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Phytochemical Investigation and Antimicrobial Activity of Hexane, Ethyl Acetate and Methanol Fractions from Stem Bark of *Icacina Trichantha* Oliv. (Icacinaceae)

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ABSTRACT

Background: Micro-organisms are responsible for the transmission of a large number of diseases. It is hard to comprehend the amount of diseases, deaths and economic losses caused by micro-organisms alone. Plants are good sources of eco-friendly and readily available antimicrobial agents.

Purpose: The aim of this study was to evaluate the chemical constituents and antimicrobial characteristics of three fractions from ethnomedicinal *Icacina trichantha*. Oliv. (Icacinaceae).

Methods: Methanol extract from *Icacina trichantha*. Oliv was obtained by maceration and fractionated successively using hexane, and ethyl acetate. The antimicrobial properties of Icacina trichantha. Oliv was assessed using agar cup diffusion method on *MRSA, P. aeruginosa, S. typhi, C. krusei, S. dysenteriae, S. pyrogenes, E. coli, K. pneumoniae, C. albicans*, and *C. tropicalis*. Phytochemical screening on fractions was also evaluated using standard methods.

Results: Phytochemical screening on fractions revealed the presence of saponins, alkaloids, steroids, tannins, and glycosides. Agar diffusion assay on fractions showed growth inhibitory effect on all the organisms except *P. aeruginosa, S. typhi*, and *C. tropicalis*. The MIC revealed that n-hexane fraction was active against *MRSA, S. pyrogenes, E. coli, K. pneumonia, C. albicans* and *C. krusei* at 10 mg/mL while S. dysenteriae was active at 5 mg/mL. The ethyl acetate fraction was active against all the organisms at a concentration of 5 mg/mL except *P. aeruginosa, S. typhi and C. tropicalis*. Methanol fraction showed activity of 5 mg/mL against *MRSA, S. pyrogenes, E. coli, S. dysenteriae, C. albicans* and *C. krusei* except for *K. pneumoniae* with activity at 10 mg/mL. Minimum bactericidal concentration/fungicidal concentration MBC/MFC evaluated on the n-hexane fraction revealed that *MRSA, S. pyrogenes, E. coli, K. pneumoniae, S. dysenteriae, C. albicans*, and *C. krusei* were active at 20 mg/mL, while the ethyl acetate fraction had MBC/MFC of 10 mg/mL against all the organisms except *P. aeruginosa, S. typhi, C. tropicalis*. Methanol extract had MBC/MFC of 10 mg/mL against *MSRA, E. coli* and *S. dysenteriae* whereas *S. pyrogenes, K. pneumoniae, C. albicans* and *C. krusei* had MBC/MFC at 20 mg/mL.

Conclusion: *Icacina trichantha*. Oliv. contain constituents with concentration dependent antimicrobial properties based on type of organism. The plant could be useful in the prevention and treatment of multi-resistant disease causing microorganisms.

1. Introduction

Medicinal plants have been used to treat health disorders for centuries all over the world in order to develop low cost and readily available therapeutic compounds (Elisabetsky, 1991). These bioactive constituents from plant fashion are potent antimicrobial agents used for combating microorganisms such as bacteria, fungi and protozoa (Krishnaiah et al., 2009). People are becoming increasingly attracted to the use of these plants because of their good therapeutic performance, toxicity, and affordability. Many research efforts have been directed towards the provision of empirical proofs to back up the use of plants species in medicinal practices (Ojo et al., 2005). Researchers have examined the effects of plants used

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traditionally by indigenous healers to support treatment of various diseases; scientific validations are being made globally to get evidence for traditionally reputed herbal plants. However, there are several plants with tremendous medicinal potentials that have not been investigated. In many developed countries, 70-80% of the population have used some forms of alternative or complementary medicine e.g. acupuncture, while in most African countries 80% of the population depend on traditional medicine for primary health care (WHO, 2002).

Icacina trichantha Oliv. an Icacinaceae, is a drought-resistant shrub indigenous to West and Central Africa. Natives of indigenous tribes in Nigeria, commonly use it as a medicinal plant. Among the Igbos in Nigeria, it is known as "Urumbia" or "Eriagbo" especially with respect to its emetic effect while Yorubas in western Nigeria, refer to it as "Gbegbe" which means to cleanse (Asuzu & Abubakar, 1995). The *plant* is a shrub that can grow up to two meters high (Burkill, 1994). It is characterized by large, fleshy, yam-like underground tubers, as big as several kilograms in weight, which are often consumable. The tuber is said to be rich in starch and can be eaten fresh or processed into flour to make soup, pastes or porridges (Umoh & Iwe, 2014). It has been reported to serve as a source of emergency food energy during long periods of drought or famine. Both the nutrient composition and "anti-nutritional factors of the flour have been analyzed, showing the presence of not only carbohydrates (mostly starch), lipids and proteins, but also mineral elements such as potassium, sodium and calcium (Umoh, 2013; Umoh & Iwe, 2014; Udofia et al., 2014). The fruit of this plant is a drupe with a soft sweet outer pulp which is edible (Burkill, 1994).

