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Comparative phytochemistry and antimicrobial activities of aqueous and hydro-ethanolic extracts of *Icacina oliviformis* root tuber and fruit husk commonly used among traditional folks in Northern Ghana

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ABSTRACT

Background: Given the alarming rise in the prevalence of new and re-emerging infectious illnesses, there is a pressing need to find new antimicrobial agents with a variety of chemical structures and unique modes of action. The results of research conducted in the late 19th and early 20th centuries, as well as the introduction of streptomycin and other antibiotics, provide the foundation for testing a large number of plants for antibiotic or antimicrobial properties that are beneficial to humans. The World Health Organization (WHO) defined a plant as one that has one or more organs that contain compounds that are either therapeutically beneficial or precursors to the production of valuable medications. The global quest to develop novel drugs from natural products, particularly, has brought our attention to investigate the antimicrobial activities of *Icacina oliviformis* (*I. oliviformis*) root tubers and fruit husks against selected bacteria. **Aims and Objective:** This study evaluates the phytochemical compositions in aqueous and hydro-ethanolic extracts of *I. oliviformis* root tubers and fruit husks and compares the anti-microbial activities among the root tubers and fruit husks. **Methodology:** The phytochemical screening of the *I. oliviformis* root tubers and fruit husks was conducted using standard protocols, the antimicrobial susceptibility was conducted using the agar well diffusion methods, and the nutrient broth was used for the minimum inhibitory concentration. All laboratory analysis was conducted in triplicate to ensure consistency and precision of test results. Laboratory quality control measures were followed to ensure repeatability and prevent cross-contamination of laboratory procedures. **Results:** The phytochemical screening revealed the presence of Alkaloids, Tannins, Terpenoids, triterpenoids, saponins, anthraquinones, and cardiac glycosides in the aqueous and hydro-ethanolic extracts of *I. oliviformis* root tubers. Saponins and phenols were absent in the aqueous and hydro-ethanolic extracts of *I. oliviformis* fruit husks. The highest zone of inhibition was observed in the aqueous extract of *I. oliviformis* root tuber extract at 29 mm against Proteus mirabilis, and the lowest zone of inhibition was observed in the aqueous extract of *I. oliviformis* at 3.15 mm against Salmonella typhi. The minimum inhibitory concentrations were 1.95 mm, 3.91 mm, and 7.81 mm for *I. oliviformis* aqueous and hydro-ethanolic extracts and aqueous extract of *I. oliviformis* fruit husks, respectively. **Conclusion:** The aqueous extract showed greater antimicrobial activity than the hydro-ethanolic extract for both root tubers and fruit husks of *I. oliviformis*. The findings in the current study have validated the rationale behind its traditional use by rural folks in treating several health conditions. The current research also provides novel information on the medicinal properties of the fruit husks, even though *I. oliviformis* has been known for its root tuber medicinal properties, but the fruit husks have never been considered for use as a traditional remedy. Future research also aims to explore the safety profile of the root tubers and fruit husks as potential drugs for treating resistant bacterial strains.

Keywords: *Icacina oliviformis*, Traditional medicine, Plant extract, Phytochemistry, Antimicrobial activity.

INTRODUCTION

Infectious diseases remain the leading cause of death globally. Infectious diseases account for one-third of all deaths in tropical countries [1]. Morbidity and mortality from infectious diseases result from increased resistance of microbes [2]. Moreover, antimicrobial resistance (AMR) is a public health issue that results in huge socio-economic losses [3,4]. According to the World Health Organization (WHO), 4.95 million deaths in 2019 occurred as a result of AMR (WHO, 2023). It is estimated that by 2050, the death rate of AMR will rise to ten (10) million lives per year [3,4]. This has redirected the focus of research on alternative treatment option from plant sources [5]. Medicinal plants have been central to the healthcare practices of many cultures around the world, especially in Africa, where traditional medicine

plays a crucial role in treating various diseases [1]. Medicinal plants serve the fundamentals of traditional medicine and in the development of new drug molecules [6].

