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Phytochemical and antimicrobial screening of crude methanolic leaf extract of *Peucedanum winkleri* H. Wolff.

Madumelu M[1*], Ndukwe IG[2] and Ayo RG[3]
[1]Department of Chemistry, Federal College of Education, Zaria Nigeria.
[2]Department of Chemistry, Ahmadu Bello University, Zaria Nigeria.
[3]College of Agriculture Samaru, Ahmadu Bello University, Zaria Nigeria.

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ABSTRACT

Phytochemical investigation of the leaves of *Peucedanum winkleri* H. Wolff, revealed the presence of secondary metabolites. The extract from total extraction with methanol was screened for its antimicrobial activity against *Staphylococcus aureus, Methicillin resistant Staphylococcus aureus, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Klebsiella pneumoniae, Streptococcus pyogenes, Proteus mirabilis, Candida albicans* and *Candida krusei* using agar-well difussion method. The result indicated that the extract inhibited the growth of one or more test pathogens and were compareable with those of the standard drugs used. The minimun inhibitory concentration (MIC) ranges from 5-10 mg/ml and the minimun bactericidal/fungicidal concentration (MBC/MFC) ranges from 20-40 mg/ml. The result of the study shows justification for the use of the plant for the treatment of infectious diseases caused by these bacteria and fungi pathogens. It was concluded that *P. winkleri* H. Wolff could be a potential source of active antimicrobial agents and a detailed assessment of antimicrobial activity of the plant material in other solvents extract, isolation and characterization of active compounds from the most active extract is on-going.

INTRODUCTION

Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contribution to human health and well-being (Igbinosa *et al.,* 2009). Nearly 80% of the world population relies on traditional medicine for primary health care most of which are plant extract (WHO, 2002; Akindele and Adeyemi, 2007a). Of about 300,000 plants species acclaimed world-wide, only about 5% have been investigated scientifically for their medicinal properties (Sanusi and Rabo, 2004).

Pencedanum winkleri H. Wolff belongs to the family of Apiaceae. Alternative name is Umbelliferae. It is an annual herb that is widely spread in Asia, Europe and Tropical Africa including Nigeria. In northern part of Nigeria, it is useful among local medicine practitioners for the treatment of typhoid fever, high fever, intestinal disorder and as an analgesic. Prior to now, there is no phytochemical and antimicrobial report on this plant species. Many efforts have been made to discover new antimicrobial compounds from various sources including plants that could be used as a remedy for a variety of ailments of microbial origin. In this present study, the extract from total extraction of leaves of *P. winkleri* H. Wolff using methanol was screened for phytochemical constituent and antimicrobial properties against *Staphylococcus aureus, Methicillin resistant Staphylococcus aureus, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Klebsiella pneumoniae, Streptococcus pyogenes, Proteus mirabilis, Candida albicans* and *Candida krusei.* as part of the exploration for new and novel bio-active compounds.

MATERIALS AND METHODS

The leaves of *P. winkleri* was collected fresh from Shika village in Zaria, Kaduna state of Nigeria. Plant materials were identified at the herbarium unit of Biological Science Department, Ahmadu Bello University Zaria, Nigeria and a voucher specimen number was deposited in the herbarium.

* *Corresponding Author*
Madumelu Mark. +2348034641173. E-mail: maksyno@yahoo.com

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Preparation of plant extract

The plant materials were dried at room temperature and then pulverized. The pulverized sample 300 g was packed into a thimble in a soxhlet extractor and extracted exhaustively using methanol. The resulting extract was concentrated at 40 ^{0}C in *vacou* using rotary evaporator and further air dried to a constant weight of 41.8 g (13.93 %).

Phytochemical screening

The extract was subjected to phytochemical test for plant secondary metabolites using standard methods described by Trease and Evans, 1989 and Soforawa, 1993.

Test organsms

Microbial strains of pathogens tested includes *Staphylococcus aureus, Methicillin resistant Staphylococcus aureus, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Klebsiella pneumoniae, Streptococcus pyogenes, Proteus mirabilis, Candida albicans* and *Candida krusei*. The pure isolates of these microorganisms were obtained from the Department of Medical microbiology Ahmadu Bello University Teaching Hospital Zaria Nigeria.

Media used

Muller- Hinton Agar and Sabouraud dextrose agar (SDA) were used to test for antibacterial and antifungal respectively.

Antimicrobial activity

The antimicrobial activity of the crude methanolic extract of *P. winkleri* was determined by agar-well diffusion method using some pathogens. Their clinical isolates were checked for purity and maintained in slants of agar for the bacteria and in slant of SDA for the fungi.

The medium was prepared according to the manufacturer's instruction, sterilized at 121 °C for 15 mins, poured into sterile petri dishes and were allowed to cool and solidify. The sterilized medium was then seeded with 0.1ml of the standard inoculum of the test microbes, the inoculum was spread evenly over the surface of the medium by the use of sterile swab. Using a standard cork borer of 6 mm in diameter, a well was cut at the centre of each inoculated medium. 0.1ml of 40 mg/ml solution of the extract was then introduced into each well on the inoculated medium.

