PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF *MANIHOT ESCULENTA* LEAF EXTRACTS AGAINST SOME CLINICAL ISOLATES

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ABSTRACT
Plant extracts are natural products with active substances which are readily available and effective as powerful therapeutic compounds against bacteria and pathogens, acting as antibiotics and antimicrobiotics. This study evaluates the phytochemical profile and antimicrobial activity of cassava manihot esculenta leaf extracts against some clinical isolates (C. albicans, S. aureus, P. aeruginosa, Salmonella sp) with a view of its potential therapeutic and justifying it use in traditional medicine. Phytochemical screening, biochemical and agar diffusion standard procedures were employed. Phytochemicals namely alkaloids, cyanide, flavonoids, saponins, phytate and tannins were found in manihot esculenta leaf extract with absence of terpenoid and glycosides. Manihot esculenta leaf extract was found promising against S. aureus, P. aeruginosa and Salmonella spp at concentration of 25, 50 and 100 mg/ml. No activity was exhibited against, Candida albicans at all the test concentration. Furthermore, the extract was also found likely to act against both S. aureus and P. aeruginosa with a similar MIC of 50 mg/ml and an MBC of 25 mg/ml. These findings suggest the potential of Manihot esculenta ethanolic leaf extract to be used in the management of infections caused by P. aeruginosa and S. aureus. Manihot esculenta (cassava) plant can thus, serve not only as source of food source but, also as an antimicrobial agent.
Keyword: Antimicrobial, Cassava leaf, Ethanol, Inhibitory zone, Salmonella.

INTRODUCTION

Manihot esculenta, known as cassava, tapioca in Asia; *manihot* in Africa; *manioca Yucca* and *mandioca* in Latin America, is both a food source and an important medicinal plant that belong to the family *Euphorbiaceae* (Imane *et al*, 2022). It is a perennial woody shrub of the savanna that has been extensively captivated as an annual crop in tropical and subtropical regions for it edible starchy and tuberous root (Ojuku and Chidiebere, 2018). As food products, cassava tuber has been processed as tapioca (cassava starch), fufu (cassava flour boiled in water), garri (ferment cassava mash) and ethanol production (Abdulahi and Haque, 2020). Its leaf is useful as vegetable, ethanol, insecticide, feed additives and medicinal preparation (Imane *et al*, 2022). Thus, may have application in improvement of animal health and welfare. Plant extracts are of great importance to the body as a natural antimicrobial agent against bacterial (Ahmed *et al*, 2022)

Plants with various medicinal properties have been source of attraction for many scientists all over the world since thousands of years ago. Millions of plants have been studied extensively since ancient time for various phytochemicals and their possible medicinal uses in various disease conditions (Germaine and Francois, 2021). They have been employed as anti-fungi, anti-bacterial, anti-cancers and antispasmodics (Oladunjoye *et al*, 2022). As such, modern-day treatment strategies do not underestimate potential of herbs for various chronic illness. Recently, there has been a tremendous increase in the use of plant-based health product in developing as well as develop countries resulting in an exponential growth of herbal products globally (Pravin *et a*l, 2012). There is therefore, the need for researchers to evaluate and characterize various plants and their constituents against large array of microbial pathogens (Ojuku and Chidiebere, 2018). Previous studies have reported the antimicrobial activity of extracts of *Manihot esculenta* against both gram Positive bacteria (*Staphylococcus aureus, Streptococcus mutans, Bacillus subtilis, Bacillus megaterium*), Gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae*) and fungi (*Aspergillus niger, Candida albicans and Penicillium chrysogenum*) (Ahmed *et al.,* 2022). Therefore, this study aimed at assessing antimicrobial efficacy of *ethanolic extract from Manihot esculenta* leaf against *some microorganism isolate* as an alternative to synthetic antibacterial drugs.

MATERIALS AND METHODS

Experimental Location

The experiment was conducted at the Department of Animal Health and Production Technology Laboratory, School of Agricultural Technology, Itakpe, Kogi State while, the cultured sample was carried out at the Science Laboratory, Kogi State Polytechnic, Lokoja.