Ghana is currently working on the full formal integration of herbal remedies into the main health delivery system [7]. The integration of herbal remedies into the main healthcare delivery system is part of the World Health Organization's effort to strengthen the fight against drug resistance and to make healthcare delivery closer to the doorsteps of the ordinary citizen [7]. Among the Ghanaian public, herbal remedy is widely recognized as a complementary and alternative form of treatment alongside conventional medical care. Numerous private herbal clinics operate throughout the country, offering natural remedies for a range of health conditions [1].

Among the plants of interest is *Icacina oliviformis* (*I. oliviformis*), commonly known as false yam, which is native to West and Central Africa and is known for its ethnomedicinal uses [1]. In Ghana, *I. oliviformis* is predominantly found in the Savannah, Northern, North East, Upper East, and Upper West regions [8]. The leaves are frequently used to address a wide array of health conditions. A decoction prepared from the leafy twigs is commonly administered to treat internal bleeding, respiratory infections such as coughs and chest congestion, snake bites, and fever [9]. In cases of fever, it is traditionally recommended that patients sleep on a bed of freshly harvested leaves to enhance recovery. In Senegal, decoctions of the twigs and roots are given to adults suffering from unexplained physical weakness. Heated leaves are applied to painful areas, particularly in cases of elephantiasis, to relieve discomfort. The sap from the leaves is also employed in treating eye infections [9]. Its tubers are inherently toxic due to a bitter compound called gum resin, ranging from 0.9 % to 2.8 % [10]. It provides essential minerals and vitamins, including calcium (150 mg/100 g), iron (7 mg/100 g), thiamine (0.04 mg/100 g), riboflavin (0.18 mg/100 g), and niacin (1.4 mg/100 g) [9]. Although considered toxic under normal circumstances, the kernels can be made safe by soaking in water, changing the water daily, followed by sun drying for two days and grinding into flour. When combined with millet or beans (locally referred to as enap), this flour produces a nourishing dish regarded as suitable for guests [9,11]. Further Studies on *I. oliviformis* root tuber extracts revealed the presence of phytochemicals such as alkaloids, saponins, phenols, phytosterols, and glycosides [12]. The production of phytochemical constituents in plants is influenced by a range of natural factors [13]. These factors include the stage of development, the presence of secretory structures, climate change, geographical location, and mechanical or chemical injuries. These diverse factors collectively determine the overall yield and therapeutic efficacy of the bioactive compounds produced by medicinal plants [14].

Although the root tubers of *I. oliviformis* have been the primary focus of phytochemical and pharmacological investigations, the fruit husk remain largely underexplored despite several evidence supporting their traditional uses. The current study is therefore aimed to evaluate the scientific evidence supporting the ethnomedicinal uses of *I. oliviformis* root tuber and fruit husk among some rural communities in Northern Ghana. The findings of this study will provide scientific data for future development of novel bioactive compounds.

MATERIALS AND METHODS

Materials/Reagents/Chemicals used in the study

The materials used in this study included: Mueller Hinton agar, Nutrient broth, Ethanol, Methyl sulfoxide, Dragendorff reagent, Fehling solutions A and B, sulphuric acid, peptone water, nutrient. Nitric acid, autoclave, vortex apparatus, rotary evaporator, separating funnel, Volumetric flask, Conical flask, stirring rod, spatula, wash bottle, electronic weighing bbalance, Whatman no. 42 filter paper, Hydrochloric acid, methanoll, sulphuric acid, Chloroform, 20 % sodium hydroxide.

Identification and collection of plant materials

The root tubers and mature fruit husks of *I. oliviformis* were identified in Damango, Savannah region, and authenticated by a botanist and the root tubers and fruit husks were harvested. The authentication of the plants was recorded under the authorization number DHT0202502/12. The plant materials were washed under running water. The root tubers were chopped into smaller parts and air-dried at room temperature for twenty-one (21) days, and the fruit husks were sliced and air-dried for fourteen (14) days. The dried plant materials were pulverized and kept in air-tight containers until used. The extraction was conducted using two solvents: water and hydro-ethanol. For the hydro-ethanolic extraction, 100 g of pulverized root tubers were macerated in 1000 mL of 70 % ethanol for 72 hours with occasional shaking. For the aqueous extraction, 100 g of pulverized root tubers was weight into 1000 mL of water and extracted for 72 hours. The mixtures were filtered using Whatman filter paper No. 42. The filtrate was dried in a Freeze dryer. The process was repeated for the fruit husks of *I. oliviformis.* The dried crude extracts were kept in clean containers until used.