Phytochemical screening of the leaf extract of *I. trichantha* has shown the presence of alkaloids, tannins, phenols and saponins (Timothy & Idu, 2011; Shagal & Kubmarawa, 2013; Otun et al., 2015). Fatty acid such as stearolic acid, oleic acid and erucic acids have also been reported (Otun et al., 2015). The presence of alkaloids, tannins, saponins, steroids, carbohydrates and cardiac glycosides has been reported in the tuber (Shagal et al., 2014; Edori & Ekpete, 2015).

Ethnomedicinally, *I. trichantha* is widely used as a common household medicine for emergency and first-aid treatment for food poisoning (Mbatchou & Dawda, 2012). Tubers and leaves of the plant are allegedly aphrodisiacs (Burkill, 1994 & Quattrocchi, 2012). The leaves and seeds, when crushed and macerated in local gin, can be used for the treatment of hypertension and asthma (Ajibesin et al., 2008). Tubers are said to be used by traditional healers to treat various medical conditions including constipation, poisoning, malaria, rheumatism, toothache, as well as to induce emesis and abortion (Asuzu & Abubakar, 1995; Ariwaodo et al., 2012). Ubom (2010), reported that the tuber juice can be used for treating mumps. Leaf methanol extract have anti-convulsion effect in mice pretreated with pentylenetetrazole, as well as increased pentobarbitone-induced sleeping time (Asuzu & Aforonwa, 1998). The anti-inflammatory activity of the Chloroform extract of the tuber in Laboratory mammals have been reported (Asuzu et al., 1999). *In vitro* screening of the leaf, wood, and root parts of *I. trichantha* have reportedly exhibited moderate levels of antioxidant activity with respect to 2, 2-diphenyl-picryl-hydrzyl radical (Oke & Hamburger 2002; Udeh & Nwaehujor, 2011). The *in vitro* antimicrobial activity of *I. trichantha* leaf was first observed in *Pseudomonas aeruginosa* and *Escherichia coli (*Timothy and Idu, 2011); both ethanol and aquous extracts were reported to be active against *Staphylococcus aureus*, *Candida albicans*, and *Klebsiella pneumonia* (Shagal & Kubmarawa, 2013). Other reports have it that the hexane and ethyl acetate extracts were active against *E. coli*, *P. aeruginosa* and *K. oxytoca* (Otun et al., 2015).

In the light of the foregoing, this current research was focused on the stem bark of *I. tricantha* with the sole aim of evaluating its phytochemicals as well as screen different extracts (hexane, ethyl acetate and methanol) for its anti-microbial properties.

2. Materials and Methods

2.1. Collection of Plant Materials

Fresh stem bark of *Icacina trichantha* Oliv. was collected from the Forest Research Institute of Nigeria (FRIN), Ibadan Nigeria and authenticated in the Forest Research Herbarium, where voucher samples were deposited with specimen voucher number FIH-112033. The plant sample was sorted, cut and air-dried for eight weeks.

2.2. Extraction and Phytochemical Screening

The root bark was air-dried and crushed to a coarse powder. The powdered root bark (200g) was successively fractionated with hexane, ethyl acetate and methanol till exhaustion. All fractions were evaporated in a rotary evaporator at 25^0C under reduced pressure (Kokate et al., 2007) and screened. All the plant extracts were screened for the presence of various bioactive compounds using standard methods (Sofowora, 1993; Trease, 1989; Harbone, 1973).

2.3. Antibacterial and Antifungal Screening

The antibacterial and antifungal activities of the fractions were assayed as described by (Adekunle, 2009). The standardized suspensions were used to inoculate the surfaces of Muller Hinton agar plates (90 mm in diameter) using a sterile cotton swab. 6 mm diameter wells were bored using sterile cork borer in agar and filled with the desired concentrations of the plant extract of 20 mg/mL, 10 mg/mL, 5 mg/mL, 2.5 mg/mL and 1.25 mg/mL. Commercial antibiotics (ciprofloxacin 5μg/disc and fluconazol 5μg/disc) were used as a reference standard to determine the sensitivity of the isolates. The plates were allowed to stand for 5 hours at roomtemperature for extracts to diffuse into the agar and then incubated at 37°C over night. The zone of inhibition was measured using a transparent ruler.