The inoculated medium was incubated at 37 °C for 24 h after which each of the plate was observed for the zone of inhibition of growth, measured and recorded in mm.

Minimun inhibition concentration (mic)

The MIC of the extract was carried out using broth dilution method. Muller –Hinton broth was prepared according to manufacturer's instructions. 10 ml was dispensed into test tubes and sterilized at 121 °C for 15 mins, the broth was allowed to cool. MCFarlands standard turbidity scale number 0.5 was prepared to give turbid solution. Normal saline was prepared, 10 ml was dispensed into sterile test tube and the test microbe was inoculated and incubated at 37 °C for 6 h. Dilution of the test microbes in the normal saline was done until the turbidity marched that of Mc Farland's scale by visual comparison. At this point, the test microbes has a concentration of about $1.5x10^8$ cfu/ml.

Two fold serial dilution of the extract in the broth was made to obtain concentrations of 40 mg/ml, 20 mg/ml, 10 mg/ml, 5 mg/ml and 2.5 mg/ml. 0.1ml of the standard inoculum of the test microbes in the normal saline was then inoculated into the different concentrations of the extract in the broth, incubation was made at 37 °C for 24 h after which each test tube was observed for turbidity (growth). The lowest concentration of the extract in the broth which shows no turbidity was recorded as the MIC.

Minimun bactericidal/fungicidal concentration (mbc/mfc)

MBC/MFC was carried out to check whether the test microbes were killed or only their growth was inhibited. Muller-Hinton agar was prepared and sterilized at 121°C for 15 mins, the medium was poured into sterile petri dishes and was allowed to cool and solidify.

The content of the MIC in the serial dilution were then subcultured onto the prepared medium, incubation was made at 37 °C for 24 h, after which each plate was observed for colony growth. The lowest concentration of the extract without a colony growth was recorded as the MBC/MFC.

RESULT

The result of the antimicrobial test, MIC and MBC/MFC of the extract are presented in table 1 and 2.

DISCUSSION

The antimicrobial activity of crude methanolic extract shows relevant antimicrobial activity and comparable with those of the standard drugs used. It zone of inhibition was between 20-29 mm. *Klebsiella pneumoniae* had the highest zone of inhibition whereas *Salmonella typhis* and *Candida albicans* had the lowest as shown in table 1.

From table 2, the MIC of the extract ranged between 5-10 mg/ml with *Klebsiella preumoniae* having the least MIC value of 5 mg/ml compare to other microorganisms with MIC value of 10 mg/ml. Also, the MBC/MFC ranged between 20-40 mg/ml with *Staphylococcus aureus, Escherichia coli* and *Klebsiella preumoniae* having an MBC value of 20 mg/ml, impliedly meaning that these microorganisms can be exterminated at a lower concentration compared to others with higher MBC/MFC of 40 mg/ml.

The inhibitory effect of the extract of *P. winkleri* against several bacterial and fungal species is an indication of broad spectrum antimicrobial potential thus, introducing the plant as a potential candidate for drug development for the treatment of infectious diseases caused by these pathogens.

Table. 1: Zone of inhibition (mm) of crude methanol extract of leaves of *P. Winkleri*.

Microorganism	Crude Methanol extract 40 mg/ml	Cefuroxine 40 µg/ml	Sparfloxacin 40 µg/ml	Erythromycin 50 µg/ml	Fluconazole 50 µg/ml
Staphyloccocus aureus	24	22	36	0	0
Methicillin resistance S. aureus	23	30	34	0	0
Streptococcus pyogenes	0	30	30	24	0
E. coli	27	30	35	0	0
Salmonella typhi	22	0	30	0	0
Proteus mirabilis	20	0	27	29	0
Pseudomonas aeruginosa	0	0	39	22	0
Klebsiella pneumoniae	29	40	47	32	0
Candida albicans	22	0	0	0	32
Candida krusei	0	0	0	0	34

Table. 2: The MIC and MBC/MFC of crude methanol extract of leaves of *P. Winkleri.*

Microorganism	MIC (mg/ml)	MBC/MFC (mg/ml)
Staphyloccocus aureus	10	20
Methicillin resistance S. aureus	10	40
Escherichia coli	10	20
Salmonella typhi	10	40
Proteus mirabilis	10	40
Klebsiella pneumoniae	5	20
Candida albicans	10	40

Phytochemical screening

Investigation of this on the leaves of *P. winkleri*, revealed the presence of carbohydrates, free reducing sugar, cardiac glycoside, saponins, steroids, flavonoids, alkanoids, tannins and triterpenes and absence of anthracene. These compounds are known to be biological active and therefore aid the antimicrobial activity of the plant. Many triterpenes and saponins have been reported to have various uses such as antiulcerogenic, anti-inflammatory, fibrinolytic, antipyretic, analgesic and anti edmatous in action (Ndukwe *et al.,* 2005). Just *et al.,* (1998) reported the inhibitory effect of saponins on inflammed cells. Saponins and triterpenes were found to be present in *P. winkleri* and has supported the usefulness of the plant in managing several ailment.