Phytochemical screening

Test for alkaloids

Wagner's test: A mass of 1 g pulverized root tubers and fruit husks of *I. oliviformis* extracts were measured and dissolved in 2 mL of distilled water in five test tubes. The solutions were acidified with hydrochloric acid (1.5 % v/v), and a few drops of Wagner's reagent (iodine and potassium iodide solutions) were added to the test tubes. The appearance of a reddish-brown precipitate indicated the presence of alkaloids [15].

Test for glycosides

Borntrager's test. Three (3) drops of dilute sulfuric acid were added to the extract solutions of root tubers and fruit husks of *I. oliviformis* in the test tubes, and the resulting solutions were filtered and observed. Then, two drops each of chloroform and ether were added to the filtrates and shaken. An ammonia solution was added to separate the organic layer from the aqueous layer. The appearance of a pink, red, or violet colour in the organic layer indicated the presence of glycosides [15].

Test for saponins

One spatula of each sample of root tubers and fruit husks of *I. oliviformis* was placed into five separate test tubes and diluted with 20.0 mL of distilled water. One milliliter of each sample was taken into different test tubes, corked, and shaken vigorously for fifteen minutes (15 mins). A foamy layer that persisted for 5 minutes indicated the presence of saponins in the extracts [15,16].

Test for flavonoids

Alkaline reagent test. One spatula full of root tubers and fruits husks of *I. oliviformis* was placed into separate test tubes and dissolved in 2.0 mL of distilled water. Then, 2.0 mL of 2 % NaOH solution was added to the yellow precipitate. Two drops of dilute acid (HCl) were added to the solutions. The disappearance of the yellow colour indicates the presence of flavonoids [17].

Test for tannins

Two drops of potassium dichromate solution were added to 1.0 mL of solution prepared by dissolving one spatula full of root tubers and fruit husk extracts of *I. oliviformis* in 2.0 mL of distilled water in separate test tubes. The formation of yellow precipitates indicated the presence of tannins [15,17].

Test for phenols

Root tubers and fruit husk extracts of *I. oliviformis* were dissolved in 2.0 mL of distilled water in five separate test tubes. Two drops of FeC_3 solution were added, and the appearance of a blue–green colour indicated the presence of phenol [18].

Test for steroids

One spatula each of the test samples of root tubers and fruits husks of *I. oliviformis* were dissolved in 2.0 mL of distilled water. One drop of chloroform was added to the solution. Additionally, 1.0 mL of concentrated sulphuric acid was added. The appearance of a blueish colour in the chloroform layer indicated the presence of steroids [18].

Test for terpenoids

A spatula full of the extracts of root tubers and fruit husk extracts of *I. oliviformis* was dissolved in five separate test tubes. Chloroform (2.0 mL) and concentrated sulfuric acid (3.0 mL) were added to the resulting solution. A reddish-brown precipitate at the interface confirmed the presence of terpenoids [15].

Test for anthocyanin

One spatula each of the extracts of root tubers and fruits husk extracts of *I. oliviformis* were dissolved in 2.0 mL of distilled water in five separate test tubes, treated with 1.0 mL of 2.0 M NaOH solution, and heated. The formation of a bluish-green colour indicated the presence of anthocyanins [19].

Antimicrobial Susceptibility Studies

Bacteria strain used

The following bacteria strains were used in this current study: *Proteus mirabilis, Candida albicans, Klebsiella pneumoniae, Escherichia coli, Salmonella typhimurium, and Shigella flexneri.* These organisms are common within our environment and are believed to have devastating effects on the lives of humans.

