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PHYTOCHEMICAL AND ANTIBACTERIAL ACTIVITY OF *CHRYSANTHELLUM INDICUM* (LINN) EXTRACTS

Ibrahim Isah Laken[1], Musah Monday[2], Dagaci M.Z[1], Mohammed S.H[2], Paiko Y.B[1]
Baba F.H[2], Mohammed S.Y[3], Mann A[4] and Abdullahi B.M[1]

[1]Dept of Chemistry, Ibrahim Badamasi Babangida University Lapai, Niger State, Nigeria.
[2]Dept of Chemistry, Niger State College of Education, Minna, Nigeria.
[3]Dept of Food Science, Ibrahim Badamasi Babangida University Lapai, Niger State, Nigeria
[4]Dept of Chemistry, Federal University of Technology Minna, Niger State, Nigeria

ABSTRACT: *The paper dual the preliminary investigation on possibility of the claim by Traditional Healers that this plant extract could cure Scorpion sting and snake bites Infectious diseases are prevalent in developing countries and plant extracts are known to contained bioactive compounds that can be used in management of these diseases. The plant of Chrysanthellum indicum (Linn) was with reference voucher FNS/0019/ibbu/ 020 air-dried and pulverized into fine powder and then percolated to give ethanol and aqueous extracts. These extracts were phytochemically screened for metabolites and evaluated antibacterial activity against some pathogenic organisms Klebsilla, pneumonia, Bacillus subtilis, and Pseudomonas aeruginosa using agar dilution method. It was found that crude extracts of C. indicum revealed the presence of saponins, tannins, alkaloids, steroidal nucleus, cardiac glycosides and coumarin, while flavonoids and anthroquinones were absent. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the active extract of C. indicum shows that the extract could be a potential source of antibacterial agents.*

KEYWORDS: Antibacterial Activity, Chrysanthellum Indicum, Infectious Diseases, Phytochemical Screening

INTRODUCTION

INFECTIOUS diseases are cause of high morbidity and mortality in developing countries and continuous to be a global health concern. It is the world's leading cause of premature deaths resulting to about 50,000 people daily (WHO, 2000). These disease conditions are responsible high percentage of deaths in tropical countries where bacterial infections are most prevalent (Iwu *et al.,* 1999). The discovery and development of antibiotics have led to significant advancement in treatment of infectious diseases. However, development of effective antibacterial agents has been accompanied by the emergence of drug-resistant organisms due to the irrational and abuse of drugs, failure to adhere to complete treatment regimen. The drug-resistant bacteria and fungal pathogens have further complicated the treatment of infectious diseases in immune compromised, AIDS and cancer patients (Pfaller *et al*., 1990; Deng *et al.,* 1996*;* Farnsworth and Soegarta, 1991). In recent years, drug-resistance to human pathogenic bacteria has been commonly reported from all over the globe

(Sangeorzan *et al*., 1992; Uaboi, 2000; Uaboi- Egbenmi, 2002). All these factors decrease the clinical efficacy of drugs (Mann *et al.*, 2014). Therefore, there is need to continuously search for new, effective and affordable antibacterial substances from other sources including plants. Antibacterial substances are substances that inhibit the growth and existence of bacteria (Cowan, 1999). Many isolates of *Escherichia coli* and *Staphylococcus aureus* are resistant to ampicillin, amoxicillin and tetracycline. The therapeutic failure of antibiotics in Nigeria, Africa and indeed all parts of the world buttresses the need for given support for the use of local medicinal plants (Oloke *et al*., 1988). Many plants possess antimicrobial activities and are used for the treatment of different diseases (Arora *and Kaur*, 1999). Medicinal uses of these plants range from the administration of the plant parts' such as root bark, stem bark, leaves, fruits and seeds, to the use of extracts from the whole plant (Akujobi *et al*., 2004). Plants have a great potential for producing new drugs of great benefit to mankind. There are many approaches to the search for new biologically active principles in higher plants (Jigna and Chanda, 2006). This search for new antibacterial properties of natural products cannot be ignored because this can be found in the most remote parts of the world (Oukemi and Kandakai, 2004). Infectious diseases are among the diseases that have been managed successfully using herbal medicine (Sofowora, 1996). Natural selection during evolution and competition between organisms has produced powerful biologically active natural products which can serve as chemicals and have been refined by modern techniques to give more specifically active drugs (Cowan, 1999). Recently, there has been renewed interest on plants as sources of antibacterial agents due to their ethnomedicinal uses to treat infectious and non-infectious diseases in developing nations.