Results

Table 1: Results of phytochemcial screening of the stem bark of *Icacina trichantha.*

Secondary Metabolites	Hexane fractions	EtOAc fractions	Methanol fractions
Alkaloids	+	+	+++
Saponins	–	+	+
Steroids	+++	++	+
Tannins	+	+	+
Flavonoid	–	–	–
Glycosides	–	++	+
Phytobatannins	–	–	–
Anthraquinone	–	–	–
Coumarins	–	–	–

Key: + = Present, – = Absent ++ =Strong +++ = Very strong

Table 2: Results of antimicrobial susceptibility test against the test microbes.

Test organism	n-HE	EA	ME	Ciprofloxacin (5μg/disc)	Fluconazol (5μg/disc)
MRSA	S	S	S	R	R
S. pyrogenes	S	S	S	R	R
E. coli	S	S	S	R	R
K. pneumoniae	S	S	S	R	R
P. aeruginosa	R	R	R	R	R
S. typhi	R	R	R	S	R
S. dysenteriae	S	S	S	S	S
C. albicans	S	S	S	R	S
C. krusei	S	S	S	R	S
C. tropicalis	R	R	R	R	S

Key: HE= Hexane fraction, EA =Ethyl acetate fraction, ME= Methanol fraction

Table 3: Results of zone of inhibition of the Stem bark extract against the test microbes

Test organism	n-HE	EA	ME	Ciprofloxacin (5μg/disc)	Fluconazol (5μg/disc)
MRSA	18	25	22	0	0
S. pyrogenes	18	24	21	34	0
E. coli	17	26	22	39	0
K. pneumoniae	18	24	20	30	0
P. aeruginosa	0	0	0	0	0
S. typhi	0	0	0	41	0
S. dysenteriae	20	26	23	40	0
C. albicans	17	23	20	0	32
C. krusei	16	24	21	0	38
C. tropicalis	0	0	0	0	35

Key: HE= Hexane fraction, EA =Ethyl acetate fraction, ME= Methanol fraction

Table 4: Result of MIC of the Stem bark extract against the test microbes.

Test organism	Concentration in mg/mL		
	HE	EA	ME
MRSA	10	5	5
S. pyrogenes	10	5	5
E. coli	10	5	5
K. pneumoniae	10	5	10
P. aeruginosa	-	-	-
S. typhi	-	-	-
S. dysenteriae	5	5	5
C. albicans	10	5	5
C. krusei	10	5	5
C. tropicalis	-	-	-

Key: HE= Hexane fraction, EA =Ethyl acetate fraction, ME= Methanol fraction

Table 5: Result of MBC/MFC of the Stem bark of *Icacina trichantha* against the microbes.

Test organism	Concentration in mg/mL		
	n-HE	EA	ME
MRSA	20	10	10
S. pyrogenes	20	10	20
E. coli	20	10	10
K. pneumoniae	20	10	20
P. aeruginosa	-	-	-
S. typhi	-	-	-
S. dysenteriae	20	10	10
C. albicans	20	10	20
C. krusei	20	10	20
C. tropicalis	–	–	–

Key: HE= Hexane fraction, EA =Ethyl acetate fraction, ME= Methanol fraction

Discussion

Phytochemical analysis carried out on the stem bark methanol extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities (Harbone, 1973). The phytochemical constituents of *Icacina trichantha* tested were summarized in table 1. Alkaloids, steroids, and tannins Flavonoid were found to be present in all the three extracts; saponins and glycosides were present in ethyl acetate and methanol only. Flavonoids, phlobotannins, free anthraquinone and coumarins were absent in all the plants extract. Previous phytochemical screening carried out on *Icacina trichantha* showed the presence of saponins, alkaloids, tannins, glycosides anthraquinone and flavonoids (Yahaya et al., 2012). Tannins have been reported to have antibacterial potential due to their basic character that allows them to react with proteins to form stable water soluble compounds thereby killing bacteria by directly damaging its cell membrane. Steroids have been reported to have antibacterial properties and they are very important compounds especially due to their relationship with compounds such as sex hormones. The antibacterial activities of alkaloids have been reported by a number of authors (Hassan et al., 2005; Aliero et al., 2008; Yesmin et al., 2008). Generally, the antimicrobial properties observed in plant are attributed to the secondary metabolites (Okoli & Iroegbu., 2005; Akinyemi et al., 2005).