Preparation of Bacterial Suspension

The bacterial suspensions were prepared according to the methods of Vijayaram S *et al*., 2016 with slight modifications [20]. In this method, Pure colonies of *Proteus mirabilis, Candida albicans, Klebsiella pneumoniae, Escherichia coli, Salmonella typhimurium, and Shigella flexneri* were prepared in freshly prepared nutrient broth under sterile conditions. The prepared colonies in the nutrient broth were kept in an incubator at a temperature of 37°C for twenty-four (24) hours. The prepared bacterial suspensions were preserved in a 100 mL sterile flask for use. Bacteria were grown in nutrient agar broth for 24 hours at 37°C. Optical density (OD) of the cultures was measured at 600 nm. The cultures were diluted with nutrient broth to bring the OD value to 0.257, which is equivalent to a turbidity of 0.5 McFarland units.

Preparation of plant extracts

The aqueous extracts of *I. oliviformis* root tubers and fruit husk were prepared by dissolving 1000 mg/mL in Dimethylsulfoxide (DMSO) solution. The stock solution of each extract was further diluted serially to obtain concentrations of 500 mg/mL, 250 mg/mL, and 125 mg/mL for *I. oliviformis* root tubers and fruit husks. These extract concentrations were used for the antimicrobial susceptibility studies of the extracts by agar well diffusion method. Ciprofloxacin was used as positive control and Dimethylsulfoxide (DMSO) was used as a negative control.

Antimicrobial susceptibility testing of Icacina oliviformis root tubers and fruit husks by Agar well diffusion

The antimicrobial activity of *I. oliviformis* root tuber and fruit husk extracts was determined using the agar well diffusion method by [21,22] and with some slight modifications. In this method, sterilized Muller-Hinton Agar (20 mL) were poured into the petri-plates and allowed to solidify. After solidification of the media, plates were streaked with bacterial culture using a sterile cotton swab. Wells of 7 mm were bored in each Petri-plates with a sterile cork borer. The wells were filled with varying concentrations (1000 mg/mL, 500 mg/mL, 250 mg/mL, 125 mg/mL) of aqueous extracts of *I. oliviformis* root tubers and fruit husks. The extracts were allowed to diffuse into the solid agar at room temperature for about 45 minutes and then incubated at 37°C for 24 hours. The zones of inhibition of *I. oliviformis* aqueous extracts against the selected microbes were measured, and the activity indexes of the extracts on the microbes were calculated. The experiments were performed in triplicate. Ciprofloxacin and fluconazole were used as positive controls, while Dimethylsulfuoxide (DMSO) was used as a negative control. All the plates were incubated at 37°C for 24 hours.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the extracts was determined according to the protocol of Devienne & Raddi. (2002) using the broth dilution method [23]. For the Minimum inhibitory concentrations (MICs), 100 µl of sterile nutrient broth was pipetted into a sterile 96-well microtiter plate. To this, 100 µl of extract solutions (31.25, 15.62, 7.81, 3.9 mg/mL) was mixed with 100 µl of nutrient broth in the well, making two-fold dilutions. Then 100 µl of this dilution was transferred and mixed with 100 µl of the diluents in the second row, making a 4:1 dilution. This proceeds consecutively down the plate, making two-fold dilutions in each well. After the dilution of the sample is completed, an aliquot of test organisms equal to a turbidity of 0.5 McFarland. The prepared microtiter plates were incubated at 37°C for 24 hours. After 24 hours, 2,5-Diphenyl-2H-Tetrazolium Bromide (MTT) was added to each well and incubated for 3-4 hours for colour change. The lowest concentration without colour change for each isolate is taken as the Minimum Inhibitory Concentration (MIC).

RESULTS AND DISCUSSION

Medicinal plants have been the main source of treatment for many ailments among the traditional folks. Medicinal plants also played a vital role in the creation of novel chemotherapeutic drugs.