Chrysanthellum indicum (Linn) is a weed that belongs to family of Asteraceae which is one of such medicinal plants. This plant is commonly called African wild daisy (Akobundun and Agyakwa, 1998). It has also been known with the following local names: oyigi / Abilere in Yoruba, Finmi wakpenye /Shani kasanni and Rariyar kasa in Nupe, Dunkufe in Hausa respectively (Yaro *et al.*, 2007). It has been reported to be of medicinal values ranging from treatment of upper respiratory tract, management of scorpion sting, lithiasis, inflammations, migraine, cirrhosis, dermatoses, and toothaches (Bisignano *et al.*, 1996; Farnsworth and Soegarta, 1991; Honore- Thorez, 1985; Ofodile *et al.*, 2010). This plant has been reported to possess some pharmacological activities such as anti-tumour, diuretic, hypoglycemic, antioxidant, gastrointestinal and anti-scorpion sting activities (Dhar *et al.*, 1973; Yaro *et al.*, 2007; Woo *et al.*, 1977). Till today, there is a growing interest in plants with antibacterial activity. Scientists are increasingly becoming involved in the screening of plants with the aim of establishing their potential antimicrobial effects and identifying the compounds responsible for the antimicrobial properties (Ndukwe *et al*., 2007). *Escherichia coli* and *Staphylococcus aureus* are intestinal bacteria often implicated in several gastrointestinal disorders. Gastrointestinal diseases caused by *E. coli* are the most frequent causes of death in developing countries. Therefore, the aim of the present research work is to scientifically screen the entire plant of *Chrysanthellum indicum* for antibacterial agents.

MATERIALS AND METHODS

The entire plant of *Chrysanthellum indicum* was obtained as described by traditional medical practitioner from the premise of the Campus of Niger State College of Education Minna, Nigeria. The plant material was identified and authenticated by a Botanist, Mr. Jonathan Nusa

Gana of the Department of Biology, Niger State College of Education Minna. The entire plant was air-dried and pulverized into fine powder and kept for future use.

Extraction of the Crude Extracts

The powdered plant material (50 g) was percolated in 200 cm^3 ethanol using 500 cm^3 capacity conical flask and stoppered and kept for 72 h with intermittent shaking. The percolate was filtered with Whatman's No 1 filter paper. The ethanol extract (EE) was concentrated at 35ºC under reduced pressure using rotary evaporator. A fresh amount of the plant material was again percolated with distilled water for 72 h and after filtration; the aqueous extract (AE) was concentrated and later evaporated to dryness (Mann *et al*., 2008). Each extract was screened for phytochemical and antibacterial properties.

Phytochemical Analysis of the Crude Extracts

Phytochemical analysis for qualitative detection of alkaloids, flavonoids, tannins, saponins, reducing sugar and flavones was performed on the extracts as described by El-Oleyi *et al.*, (1994); Harbone, (1998) and Trease and Evans, (1978).

Preparation of Microorganisms

Pseudomonas aeruginosa, Escherichia coli, Bacillus subtitles and *Klebsilla pneumonia* were obtained from General Hospital, Minna, Niger State. Cultural and morphological identification were carried out and finally biochemical characterization of isolates using protocols described by Cheesbrough was done. Pure cultures of the isolates were maintained in appropriate media for future use.

Antimicrobial Disc Preparation

Discs of about 6 mm diameter were made from Whatman's No.1 filter paper using a paper puncher. Batches of 100 discs were transferred into Bijou bottles and sterilized in the oven at 121ºC for 15 min. Stock solution (400 mg/cm^3) of the plant extract was prepared by dissolving 0.8 g of each extract in 2 cm^3 Dimethylsulphoxide (DMSO). Serial doubling dilution was carried out by adding 1 cm^3 of DMSO at each serial dilution. Four concentrations were prepared from the stock solution such that each disc would absorb 0.01 cm^3 which is equivalent to 500 µg/disc, 1000 µg/disc, 2000 µg/disc and 4000 µg/disc respectively.

