



## INTERNATIONAL JOURNAL OF SCIENCE FOR GLOBAL SUSTAINABILITY

### Studies on *In-vitro* Bioactivity of *ICACINA TRICHANTHA* Methanol Seed Extract

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Received on: January, 2023

Revised and Accepted on: February, 2023

Published on: March, 2023

#### ABSTRACT

Plants are new sources of antioxidative and antimicrobial agents, hence the need to determine and evaluate the antibacterial, antifungal properties and antioxidant activity of medicinal plants. The aim of this study was to investigate antioxidant and antimicrobial potentials of methanol extract of *ICACINA TRICHANTHA* Oliv. (ICACINACEAE) seed. In this research work, antioxidant study was done by using various *in vitro* methods such as: 1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2-azinobis (3-ethylbenzthiazoline)-6-sulfonic acid (ABTS), total antioxidant capacity (TAC), nitric oxide (NO) scavenging, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) scavenging, ferric reducing antioxidant power, superoxide radical scavenging and hydroxyl radical scavenging assays. Antimicrobial properties were determined 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*. The extract of the studied plant exhibited pronounced antioxidant activities in the various antioxidant activity assay tested. Also, the extract had remarkable susceptibility effect on the tested microorganism with the exception of *P. aeruginosa*, *S. typhi* and *C. tropicalis* that were resistant. The findings indicate that the extract was found to be effective free radical quencher, a potent source of natural antioxidants and antimicrobial agents, thus justifying its traditional use in green therapeutics.

**Keywords:** *ICACINA TRICHANTHA*, antioxidant, antimicrobial, DPPH, herbal therapy

#### 1.0 INTRODUCTION

Free radicals are unstable and highly reactive atoms/groups with unpaired electrons, which can cause membrane damage, heart complications, aging, and cancer. To protect damages caused by free radicals, antioxidants can be used (Venkatesh and Dorai, 2011). The stability of drugs, foods, and nutrients and increase antiinflammatory, antiallergic and anticancer potential of the human body can be efficiently improved by natural antioxidants. Plants with high phenolic contents, on the other hand, are a good source of powerful antioxidants (Akyuz *et al.*, 2014). One of the most serious public health problems is microbial resistance to antibiotics, especially in developing countries where infectious diseases are a major cause of human mortality (Da-Silva *et al.*, 2016). Thus, there is great interest in finding new compounds derived from medicinal plants. Brought to light by recent studies, organic herbs contain secondary metabolites that make them good

candidates for traditional or native remedies (Ojatula, 2022), especially as antimicrobial and antifungal agents (Oussalah *et al.*, 2007). Recent investigations have proved that pathogenic microbes can be controlled via medicinal plants-based drugs (Donkor *et al.*, 2014; Ojatula and Ikuesan, 2022). Accordingly, examining medicinal plant extract and constituents is essential for their antibacterial activity.

*ICACINA TRICHANTHA* Oliver belongs to the family *ICACINACEAE*. It is one of the five species (*ICACINA CLAESSENSII* De Wild., *I. GUESSFELDTII* Asch. ex Engl. *I. MANNII* Oliv., *I. OLIVIFORMIS* (Poir.) J. Raynal and *I. TRICHANTHA* Oliv.) of the genus and are found only in Africa. The plant is a perennial shrub up to 2 m with scandent growth above, and commonly found in crop fields, forest regrowths and waste areas in the forest and savannah. The leaves are broadly elliptic, abruptly acute at the apex and rounded at the base.