Another secondary metabolite found in the plant extract was flavonoid. Flavanoid provide many health promoting benefits. They act as antihistamine (Guardia *et al.,* 2001). Flavanoids have been reported to exhibit a wide range of biological activities like antimicrobial, antiinflamatory, antiangionic, analgesic, anti-allergic, cytostatic and antioxidant properties (Hodek *et al.,* 2002). Tannins is another constituent of *P. winkleri*, Tanins has been used in medicine to aid the healing of burns and wounds. They are equally claimed to have antiviral and antitumour properties (Herbone, 1993).

Tannins has been found to form irreversible complexes with proline-rich protein (Shimada, 2006) resulting in the inhibition of cell protein sythesis. Perekh and Chanda (2007) reported that tannins are known to react with proteins to provide the typical tannins effect which are important for the treatment of inflammed tissues. Li *et al.,* (2003) reported that tannins have anticancer activity and can be used in cancer prevention, thus suggesting that *P. winkleri* has potential resource of important bioactive molecules for the treatment and prevention of cancer. Similarly, steroids founds in *P. winkleri* is another important plant secondary metabolite. Okwu (2001) reported the relationship of steroidal compounds with various anabolic hormones including sex hormones. Steroids has equally been reported to have antimicrobial activity (Quinlan *et al.,* 2001) and confirmed to have antiviral properties (Neumann *et al.,* 2004).

CONCLUSION

The result of the experiment showed that the leaf of *P. winkleri* H. Wolff could be a potential source of active antimicrobial agents and a detailed assessment of antimicrobial activity of the plant material in other solvents extract, isolation and characterization of active compounds from the most active extract is on-going.

REFERENCES

Akindele AJ, Adeyemi OO. Anti-inflammatory activity of the aqueous leaf extract of Byrsocarpus coccineus. *Fitoterapia*, 2007a;78: 25-28.

Guardia T, Rotelli AE, Juarez AO, Pelzer LE. Anti – inflamatory properties of plant flavanoids. Effect of rutin, quercetin and hesperidin on adjuvant arthritis in rat. *Farmaco*, 2001; 56(9): 638 – 687.

Harborne JB, Baxter H. 1993. Phytochemistry dictionary; a hand book of Bioactive compounds from plants. Published by Tailor and Francis Ltd.: 2–3, 127–128.

Hodek P, Trefil P, Stiborova M. Flavonoids - Potent and versatile biologically active compounds interacting with cytochrome P450. *Chemico-Biol. Intern.*, 2002; 139(1): 1-21.

Igbinosa OO, Igbinosa EO, Aiyegoro OA. Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* (Linn). *African Journal of Pharmacy and Pharmacology*. 2009; 3(2): 58-62.

Just MJ, Recio MC, Giner RM, Cueller MJ, Manez S, Bilia AR, Rios JL. Anti-inflammatory activity of unusual lupine saponins from Bupleurum fruticescens. *Planta Medica.*, 1998; 64: 404-407.

Li H, Wang Z, Liu Y. Review in the studies on tannins activity of cancer prevention and anticancer. *Zhong Yao Cai*., 2003; 26(6): 444-448.

Ndukwe IG, Achimugu MO, Amako NF. Phytochemical and antimicrobial screening of crude extracts from the stem bark of *Irvingia gabonensis*. *Journal of Pest, Disease and Vector Management,* 2005; 6: 391-397.

Neumann UP, Berg T, Baha M, Puhl G, Guckelbeger O, Langreh J, Mneuhaus P. Long-term outcome of liver transplant for hepatitis C: A 10 year follow-up. *Transplantation,* 2004; 77(2): 226-231.

Okwu DE. Evaluation of the chemical composition of indigenous Spices and flavouring agents. *Global J. Appl. Sci*., 2001; 7(3): 455-459.

Parekh J, Chanda S. *In vitro* antibacterial activity of crude methanol extract of Woodfordia fruticosa Kurz flower (Lythacease). *Braz. J. Microbiol*, 2007; 38: 2.

Quinlan MB, Quinlan RJ, Nolan JM. Ethnophysiology and herbal treatments of intestinal worms in Dominica, West Indies. *J. Ethanopharmacol*, 2000; 80: 75-83.

Sanusi SS, Rabo ET. 2004. An inventory of Medicinal plant the Nigeria Savannah Leviathan book Lagos. pp. 21- 24.

Shimada T. Salivary proteins as a defense against dietary tannins. *J. Chem. Ecol*., 2006; 32(6): 1149-1163.

Sofowara A. 1993. Medicinal plants and Traditional medicine in Africa. John Wiley and Sons Limited. Chinchester,: 145, 148, 135 – 153.

Trease GE, Evans WC. 1989. Textbook of Pharmacognosy. 12th Edn. Balliere, Tinadl London.

WHO. 2002. Traditional Medicine: Growing Needs and Potential. WHO Policy Perspectives on Medicines. World Health Organization, Geneva. 1-6.

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