency when compared to the standard drug tetracycline. Even at the least tested concentration of 3.125 µg/mL, Aq was shown to have more than double (15mm) the potency (as indicated by the size of the zone of inhibition) of tetracycline (6mm) against *Staphilococcus aureus*. Within the dose range of 3.125-12.5 µg/mL, both Met and Met:Aq were similar to the standard drug in sensitivity to this bacteria. Met showed a dose dependent sensitivity to *Shigella spp* (3.125-12.5 µg/mL) and was found to be more potent than tetracycline. At a higher

dose range (25-100 µg/mL) Aq and Met:Aq also displayed comparable sensitivity to *Shigella spp.* Aq and Met:Aq were found to be more potent (dose dependently) than tetracycline at all dose ranges against *Salmonella typhi* while Met was similar to tetracycline at all doses. For *Serratia marcescens*, all the extracts (17-27mm) had comparable activity with the standard drug (20mm). For *Pseudomonas aeroginosa* and *E. coli*, the story was different as none of the extracts (6-23mm) was as potent as the drug (25mm).

3.3. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Extracts

From the results shown in Table 3, Aq seemed to be the most potent as it had the least MIC (1.41-11.25 µg/mL) and MBC (5.63-22.5 µg/mL) against the test organisms. Similar values for Met and Met:Aq were (3.125-6.25; 12.5-50 µg/mL) and (3.125-25; 25-50 µg/mL) respectively. Therefore, the antibacterial potencies of the extracts seemed to be in the order of Aq > Met> Met:Aq.

Table 1

Comparative Qualitative Phytochemical Composition of Different Polar Solvents Extracts of Tuber Sample of *Icacina senegalensis*.

Components	Met	Aq	Met:Aq (1:1)
Alkaloids	++	++	+++
Tannins	ND	ND	ND
Saponins	ND	+	ND
Terpenoids	ND	ND	ND
Phlobatannins	ND	ND	ND
Flavonoids	ND	ND	ND
Phenols	+++	++	+++
Steroids	ND	ND	+
Cardiac glycosides	ND	ND	+
Anthraquinones	ND	ND	ND
Cyanogenic glycosides	ND	ND	ND
Phytates	+	-	-
Oxalates	+++	-	-

Keys:
- = Absent
+ = Slight presence
++ = Medium presence
+++ = Heavy presence
ND = Not detected

Table 2

Antimicrobial Activities of Methanol, water, and Methanol: Water extracts of Icacina senegalensis Tuber

Test Organisms	Tetracycline (10µg/mL)	Extract (µg/mL), Zone of inhibition (mm)																
		3.125			6.25			12.5			25			50			100		
		Met	Aq	Met: Aq	Met	Aq	Met: Aq	Met	Aq	Met: Aq	Met	Aq	Met: Aq	Met	Aq	Met: Aq	Met	Aq	Met: Aq
Staphylococcus aureus	6	6	15	6	6	12	6	6	18	6	6	17	10	6	13	12	6	11	6
Shigella dysenteriae	16	17	6	10	20	6	12	26	14	12	16	21	14	16	19	17	6	19	20
Salmonella typhi	6	6	10	10	6	13	15	6	16	16	6	14	19	6	12	16	6	13	11
Peudomonas aeroginosa	21	12	12	13	10	13	14	10	14	15	10	16	16	8	11	16	13	10	16
Serratia marcescens	20	18	20	18	20	22	20	22	24	22	23	27	17	25	27	17	27	28	17
Escherichia coli	25	6	9	6	12	12	6	18	11	6	20	11	12	22	18	18	23	12	19

Table 3

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentrations (MBC) of Tuber Extracts

Test organisms	MIC (μg/mL)			MBC (μg/mL)		
	Met	Aq	Met: Aq	Met	Aq	Met: Aq
Staphylococcus aureus	6.25	1.41	25	25	5.63	25
Shigella dysenteriae	3.125	11.25	3.125	12.5	22.5	25
Salmonella typhi	3.125	2.81	3.125	12.5	11.25	50
Peudomonas aeroginosa	3.125	2.81	3.125	25	5.63	25
Serratia marcescens	3.125	1.41	3.125	50	11.25	50
Escherichia coli	6.25	2.81	25	12.5	22.5	50

4.0. Discussion

Medicinal role of plants is attributable to their respective proximate composition and phytochemical profiles. The quality and quantity of these secondary metabolites in any concoction is also dependent upon the medium and method of extraction. In this study, only water extract had saponins in addition to alkaloids and phenols that were also extracted by methanol and methanol:water solvents. It therefore appears that saponins contributed to the higher antibacterial potency demonstrated by the water extract. This notion is in agreement with previous studies [7,16] that ascribed antibacterial potency in plants to the presence of saponins, alkaloids, and phenols, among other phytochemicals. It is interesting to note also that water was unable to extract the anti-nutrients phytates and oxalates from the tuber but methanol did. This raises a concern and questions the ability of water soaking to reduce or completely eradicate these anti-nutrients from *Icacina senegalensis* tuber meals. It is doubtful if boiling can achieve this as an earlier experiment [17] failed to improve the nutritional well-being of birds fed with diets supplemented with boiled *I. senegalensis* tuber. It is equally important to note that methanol:water solvent extraction yielded steroids and cardiac glycosides in addition to alkaloids and phenols commonly extracted by all polar solvents under study. Extracts from steroids containing plants are used traditionally to improve lactation and hormonal balance in expectant and breast-feeding mothers [18] while cardiac glycosides, though useful in the treatment of heart failure, can cause cardio-toxicity when large doses are consumed [19]. However, the level of presence of cardiac glycosides reported for *Icacina senegalensis* tuber in this study may be considered too low to cause any serious damage except under chronic consumption.

Antimicrobial potentials of several medicinal plants has received much attention [20–23] and efforts have been made to manufacture herbal based antibacterial drugs [1,5]. In this study, all the polar extracts of *Icacina senegalensis* tuber exhibited anti-bacterial potencies comparable to the standard drug, tetracycline. The ranges of MICs and MBCs of the various tuber extracts against the test organisms indicate wide spectrum antibacterial activities that cannot be over looked in the light of the present quest for herbal sources of potent and safe drugs [24,25]. Anti-plasmodial [10] and antibacterial [7] potentials have been reported for *Icacina senegalensis* leaves. The discovery of antimalarial [12] and now, antibacterial role for the tuber of this plant has opened wider the door for maximum utilization of a hitherto neglected agro-forest resource [9].

4.1. Conclusion

The different polar solvent extracts of *Icacina senegalensis* tuber exhibited wide spectrum antibacterial potencies against clinical isolates of of *Staphylococcus aureus, Shigella dysenteriae, Salmonella typhi, Peudomonas aeroginosa, Serratia marcescens*, and *Escherichia coli* in the order of water > methanol > methanol:water. This pharmacologic potential is attributed to the phytochemical profiles of these extracts. The result of this study suggests the combination of these extracts in antimicrobial drug formulation for enhanced efficacy.

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Conflict of interest: The authors declare that there is no conflict of interest regarding the publication of this paper

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