Sometimes, they may be thinly pilose with simple, fascicled hairs beneath. Leaf length is about 8.0 - 10.0 cm. while the width is up to 17.0 cm. Flowers are densely crowded and subsessile with calyx nearly as long as the petals which are usually villous outside. The fruits are tomentose on the surface, ellipsoid to globose in shape and they are about 2.5 cm long (Agyakwa and Akobundu, 1998). It is sometimes locally abundant and troublesome as a weed in some parts of Nigeria. It is called as "Gbegbe" in Yoruba in the south west Nigeria and "Ibugo" in Igbo in the eastern Nigeria. The plant is extensively used in the rural areas; the leaves and tuber have folkloric uses in the treatment of malaria, constipation and food poisoning in Nigeria (Che *et al.*, 2016). To date, the chemical composition and pharmacological effects of

## 2.0 MATERIALS AND METHOD

### 2.1 Material

#### 2.1.1 Chemicals and Reagents

Chemicals such as 1,1-diphenyl-1-picrylhydrazole (DPPH), hydroxytoluene butylated (BHT), 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4',4'-disulphonic acid, hydrogen peroxide, ferrous chloride, nicotinamide adenine dinucleotide

### 2.2 Method

#### 2.2.1 Collection of Plant Material Sample

The plant material (seed of *Icacina trichantha*) were collected in November 2020 within the campus of Olusegun Agagu University of Science and Technology, Okitipupa, Ondo State, Nigeria. The samples were identified by the herbarium Curator, Botany Programme, Department of Biological Sciences, Olusegun Agagu University of Science and Technology, Okitipupa, Ondo State, Nigeria.

#### 2.2.2 Preparation of Plant Extract

The seeds were air dried at room temperature to a constant weight. The dried seeds were then pulverized to powder using an electric grinding machine (Panasonic MX-337N, China). The powdered material was stored in air-tight containers. Two hundred and fifty grams (250 g) of the powdered seed sample was extracted using 1000 ml of distilled water for 48 hrs with continuous stirring. The mixture was filtered using Whatman paper No1. The filtrate was evaporated

various species of *Icacina tricantha* have been reported. However, to our knowledge, *Icacina tricantha* seed chemical components and extract(s)-related activities have not been shown. The focus of this study is on the antioxidant, antibacterial, and antifungal activities of methanol extract of *Icacina tricantha* seed.

The objective of the research was to carried out *in vitro* bioactivity screening on the methanol seed extract of *I. tricantha* using standard procedures thereby evaluating antioxidative properties and antimicrobial effect of methanol seed extract of *I. tricantha* against some bacterial and fungal isolates using agar cup diffusion method.

(NADH), trichloroacetic acid (TCA), phosphate buffer, sulfanilic acid, glacial acetic acid, and naphthylethylenediamine dichloride will be used. Sigma provided the foline-ciocalteum reagent, sodium carbonate, vanillin, aluminum chloride, ascorbic acid, and potassium acetate (Sigma-Aldrich GmbH, Sternheim, Germany). All of the other chemicals and reagents used, including the solvents, were of analytical grade.

to dryness using a water bath to obtain 30% yield of the extract.

#### 2.2.3 Determination of *In-vitro* Antioxidant Activities of The Studied Plant Extract

##### 2.2.3.1 1,1, Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activities

The DPPH radical scavenging assay was performed using 1,1 diphenyl-2-picrylhydrazyl (DPPH)

According to the method described by Brand-Williams *et al.* (1995) with some modifications. Briefly, five different concentrations of the studied plant extract (0.0625, 0.125, 0.25, 0.5, and 1 mg/ml) were prepared in methanol (analytical grade). &e same concentrations were also prepared for L-ascorbic acid, which was used as a standard antioxidant. 1 ml of the studied extract was transferred into a clean test tube into which 0.5 ml of 0.3mM DPPH in methanol was added. &e mixture was shaken and left to stand in the dark at room temperature for 15 minutes. Blank solutions comprising of the studied extract solutions (2.5 ml) and 1 ml of methanol were used as baseline. The negative control comprised 2.5 ml of DPPH

solution and 1 ml of methanol, while L-ascorbic acid at the same concentrations as the studied extract was used as the positive control. After incubation in the dark, the absorbance values were measured at 517 nm using a spectrophotometer. The experiments were performed in triplicate. The DPPH radical scavenging activity was estimated using the equation described by Brand-Williams *et al.* (1995).

$$\% \text{ Radical scavenging activity} = \frac{A_c - A_s}{A_c} \times 100$$

where  $A_s$  is the absorbance of the sample, and  $A_c$  is the absorbance of the control. The half maximal inhibitory concentration ( $IC_{50}$ ) of the extract was computed from a plot of percentage DPPH free radical inhibition versus the extract concentration.