In addition to being a source of traditional medicine, plants-based chemicals are also component of majority of pharmaceutical substances [24]. The current study revealed varying phytochemicals in the aqueous and hydro-ethanolic crude extracts in *I. oliviformis* root tubers and fruit husks as evidence in Table 1. The phytochemical constituents identified in the current study are comparable to the research finding reported by [22] in *P. kotschyi* root and leaf extracts. The results of the current study are also consistent with research findings of [25], who reported the presence of flavonoids, tannins, and alkaloids in the aqueous extract of *Allium sativum*.

The variations in phytochemical constituents observed in table 1 and previous research findings may be attributed to several factors, including the maturity stage of the plant material, geographical location, processing stages, environmental conditions, and the type of solvent used for extraction [26]. Research has shown the importance of phytochemical constituents in treating several diseases in humans [26]. The antibacterial properties of *I. oliviformis* revealed in the current study may be due to the presence of these phytochemical constituents [27]. As seen in figures 1 & 2, the root tuber extracts demonstrated higher antimicrobial activities than the fruit husks of *I. oliviformis*.

The findings in figures 1 & 2 showed that the hydro-ethanolic extracts possess higher antimicrobial activities than the aqueous extracts of both root tubers and fruit husks of *I. oliviformis*. These findings are consistent with research findings by [28], who reported on similar antimicrobial activities of ethanol and aqueous extracts of common

medicinal plants used by traditional folks. Figures 1 and 2 demonstrate that the antimicrobial activities of the current study are concentration dependent; an increase in concentration demonstrated a rise in the zone of inhibitions, and vice versa [29]. According to [30], the emergence of antibiotic resistance in contemporary clinical usage is another major worry. Numerous chemical compounds with varying biological functions range from hundreds to thousands in higher plants have been profiled as potential lead compounds for the development of higher efficacy drugs [31]. The recorded antimicrobial activities of the aqueous and hydro-ethanolic extracts in the current study may be traced to the presence of the phytochemical constituents in these extracts [30]. Research findings reported by [33,34] revealed that tannins, saponins, glycosides, and alkaloids, inhibited the growth of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pyogenes* [34].

Also, the varying inhibitions recorded among the aqueous and hydro-ethanolic extracts may be due to the levels of phytochemical constituents and not necessarily the type of phytochemical constituents present [35]. According to [34], extracts from *I. oliviformis* have been shown to have antimicrobial qualities against a variety of pathogens.

Additionally, an analysis of the use of extracts from *I. oliviformis* in liquid soap compositions revealed improved antibacterial activity [36]. Tannins and flavonoids are known for their antimicrobial activities through their capacity to bind bacterial cell walls, soluble enzymes, and extracellular proteins. By breaking down the membranes of microorganisms, lipophilic flavonoids may further suppress them [37,38]. A noteworthy example is berberine, which has been shown to have anti-Trypanosoma [39] and anti-Plasmodium species properties. Alkaloids also disrupts essential cellular processes through DNA intercalation as part of its antibacterial action [40]. The aqueous extract showed higher antibacterial activity. The zones of inhibitionn for each of the extracts were compared with standard drug (positive control) (Ciprofloxacin). It was observed that inhibition zones of the extracts were lower than the inhibition zones of the standard drug (Ciprofloxacin) against all tested bacteria (Figures 1 & 2). Among the parts tested, the root tubers exhibited greater inhibition zones than the fruit husks extracts. The zone of inhibitions presented in figures 1 & 2 showed that the aqueous extracts exhibited higher inhibition against all the bacteria tested compared to the hydro-ethanolic extracts.