Standardization of Inoculum

The inocula were prepared from the stock cultures which were maintained in nutrient agar slant at 4ºC and subculture in nutrient broth using a sterilized wire loop. The density of suspension inoculated unto the media for susceptibility test was determined by comparison with 0.5 McFarland standard of Barium sulphate solution. Mc-Farland's turbidity standard scale (0.5) was prepared by adding 9.95 cm^3 of 1% H_2SO_4 and 0.05 cm^3 of 1% $BaCl_2$ to give a turbid solution. Ten cm^3 sterile normal solution was used to make a turbid suspension of the micro-organism. Dilution of the organism suspension was done continuously using normal saline until the turbidity marched that of Mc-Farland's scale by visual comparison.

Susceptibility Testing

Agar dilution method described by Bauer and Kirby was employed for antibacterial bioassay. The preparation was incubated at appropriate temperatures. After incubation, zone of inhibition diameter formed in the medium was measured to determine antibacterial effectiveness of the different concentrations of the extracts.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration for bacterial isolates was carried out using tube dilution as described by Akinyemi *et al.* Stock solution of 80,000 µg in 10 cm^3 sterilized distilled water was serially diluted to arrive at concentrations of 500, 1000, 2000 and 4000 µg/ cm^3 respectively. At that point, the concentration of the micro-organisms was about 1.5 x 108 cfu/ cm^3. Two-fold dilution of the extracts with nutrient broth was done to give concentrations of 40, 20, 10, 5 and 2.5 mg/cm^3. 0.2 cm^3 of the micro-organism suspension was inoculated into the different concentration of the extract in test tubes. The tubes were incubated at 37°C for 24 h and at 25°C for 48 h for bacteria after which the plates were observed for growth. The MIC was defined as the lowest concentration of the extract inhibiting the visible growth of each microorganism.

Determination of minimum bactericidal concentration (MBC)

Blood agar plates were prepared according to the manufacturer's instructions. The contents of the MIC tubes and the following tubes in the serial dilution were sub-cultured into appropriately labeled blood agar plates by dipping a sterile wire loop into each test tube and streaking the surface of the labeled blood agar plates. The plates were then incubated at 37°C for 24 h after which they were observed for growth. The MBC was the plate with the lowest concentration of the extract in serial dilution without growth.

Nutrient Agar

2.8 g of the powdered commercially prepared nutrient agar was weighed and dissolved in 100 cm^3 of distilled water by boiling and autoclaved at standard conditions. The nutrient agar was cooled to 50ºC. After the solidification of the agar, the plates were placed in the oven to dry up any available water droplet after which they were inoculated (Black and Mould, 1991).

Plate Inoculation Method

The method of inoculation used in this work is the Cork Plate Method. It involves the use of sterile loop to smear a loop full of the test organism on the surface of the medium. The loop was sterilized in the Bunsen flame and when cools, streaked all over the plate to cover the plate surface. Holes were bored on the solidified agar using a sterile cork borer of 7.0 mm in diameter. 0.2 cm^3 of each extract was dispersed in the holes made on the agar. After dispensing, the extract was allowed to diffuse into the agar for 2 h and finally incubated (in an incubator) at 35ºC for 16-18 h, or in an anaerobic jar for 48 h in the case of an anaerobic jar. After incubation, the diameter of zone of inhibition was measured to the nearest millimeter (Black and Mould, 1991; Cherkasov *et al.*, 2008; Harbone, 1998; Trease and Evans, 1978).

RESULTS AND DISCUSSION

Table 1: The Phytochemical Screening of *Chrysanthellum Indicum*

Group Constituent	Test	Observation	A E	E E
Alkaloids	Hager's test	Yellow precipitate	+	+
	Mayer's test	Yellow precipitate	+	+
	Marquis's test	Black precipitate	+	+
Tannins	Ferric chloride	Dark green colour	+	+
Flavonoids	Sodium hydroxide	Yellow colour	–	–
Saponins	Extract with water	Foaming	+	+
Steroids	Acetic anhydride and conc. H_2SO_4	Green colour	+	+
Glycosides	Fehling's test dil H_2SO_4 / water	Reddish brown precipitate	+	+
		Reddish brown precipitate	+	+
Anthroquinones	Borntrager's Test	Pink colour	–	–
Coumarin	sample and NaOH	Yellow green fluorescence	+	+