### 2.2.3.2 ABTS (2,2-azinobis (3-ethylbenzthiazoline)-6-sulfonic acid) radical scavenging assay

The 2, 2-azinobis (3-ethylbenzthiazoline)-6-sulfonic acid (ABTS) free radicals scavenging activity can be assayed by determine the antioxidant potential of extract. Solutions in 100 ml methanol were prepared for ABTS (7 mM) and potassium persulphate (2.45 mM). These two solutions were thoroughly mixed for the preparation of free radicals and kept in the dark overnight. Around 3 ml of stock solution was taken and its absorbance at 745 nm was set to 0.76 (control solution). Approximately 300 $\mu$ l of the test sample was mixed with 3 ml of 2,2-azino-bis(3 ethylbenzothiazoline-6-suplhonic acid solution and kept at 25°C for 15 min. The optical density of the solution was read at 745 nm. For the preparation of various ascorbic acid dilutions, same method was used as for sample (Re *et al.*, 1999). The data was collected in triplicates, and the formula used to measure the percentage of ABTS free radicals scavenging activity was:

$$\% \text{ Inhibition} = \frac{A_c - A_s}{A_c} \times 100.$$

### 2.2.3.3 Total antioxidant capacity (phosphomolybdenum method)

In this assay, the method of Prieto *et al.* (1999) was used with slight modification. The obtained data was expressed as mg of  $\alpha$  tocopherol equivalents per g dry weight. All tests were performed in triplicate.

### 2.2.3.4 Nitric oxide (NO) Scavenging Activity

The method described by Lee *et al.* (2003) was used to determine the degree of inhibition of nitric oxide radical generation *in vitro*. At physiological pH, sodium nitroprusside in aqueous solution spontaneously produces nitric oxide, which reacts with oxygen to produce nitric ions at 546 nm spectrophotometrically. As reagents, sodium nitroprusside (100 mM), phosphate buffered saline (pH 7.4), and gray reagent (1 percent sulphanilamide, 2% H<sub>3</sub>PO<sub>4</sub>, and 0.1 percent naphthylethylene dihydrochloride) were used. The reaction was triggered by the addition of 2.0 ml of sodium nitroprusside, 0.5 ml of PBS, and 0.5 ml of seed extract (50 mg), followed by 30 minutes of incubation at 25°C. Griess reagent (0.5 ml) was added and incubated for an additional 30 minutes. Control tubes were made without the extract. A spectrophotometer was used to measure absorbance at 546 nm in comparison to a blank reagent (Genesys 10-S, USA).

### 2.2.3.5 Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) Scavenging Activity

The percentage of H<sub>2</sub>O<sub>2</sub> scavenging was calculated using the following equation as described by Lee *et al.* (2003): percentage of H<sub>2</sub>O<sub>2</sub> scavenging =  $[(A_0 - A_1) / A_0] \times 100$ , where  $A_0$  and  $A_1$  were the absorbance of the control and test extract respectively.