The minimum inhibitory concentration (MIC) was determined to be the concentration at which no bacterial growth was visually evident. The Minimum inhibitory concentration of the extract that showed activities in the agar well diffusion was considered for the minimum inhibitory concentration (MIC) studies. In this study, the concentration showing inhibition against the selected organisms were further reduced to observed the efficacy of the extract in treating bacterial infection. The MIC values vary across the organisms and extracts. The minimum inhibitory concentrations were 1.95 mg/mL, 3.91 mg/mL, for aqueous and hydro-ethanolic extracts of *I. oliviformis* root tubers respectively, 7.81 mg/mL for *I. oliviformis* aqueous extract of *I. oliviformis* fruit husks. These MIC values were the concentrations that inhibited the growth of the tested organisms. The current findings in table 2 showed higher inhibitory activities of the *I. oliviformis* extracts against the tested organisms than the research findings by [34], who reported MIC of 15.mg/mL against *Salmonella spp* and 1.56mg/mL against *Candida albicans* in his report. The current findings were found to be similar to the research findings by [36], who observed similar MIC of his crude extracts against *Pseudomonas aeruginosa* and *Escherichia coli,* and Candida *albicans.* This might be due to similarity in the permeability of the bacteria cell wall structure invaded by the different plant extracts, and also as a result of the complexity of the phytochemical constituents present in the crude extracts. The lower concentration that was able to inhibit the organisms from growing demonstrate that the extracts are very efficacious and potent against the selected bacteria.

Successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional healers or practitioners make use of water primarily as a solvent, and our studies confirmed the water (aqueous) extracts of the studied plants parts were certainly much better and powerful than the hydro-ethanolic extracts.

Table 1: Comparative phytochemical constituents of root tuber and fruits husks of *Icacina oliviformis* extracts and pulverized sample

Test	***Icacina oliviformis* root tuber**			***Icacina oliviformis* fruits husks**		
	Pulverized sample	**Aqueous extract**	**Hydro-ethanolic**	**Pulverized sample**	**Aqueous extract**	**Hydro-ethanolic**
Alkaloids	+	+	-	+	+	-
saponins	+	+	+	+	-	-
Flavonoids	-	-	-	-	+	+
Anthraquinones	+	+	+	+	-	-
Terpenoids	+	+	`	+	-	+
Triterpenoids	+	+	`	+	+	-
Cardiac glycosides	+	+	+	+	+	+
Phenol	-	-	-	-	-	-
Tannins	+	+	+	+	+	+

+: Present, - : Absent

Table 2: Minimum inhibition concentration of *Icacina oliviformis* aqueous and hydro-ethanolic extract

Concentration (mg/mL)	*Icacina oliviformis* root tuber		Icacina oliviformis fruits husks	
	Aqueous extract	Hydro-ethanolic extract	Aqueous extract	Hydro-ethanolic
31.25	+	+	+	-
15.63	+	+	+	-
7.81	+	+	+	-
3.91	+	+	-	-
1.95	+	-	-	-
0.98	-	-	-	-

+= means it is susceptible, -= means it is not susceptible

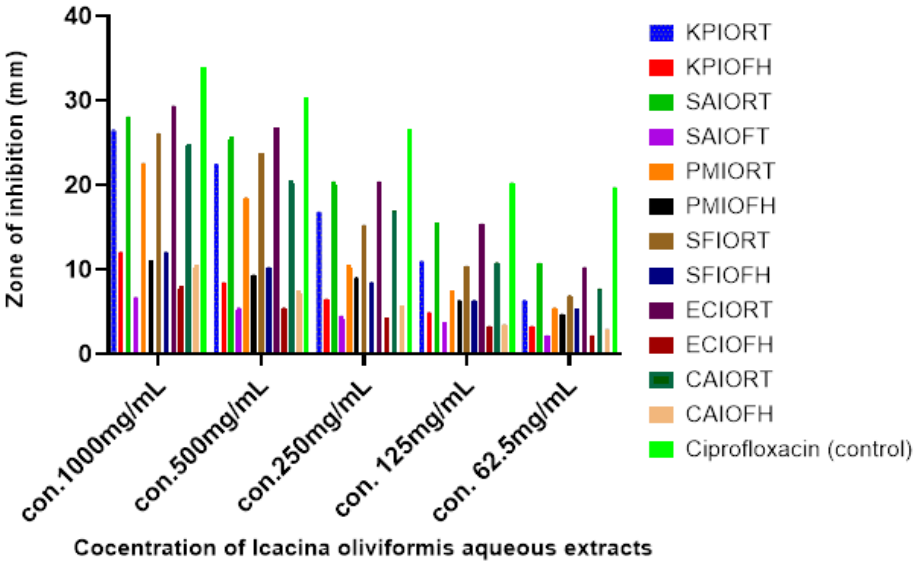

Figure 1: Comparative antimicrobial activities of aqueous extracts *Icacina oliviformis* root tubers and fruit husks