Key AE = Water Extract, EE = Ethanol Extract, + = Present, - = Not present

The phytochemical screening of the extract as presented in the above table1 and revealed that saponins, tannins, alkaloids, steroidal nucleus, cardiac glycosides and coumarin are present; flavonoids and anthroquinones are absent. The medicinal properties of plant extracts could be based on the antibacterial effects of the phytochemicals in them (Cowman, 1999). This is similar to the studies reported by Yaro *et al.* (2007) and Ofodile *et al*. (2010) that *C. indicum* possess antifungal and antipsychotic properties respectively. Phytochemical studies have also shown that the antibacterial properties of these plants depend on certain active ingredients such as saponins, tannins and alkaloids. The presence of the aforementioned secondary metabolites in *C. indicum* extracts supported its traditional use by the inhabitant of Minna and environs. Since their presence in plants probably explains the various uses of plants for traditional medicine. Most of these phytochemical constituents are potent bioactive compounds found in medicinal plant parts, which are precursors for the synthesis of useful drugs (Sofowora, 1993). Some of the simplest bioactive phytochemicals consist of single phenolic compounds. Catechol and pyrogallol are shown to be toxic to microorganisms. These groups of compounds exhibit inhibitory activities (Table 2) that could be attributed to the fact that sites and number of hydroxyl groups are related to the toxicity to microorganisms (Scalbert, 1991). Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties (Harborne and Baxter, 1995; Honore- Thoerez and Irabor, 1985). The substances that can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells are considered candidates for developing new antibacterial drugs. Antibacterial properties of medicinal plants are reported from different

parts of the world (Saxena and Sharma, 1999). It is expected that plant extract showing target sites other than those used by antibiotics will be active against bacterial pathogens.

Table 2: The Antibacterial Screening of *C. Indicum* Susceptibility Test

Solvent	Diameter of Zone of Inhibition (mm)	Organism
Water Ethanol	Resistant 18	*Klebsilla pneumonia*
Water Ethanol	Resistant 27	*Bacillus subtilis*
Water Ethanol	Resistant 18	*Escherichia coli*
Water Ethanol	Resistant 13	*Pseudomonas aeruginosa*

The susceptibility test carried out on *C. indicum* against *Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis and Klebsilla pneumonia* microorganisms exhibit the resistant to aqueous extract as presented in table 2 which is corroborated with the studies reported (Darouiche, 2004; Igboanugo, 2006; Ofodile *et al.*, 2010).

Table 3: The Antibacterial Screening Chloroform Extract of *C. Indicum* MIC Test

Solvent	Diameter of Zone of Inhibition (mm)	Organism
Water Ethanol	10-3 10-2	*Klebsilla prenmonia*
Water Ethanol	NA or Resistant 10-2	*Bacilus subtilis*
Water Ethanol	NA or Resistant 10-2	*Escherichia coli*
Water Ethanol	Resistant 10-3	*Pseudomonas aeruginosa*

Table 4: The Screening MBC test on extract of *C. indicum* plant from preparatory plate

Solvent	Diameter of Zone of Inhibition (mm)	Organism
Water Ethanol	10-3 10-2	*Klebsilla prenmonia*
Water Ethanol	NA or Resistant 10-2	*Bacillus subtilis*
Water Ethanol	NA or Resistant 10-2	*Escherichia coli*
Water Ethanol	Resistant 10-3	*Pseudomonas aeruginosa*

The results of MIC and MBC tests as presented in the tables 3 and 4 revealed that the crude ethanolic extract of *C. indicum* plant inhibits the growth of the microorganism screened against, which consequently justifies the claim of its medicinal value as causative agent. *C. indicum* plant is very rich in secondary metabolites as presented in Table 1 most especially *E. coli* and *P. aeruginosa* that have been implicated to be very resistant to anti-diarrhoea and some other antibiotics. The results from this study further supports earlier reports of studies of *in-vitro* inhibitory activities as very reliable and potent antimicrobial agent (Alam and James, 1998; Amos *et al.,* 2001; Babayi *et al.,* 2007; Fried and Sherma, 1999; Nazemi *et al.,* 2010; Sherma, 2005; Iroegbu and Nkere, 2005; Fadipe *et al.*, 2012).

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