### 2.2.3.6 Superoxide Radical Scavenging Activity

The scavenging activity against chemically generated superoxide radicals of the crude extract was measured by means of spectrophotometric measurement of the product on reduction of nitro blue tetrazolium (NBT) (Robak and Gryglewski, 1988). Test samples were dissolved in DMSO and diluted in water to give a final concentration of 12% (v/v) for DMSO (Hatano *et al.*, 1991). Superoxide anions were generated in a nonenzymatic [phenazine methosulfate (PMS)/NADH] system. The reaction mixture contained 1 ml of test solution, 1.9 ml 0.1 M phosphate buffer, pH 7.4, 1ml of 20mM PMS, 156 mM NADH, and 25mM

of NBT in phosphate buffer, pH 7.4. After 2 min of incubation at 25 °C, the colour was read on a Hitachi U-2000 spectrophotometer at 560 nm against blank samples, which contained no PMS. The percentage of scavenging activities (%) was calculated as follows:

Scavenging activities % (capacity to scavenge the superoxide radical) =  $[1 - (\text{absorbance of sample at 560 nm}) / (\text{absorbance of control at 560 nm})] \times 100$ .

### 2.2.3.7 Ferric Reducing Antioxidant Power Assay

The reducing power of the extracts was determined according to the method described by Oyaizu (1986), with some modifications. Briefly, five different concentrations of methanol extract (0.2, 0.4, 0.6, 0.8, and 1 mg/ml) and L-ascorbic acid at same concentrations were mixed with 2 ml phosphate buffer (0.2 M, pH 6.6) and 2 ml of 1% potassium ferricyanide (K<sub>3</sub>Fe (CN)<sub>6</sub>). The mixture was incubated at 50°C for 20 minutes. Then, 2 ml of 10% trichloroacetic acid (TCA) was added, and the mixture was centrifuged at 1000 revolutions per minute (rpm) for 10 min. The supernatant (2 ml) was aspirated and mixed with 2 ml of distilled water and 1 ml of 0.1% ferric chloride (FeCl<sub>3</sub>). In each case, the experiment was performed in triplicate. Afterward, the absorbances were measured spectrophotometrically at 700 nm using a UV-vis spectrophotometer and recorded. The concentrations of each extract able to yield an absorbance value of 0.5 were determined from the graph of absorbance at 700 nm against extract concentrations and considered as the median effective concentration (EC<sub>50</sub>).

### 2.2.3.8 Hydroxyl Radical Scavenging Activities

The hydroxyl radical scavenging activity was performed as per the method described by Klein *et al.* (1981) with minor modifications. The reaction mixture was constituted by adding 2.4 ml of phosphate buffer (pH 7.8) into test tubes. To the same test tubes, 90 µl of 1mM 1, 10 phenanthroline, 150 µl of 0.1mM hydrogen peroxide, 60 µl of 1mM iron (III) chloride, and 1.5 ml of the Phytexponent and the standard (L-ascorbic acid) at different concentrations (100%, 10%, 1%, 0.1%, and 0.01%) were added except in the controls, followed by incubation at room temperature for 5 minutes. &e increase in absorbance at 560 nm was measured,

and radical scavenging activity was calculated using the following formula as described by Bhart *et al.* (2015):

% Radical scavenging activity =  $[\text{Abs. of control} - \text{Abs. of sample} / \text{Abs. of control}] \times 100$ .

### 2.2.4 Antimicrobial Screening

The antibacterial and antifungal activities of the fractions were assayed as described by (Adekunle and Adekunle, 2009; Ojatula and Ikuesan, 2022). 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 corks 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 fluconazole 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 room temperature for extract to diffuse into the agar and then incubated at 37°C over- night. The zone of inhibition was measured using a transparent ruler.

## 3.0 RESULTS

### 3.1 Antioxidant Study

The maximum effective concentration (MEC) of the various chemical indexes used to assay the *in-vitro* antioxidant potentials of the studied plant is shown in Table 1 using AA (ascorbic acid) as reference. The various indexes exhibited effective concentration of antioxidant activity with EC<sub>50</sub> of 5.20<sup>±</sup>1.39 µg/ml - 29.91<sup>a±</sup>7.96 µg/ml which were higher than that of the positive control (4.51<sup>±</sup>0.02 µg/ml) by comparison (Table 1). A good correlation has been reported in literature between the polyphenol of medicinal plants and their antioxidant activity (Ojatula, 2021); acting as antioxidant agents and scavenge free radicals (Milliauskas *et al.*, 2004). The concentration of phenol and flavonoids observed in the literature of published researched works, contributed the antioxidant activities of many medicinal plant extracts.