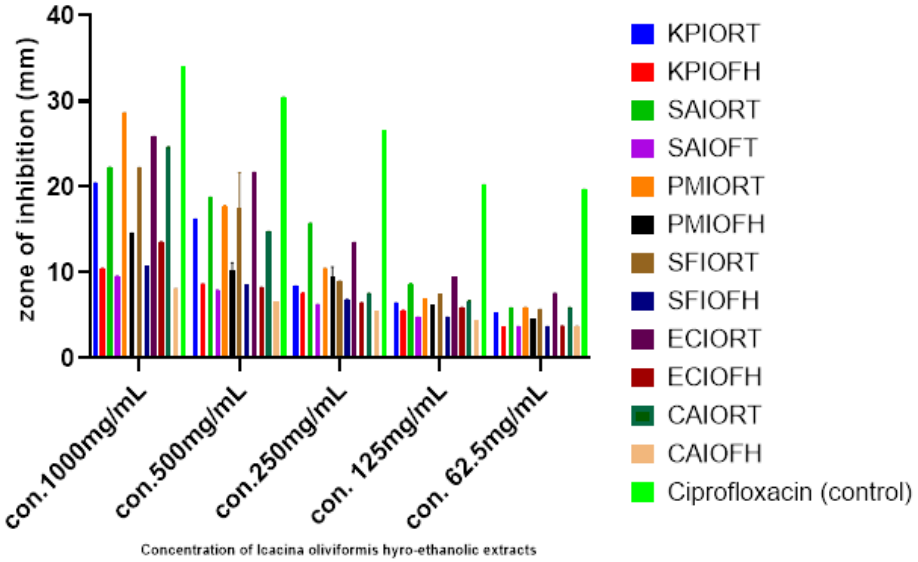

Figure 2: Comparative antimicrobial activities of hydro-ethanolic extracts of *Icacina oliviformis* root tubers and fruits husks

CONCLUSION

The current study revealed the presence of alkaloids, terpenoids, triterpenoids, saponins anthraquinones, cardiac glycosides in the aqueous extract of *I. oliviformis root* tuber while flavonoids and phenols were absent in the hydro-ethanolic extract of root tuber. The fruit husks aqueous extract also revealed the presence of saponins, terpenoids, triterpenoids, flavonoids, tannins, but flavonoids, saponins, phenols were absent in the hydro-ethanolic extract. The antimicrobial susceptibility studies demonstrated varying zones of inhibitions of *I. oliviformis* extracts against the tested bacteria strains with the highest zone of inhibition at 29 mm against Proteus mirabilis and the lowest zone of inhibition observed as 3.15 mm against *Salmonella typhi*. The minimum inhibitory concentrations (MIC) were 1.95 mm, 3.91 mm, 7.81 mm for *I. oliviformis* aqueous and hydro-

ethanolic extracts and aqueous extract of *I. oliviformis* fruit husks respectively. The findings suggest aqueous extracts demonstrated greater antimicrobial activities than the hydro-ethanolic extracts. The extracts' minimum inhibitory concentrations were rather high, which may be due in part to their crude nature and the variety of unidentified compounds that made up the weight for concentrations. To ascertain their safety in conventional usage, more study is necessary. However, certain extracts shown cytotoxicity, which may be connected to distinct chemicals than those that provide the antimicrobial properties. The current findings have provided enough information on the phytochemical composition and antimicrobial profile of the root tubers and fruit husks. This has validated the rationals behind its traditional used by rural folks in treating several health conditions. The current research also provides novel information of the medicinal properties of the fruit husks. Even-though *I. oliviformis* has been known purposely for its root tuber medicinal properties, the fruit husks have never been considered for use as a traditional remedy.

Conflict of interest

The authors declared no conflict of interest.

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