Sample collection and preparation.

Cassava (*Manihot esculenta*) leaves were gotten from farmers in Itakpe farm, Okehi Local Government, Kogi State. The taxonomic identity of the plant materials was confirmed by a Botanist in the Department of Horticulture and Landscaping, School of Agricultural Technology, Itakpe, Kogi State. A voucher specimen with reference number M123 was deposited in the herbarium of the Department of Horticultural Science. The leaves were air dried at room temperature of 29ºC for two weeks and then pulverized into powder using clean mortar and pestle. The powdered leaf meal was stored in air tight container prior to extraction.

Test isolates Sample

S. aureus, Salmonella spp, P. aeruginosa and C. albicans cultured isolates were obtained from Microbiology laboratory, Kogi State Polytechnic, Lokoja. The taxonomic identity of the isolates was confirmed using grams staining and biochemical tests. Inocula of the test isolates were prepared and standardized using McFarland standard (Abdulahi and Hague, 2020).

Extraction of Cassava leaf

Fifty grams of pulverized cassava leaf were weighed using digital weighing balance (Shimdzu, Japan), percolated in 500ml ethanol in a 1000ml conical flask, covered with cotton wool and aluminum foil and the preparation was allowed to stand for 72 hours after which, it was filtered using Whatman's No 1 filter paper. The filtrate was concentrated on water bath set at 78ºC. The extract was transferred into a sterile, airtight bijou bottle and stored in the refrigerator at 4ºC until use.

Phytochemical screening of manihot esculenta leaf extract.

The cassava leaf extract was screened for the presence of alkaloids, cyanide, flavonoids, anthraquinone, saponins, phytate, terpenoids, glycosides and tannins according to the method of Ahmed *et al*. (2022).

Antimicrobial activity of Cassava Ethanol Leaf Extract

This was done using agar well diffusion method as described by Abdulahi and Haque (2020). Three concentrations (100 mg/ml, 50 mg/ml and 25 mg/ml) of the plant extract were prepared and introduced into different holes bored onto sterile medium containing the test isolates. The plates were incubated uninvertedly at 37ºC for 24 hours. Areas that showed clear zones around the bored holes indicate the susceptibility of the test isolates to the extract and these were measured using centimeter rule and recorded in mm.

Minimum Inhibitory Concentrations

Three concentrations namely 100m/ml, 50m/ml and 25 m/ml of the cassava ethanol leaf extract were introduced into different test tubes in the sterile medium containing the standardized inocula of the test isolates. The test tubes were incubated at 37ºC for 24 hours. The dilutions that first showed no sign of visible turbidity were taken as minimum inhibitory concentration.

Minimum fungicidal concentrations

The test tubes with fungal standardized inocula that showed no visible turbidity after overnight incubation were subcultured on sterile potatoes dextrose agar plates and incubated at 35ºC for 24 hours. The concentration that showed no visible growth after incubation was considered as minimum fungicidal concentration (Abdulahi and Haque, 2020).

Minimum Bacterial Concentrations

The test tubes of the MIC that showed no visible inocula turbidity were sub cultured on sterile nutrient agar plates and incubated at 37ºC for 24 hours. The plates that showed no visible growth were the minimum bactericidal concentrations.

RESULTS AND DISCUSSION

Phytochemical profile of Cassava ethanol leaf extract

The result of phytochemical screening of cassava ethanol leaf extract demonstrated the presence of alkaloids, cyanide, flavonoids, anthraquinone, saponins, phytate and tannins with the absence of terpenoid and Cardiac glycosides (Table 1). The occurrence of these phytochemicals suggest potential of being harnessed as candidate for drug development to be employ in the management of infections. This agrees with previous findings of vast arrays of phytochemicals in the tropical plants (Pravin *et al*, 2012 & Ahmed *et al*, 2022). It also revealed anti-inflammatory nature, antioxidant and antibacterial profile of cassava leaf extract. Ojuku and Chidiebere (2018) affirmed that Cassava leaf extract possess potent bioactive compounds potent as antifungal and antibacterial due to ability of its extract to elicit protein denaturation of microbial and bacterial isolates cell wall. The antibacterial properties may be as a result of their free radical scavenging and metal ion chelating activities (Germain and Francois, 2021).