**Table 1: *In vitro* antioxidant indices of *Icacina trichantha* methanol seed extract**

Chemicals	Level of EC <sub>50</sub> (µg/ml)
ASCORBIC ACID (STANDARD)	4.51±0.02
DPPH SCAVENGING ACTIVITIES (% INHIBITION)	29.91 <sup>a</sup> ±7.96
NITRIC OXIDE (% RADICAL SCAVENGING ACTIVITY)	28.99 <sup>a</sup> ±12.72
FRAP	7.60 <sup>c</sup> ±1.65
TOTAL ANTIOXIDANT CAPACITY	5.20 <sup>c</sup> ±1.39
HYDROGEN PEROXIDE SCAVENGING ACTIVITIES	6.15 <sup>c</sup> ±0.02
HRSA	15.32 <sup>b</sup> ±2.42
ABTS	16.23 <sup>b</sup> ±2.62
SSA	12.15 <sup>b</sup> ±1.32

\**P* < 0.05 - Significant; Different letters in superscript down column showed significant differences in the chemical response when compared with value of the standard compound.

### 3.2 Antimicrobial Study

As regards the antimicrobial activities of the studied plant material, **table 2** shows the activity of the plant extract 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 fraction whereas *P. aeruginosa*, *S. typhi* and *C. tropicalis* were found to be resistant to the fraction. This is a clear indication that the plant could be used to treat dysentery, coughs, stomach aches, diarrhea and new wounds. The result showing zone of inhibition **table 3** revealed that the methanol extract had considerable zone of inhibition of **20 mm to 23 mm**. The fraction of the studied plant 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 fraction; this is possibly

because of impurities which interfere with the various activities. However, when the active compounds are isolated the fraction 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 extract revealed activity against gram positive and gram negative bacteria as well as some fungi species. The result of the MICs and MBC/MFC **table 4** shows that the methanol fraction of the studied plant had MICs of 5 mg/mL against all the microorganisms except *K. pneumonia* with MICs at 10 mg/mL. Also, the methanol fraction of the studied plant 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

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

Test Organism	Methanol Extract of <i>I. trichantha</i> seed	Ciprofloxacin (5µg/disc)	Fluconazol (5µg/disc)
<i>MRSA</i>	S	R	R
<i>S. pyrogenes</i>	S	R	R
<i>E. coli</i>	S	R	R
<i>K. pneumonia</i>	S	R	R
<i>P. aeruginosa</i>	R	R	R
<i>S. typhi</i>	R	S	R
<i>S. dysenteriae</i>	S	S	S
<i>C. albicans</i>	S	R	S
<i>C. krusei</i>	S	R	S
<i>C. tropicalis</i>	R	R	S

Key: MRSA = Methicillin-resistant *Staphylococcus aureus*, *S. pyrogenes* = *Streptococcus pyogenes*, *E. coli* = *Escherichia coli*, *K. pneumoniae* = *Klebsiella pneumonia*, *P. aeruginosa* = *Pseudomonas aeruginosa*, *S. typhi* = *Salmonella typhi*, *S. dysenteriae* = *Shigella dysenteriae*, *C. albicans* = *Candida albicans*, *C. krusei* = *Candida krusei*, *C. tropicalis* = *Candida tropicalis*, S = Susceptible, R = Resistant.