Table 2 shows the activity of each plant extracts against the microorganisms. The result revealed that *MRSA, S. pyrogenes*, *E. coli*, K. *pneumonia, S. dysenteriae*, *C. albicans* and *C. krusei* were sensitive to the fractions whereas *P. aeruginosa*, *S. tyhi and C. tropicalis* were found to be resistant to all the fractions. This is a clear indication that the plant could be used to treat dysentery, coughs, stomach aches, diarrhea and new wounds. The success of treating diarrhoea and stomach aches attributed to *S. aureus* and *E. coli* could be due to the antibacterial effects of alkaloids, saponins and steroids (Vieira et al., 2001). The result showing zone of inhibition table 3 revealed that the n-hexane extract had significant zone of inhibition between **16** mm to **20** mm, the methanol extract had considerable zone of inhibition of **21** mm to **23** mm while the ethyl acetate fraction showed the highest zone of inhibition of between **23** mm to **26** mm. All the fractions showed lower but comparable zone of inhibition against the microorganism with the standard drugs used (Ciprofloxacin and Fluconazol). The standard drugs ciprofloxacin and fluconazol are higher in performances with greater zone of inhibition than the fractions; this is possibly because of impurities which interfere with their various activities. However, when the active compounds are isolated the fractions may give higher activity as the standard drugs. The plant showed good *in-vitro* antimicrobial profile especially against *MRSA, S. pyrogenes* and *E. coli* which are known to cause infections that are difficult to treat due to their multiple drug resistance. Furthermore, the extracts revealed activity against gram positive and gram negative bacteria as well as some fungi species, these findings indicate that the extracts had broad spectrum activity against a wide range of infectious agents that could be the basis for the folkloric use of the plant. The result of minimum inhibitory concentration (MIC) Table 4 shows that n-hexane had MIC of 10 mg/mL against *MRSA*, *S. pyrogenes*, *E. coli*, *K. pneumonia*, *C. albicans* and *C. krusei* except for *S. dysenteriae* with MIC of 5 mg/mL. The ethyl acetate fraction showed MIC of 5mg/mL against all the organisms except *P. aeruginosa*, *S. typhi* and *C. tropicalis*. The methanol fraction had MIC of 5 mg/mL against all the microorganisms except *K. pneumonia* with MIC at 10 mg/mL. The result of the MBC/MFC table 5 shows that n-hexane fraction had MBC/MFC at 20 mg/mL against all the organisms except *P. aeruginosa*, *S. typhi* and *C. tropicalis*. The ethyl acetate fraction had MBC/MFC of 10 mg/mL against all the organisms except *P. aeruginosa*, *S. typhi* and *C. tropicalis*. The methanol fraction had MBC/MFC of 10 mg/mL against *MRSA, E.coli* and *S. dysenteriae* whereas *S. pyrogenes, K. pneumonia, C. albicans* and *C. krusei* had MBC/MFC at 20 mg/mL.

Conclusion

Phytochemical screening on fractions revealed the presence of saponins, alkaloids, steroids, tannins and glycosides. Agar diffusion assay on fractions showed growth inhibitory effects on all the organisms except *Pseudomonas aeruginosa, Salmonella typhi,* and *Candida tropicalis*. The MIC revealed that n-hexane fraction was active against *MRSA*, *S. pyrogenes*, *E. coli, K. pneumonia, C. albicans* and *C. krusei* at 10 mg/mL while *S. dysenteriae* was active at 5 mg/mL. The ethyl acetate fraction was active against all the organisms at a concentration of 5 mg/mL except *P. aeruginosa*, *S. typhi* and *C. tropicalis.* Methanol fraction showed activity of 5 mg/mL against *MRSA, S. pyrogenes, E. coli, S. dysenteriae, C. albicans* and *C. krusei* except for *K. pneumoniae* with activity at 10 mg/mL. Minimum bactericidal concentration/ fungicidal concentration MBC/MFC evaluated on the n-hexane fraction revealed that *MRSA, S. pyrogenes, E. coli, K. pneumoniae, S. dysenteriae, C. albicans* and *C. krusei* were active at 20 mg/mL, while the ethyl acetate fraction had MBC/MFC of 10 mg/mL against all the organisms except *P. aeruginosa, S. typhi,* and *C. tropicalis.* Methanol extract had MBC/MFC of 10 mg/mL against *MSRA, E. coli* and *S. dysenteriae* whereas *S. pyrogenes, K. pneumoniae, C. albicans* and *C. krusei* had MBC/MFC at 20 mg/mL. The result presented a broad spectrum of reliable and non-toxic phytochemicals in *Icacina trichantha* which could be useful in the therapeutic intervention of multi-resistant microorganisms.

Conflict of Interest

The authors declare that there is no conflict of interest.

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