Table 1: Phytochemical profile of *Manihot esculenta* ethanol leaf extract

	Phytochemical Profile						
Extract	**Alkaloid**	**Cyanides**	**Flavonoid**	**Phytate**	**Saponin**	**Glycoside**	**Terp.**
Cassava leaf	+	+	+	+	+	-	-

+ = presence of phytochemicals - = absence of phytochemicals

Antimicrobial Activity of Cassava ethanol leaf extract

The cassava ethanol leaf extract was found promising as it inhibited the growth of *S. aureus* at 50 mg/ml (zone of inhibition = 15mm), *Salmonella spp and P. aeruginosa at* 100 mg/ml and 50 mg/ml, with 18.7- and 17.6-mm zones of inhibition, respectively. *C. albicans* was found to be resistant at 100 mg/ml, 50 mg/ml and 25 mg/mm. These variations could suggest difference in action of different concentration of cassava leaf ethanolic extract. Oladunjoye *et al*. (2022) observed that higher volatility of ethanol tends to extract more active compounds which may affect the rate of action on isolates. The resistance phenomenon exhibited by *C. albicans* to all the tested concentration may be due to the absence of other phytochemicals that may inhibit fungus actions due to presence of certain cell constituents present in *C. albicans* that may protect it from being inhibited by cassava ethanolic leaf extract as earlier observed by Abdullahi and Haque (2020).

Minimum inhibitory concentration (MIC), minimum bacterial concentration (MBC) and minimum fungicidal concentration (MFC) of *Manihot esculenta* leaf extract against *P. aeruginosa, S. aureus, Salmonella spp and C. albicans.* The MIC, MBC and MFC results indicated that *M. esculenta* was found promising against both *S. aureus, Salmonella spp and P. aureginosa,* with a similar MIC of 50 mg/ml and MBC of 25mg/ml. MFC also, recorded similar value of 25mg/ml for *S. aureus and Salmonella* spp. Variation in the zone of inhibition exhibited by extracts against the test bacteria can possibly be due to their relative composition of cell wall components (Imane *et al*, 2022). Similarly, results for antibacterial actions suggest Cassava leaf extract as promising against test isolates further, supporting its use in folklore medicine. This finding is similar to that of Germaine and Francois (2021) who reported that *M. esculenta* ethanol leaf extract was promising on *P. aureginosa* at the concentrated tested. Additionally, a prior study of Oladunjoye *et al*. (2022) reported that the extracts of cassava leaf showed strong antimicrobial activities against *S. aureus* and *Salmonella spp* isolates.

Table 2: Antimicrobial Effect of Ethanol Extract of Cassava Leaves against S. aureus, *Salmonella Spp and C. albicans*

	Zone of Inhibition (mm)		
Clinical isolates	**100%**	**50%**	**25%**
S. aureus	12.41	15.00	11.31
Salmonella sp	18.70	17.60	8.3
P. aeruginosa	18.72	17.63	8.2
C. albicans	0	0	0

Table 3: MIC, MBC and MFC of *M. esculenta* ethanol leaf extract against selected isolates.

		Varied concentration (mg/ml) of *M. esculenta* leaf extract.	
Clinical isolates	**MFC**	**MIC**	**MBC**
S. aureus	25	50	25
Salmonella sp	25	50	25
P. aeruginosa	0	50	25
C. albicans	0	0	0

CONCLUSION

Manihot esculenta (Cassava) leaf extract demonstrates promising antibacterial activity against *S. aureus, Salmonella spp, P. aeruginosa and C. albicans* due to the presence of a vast array of phytochemicals. There is need therefore to purify, isolate and characterize the phytochemical constituents for use as drug supplements.

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