**Table 3: Results of zone of inhibition of the Seed extract against the test microbes**

Test organism	Methanol Extract of <i>I. trichantha</i> seed	Ciprofloxacin (5µg/disc)	Fluconazol (5µg/disc)
MRSA	22	0	0
<i>S. pyrogenes</i>	21	34	0
<i>E. coli</i>	22	39	0
<i>K. pneumoniae</i>	20	30	0
<i>P. aeruginosa</i>	0	0	0
<i>S. typhi</i>	0	41	0
<i>S. dysenteriae</i>	23	40	0
<i>C. albicans</i>	20	0	32
<i>C. krusei</i>	21	0	38
<i>C. tropicalis</i>	0	0	35

Key: MRSA = Methicillin-resistant *Staphylococcus aureus*, *S. pyrogenes* = *Streptococcus pyogenes*, *E. coli* = *Escherichia coli*, *K. pneumoniae* = *Klebsiella pneumonia*, *P. aeruginosa* = *Pseudomonas aeruginosa*, *S. typhi* = *Salmonella typhi*, *S. dysenteriae* = *Shigella dysenteriae*, *C. albicans* = *Candida albicans*, *C. krusei* = *Candida krusei*, *C. tropicalis* = *Candida tropicalis*.

**Table 4: Result of Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentration/Minimum Fungicidal Concentration (MBC/MFC) of *Icacina. trichantha* seed extract against the test microbes.**

Test Organism	MICs (Concentration in mg/mL)	MBC/MFC (Concentration in mg/mL)
MRSA	5	10
<i>S. pyrogenes</i>	5	20
<i>E. coli</i>	5	10
<i>K. pneumoniae</i>	10	20
<i>P. aeruginosa</i>	0	0
<i>S. typhi</i>	0	0
<i>S. dysenteriae</i>	5	10
<i>C. albicans</i>	5	20
<i>C. krusei</i>	5	20
<i>C. tropicalis</i>	0	0

Key: MRSA = Methicillin-resistant *Staphylococcus aureus*, *S. pyrogenes* = *Streptococcus pyogenes*, *E. coli* = *Escherichia coli*, *K. pneumoniae* = *Klebsiella pneumonia*, *P. aeruginosa* = *Pseudomonas aeruginosa*, *S. typhi* = *Salmonella typhi*, *S. dysenteriae* = *Shigella dysenteriae*, *C. albicans* = *Candida albicans*, *C. krusei* = *Candida krusei*, *C. tropicalis* = *Candida tropicalis*

### 3.3 Statistics and Data Analysis

The statistical package for social sciences (SPSS) computer software version 16 was used for data analysis. The results were expressed as mean of replicate determinations  $\pm$  standard error of mean

(S.E.M), results were analysed by using one way analysis of variance (ANOVA). Differences in values between means were considered statistically significant at  $p < 0.05$ .

#### 4.0 DISCUSSION

The *in vitro* methods for evaluation of antioxidant activity have been developed to measure the efficiency of natural antioxidants either as pure compounds or as plant extracts. Therefore, measurement of antioxidant capacity of plant materials involves the use of highly sensitive methods, and because of the complex nature of phytochemicals, a single assay may not be representative of the antioxidant potential of plant extract (Chanda and Dave, 2009). In the present study, the antioxidant capacity of extract of *I. trichantha* seed against reactive oxygen species (ROS) was assessed by using various *in vitro* antioxidant assays. Plants with antioxidant activities have been reported to possess free radical scavenging activity, and they are known to be major contributors to several clinical disorders such as diabetes mellitus, cancer, liver diseases, renal failure and degenerative diseases as a result of deficient natural antioxidant defence mechanism (Parr and Bolwell, 2000). Plant secondary metabolites such as polyphenols are widely distributed in the plant kingdom and have been reported to protect the cell constituents against destructive oxidative damage associated with various degenerative diseases (Olorunnisola *et al.*, 2012). Their biological activities are believed to be due to their redox properties which play important roles in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Olorunnisola *et al.*, 2012). The DPPH radical assay serves as a quick, reliable and reproducible method for estimating the free radical scavenging activity of antioxidants. DPPH is a stable nitrogen-centered radical which can easily accept an electron or hydrogen radical to become a stable diamagnetic molecule (Mandade *et al.*, 2011). In the DPPH assay, the antioxidants are able to reduce the stable radical DPPH to non-radical form, DPPH-H. Various mechanisms have been put forward to explain the antioxidant activity of phenolic compounds including reducing capacity, prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction and radical scavenging (Vasanthi *et al.*, 2014). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Chanda *et al.*, 2011). It is a

measure of the reductive ability of antioxidants and it is evaluated by the transformation of Fe<sup>3+</sup> to Fe<sup>2+</sup> in the presence of the sample extract (Olorunnisola *et al.*, 2012). Several studies have revealed that ferric reducing power of bioactive compounds is associated with antioxidant activity and that the phenolic compounds in the plant may be acting as electron donors thereby reducing free radical generation (Olorunnisola *et al.*, 2012). The results from this study showed that methanol extract of *I. trichantha* seed have varied degree of *in vitro* antioxidant activity. The antioxidant activity of the extract may be attributed to the presence of some phytochemicals such as flavonoids, tannins, cardiac glycosides and terpenoids. Therefore, *I. trichantha* may serve as potential source of natural antioxidant which may help in preventing the progress of various oxidative stress related diseases.

In the last two decades, studies have been conducted with medicinal plants in different countries to prove their effectiveness as antimicrobial agents. Maddila and Hemalatha (2017) reported that global antimicrobial resistance is becoming a growing public health problem, as microbial resistance to most available antimicrobial has been reported. The pharmaceutical industry and new biotechnology companies are intensifying efforts to discover new antimicrobial in attempts to overcome microbial resistance (Maddila and Hemalatha, 2017). Table 6 shows the activity of the studied plant extract 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 fraction whereas *P. aeruginosa*, *S. tyhi* and *C. tropicalis* were found to be resistant to the fraction. 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 diarrhea and stomach aches attributed to *S. aureus* and *E. coli* could be due to the antibacterial effects of alkaloids, saponins, steroids and tannins (Vieira *et al.*, 2001). That tannin-rich plants are used in folk medicine as antiseptic because the basis of their mechanism of action is the ability to precipitate proteins, forming a tannin-protein complex on damaged tissues preventing the development of microorganisms (Janovik *et al.*, 2007). Thus, we can infer that the appreciable phytochemical contents in the extract

of *I. trichantha* seed are sufficient for it to have a good antimicrobial potential. The result showing zone of inhibition table 7 revealed that the methanol extract of the studied plant material had considerable zone of inhibition of 21 mm to 23 mm. The fraction showed lower but comparable zone of inhibition against the microorganisms 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 fraction; this is possibly because of impurities which interfere with their various activities. However, when the active compounds are isolated the fraction 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 extract 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 8 shows that 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 8 shows that 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. The ability of the extract to inhibit the growth of several bacterial and fungal species (caused by the presence of secondary metabolites in plant cells) is an indication of the

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broad spectrum anti-microbial potential of *I. trichantha* seed which makes the plant a candidate for bioprospecting for antibiotic and antifungal drugs.

## 5.0 CONCLUSION

The present study shows the presence of biochemical compounds in *Icacina trichantha* seed which may therefore justify both its antioxidant and antimicrobial agent of natural origin. The plant showed efficient resolution of *in vitro* oxidative stress (anti-oxidant activity) by scavenging DPPH, and improving the concentrations of other radicals. The agar diffusion assay on fraction showed growth inhibitory effects on all the organisms except *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Candida tropicalis*. The MIC revealed that methanol extract 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 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 antioxidative biomolecules in *I. trichantha* seed which could be useful in the therapeutic intervention of multi-resistant microorganisms.

## ACKNOWLEDGEMENTS

Authors acknowledged the technical support provided by Mr Efe of FUTA Central Laboratory, Federal University of Technology, Akure, Ondo State

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