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In vitro evaluation of the antioxidant and antimicrobial activity of leaf extracts of *Petroselinum cripsum* (Parsley)

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ABSTRACT

Petroselinum crispum also known as parsley belongs to the *Apiaceae* family. It is a biennial herb native to the Mediterranean region but now cultivated worldwide. It has been claimed in Arab traditional medicine to possess variety of properties including laxative, diuretic and antiurolithiatic benefit. This study investigated the antioxidant and antimicrobial activity of the leaf extracts of parsley grown in Kano, Nigeria. The results showed that the ethyl acetate extract has the highest antioxidant activity with an IC_{50} value of 49.7 and 59.9 µg/ml of ascorbic acid equivalence in the total antioxidant assay. The petroleum ether fraction showed poor radical scavenging ability and a low total antioxidant activity hence, making it the least active fraction. The antimicrobial activity was examined using micro dilution technique against six standard human pathogens (*Salmonella typhi*, *Staphylococcus aureus*, *Klebsiella pneumoniae Mucor specie*, *Aspergillus flavus* and *Candida albicans)*. Significant activity of the fractions was observed in Minimum Inhibitory Concentration and Minimum Bactericidal/Fungicidal Concentrations. The fractions were all active against all the tested microorganisms at the highest concentrations. The chloroform fraction was the most active with MIC against all the tested microbes found to be 62.5 µg/ml while the ethanol fraction was found to be the least active fraction. The result obtained justifies part of the ethno medicinal claims on the medicinal uses of parsley.

Keywords: Antimicrobial activity, Antioxidant activity, Leaf extract, *Petroselinum crispum*

INTRODUCTION

Plants are widely used in many indigenous systems of medicine for therapeutic purposes and are increasingly becoming popular in modern society as alternatives to synthetic medicines (Dey and De, 2015).

Culinary herbal extracts and essential oils have become increasingly popular as alternative sources of natural preservative agents. This is largely because herbs are widely cultivated, effective, and safe for consumption (Tsai *et al*., 2013). Parsley or garden parsley (*Petroselinum crispum*) which belongs to genus *Petroselinum* of the family *Apiaceae*, is a specie native to the central Mediterranean region, naturalized elsewhere in Europe and widely cultivated as a herb, spice and vegetable (Heinrich, 2004). Parsley is a very popular spice and vegetable in Europe. It is known to be a diuretic, smooth muscle relaxant and hepatoprotective. The most important identified active ingredients are flavonoids, coumarins and vitamin C (Heinrich, 2004). Parsley leaves are used in some parts of the world for the treatment of skin disease, hypertension, urinary tract diseases, nose bleeding and baldness (Aurelia and Negulescu, 2011).

Free radical are highly reactive chemical species which causes oxidative stress and resulted to a large number of human diseases, such as heart disease, cataracts, cognitive dysfunction, aging and cancer as well as neurodegenerative diseases like Parkinson's and Alzheimer's diseases (Svetlana and Dorina, 2012). The presence of various, natural antioxidants in herbs have recently drawn interests in medicinal plants as potential source of agents against oxidative stress for instance phenolic compounds, especially flavonoids, can donate hydrogen to the harmful free radicals to prevent the oxidative damage at the first initiation step. They are not only scavenging radicals, but inhibiting their genesis (Nijveldt *et al.* 2001). Antioxidants can either be natural or synthetic and scientists have a serious concern on their safety because synthetic antioxidants have recently been found to cause health problems such as liver damage, due to their toxicity and carcinogenic nature. Therefore, search for safer antioxidants from natural sources has increased due to potential of plants as good source of traditional medicines to treat different diseases (Ammar *et al*., 2017). Another emerging challenge in disease control is the growing number of antibiotic-resistant bacteria. This poses a possible threat due to the decline in the therapeutic options for treating infectious diseases (Liu *et al*., 2017). Therefore, much attention should be paid to natural products, which could provide alternative

and effective drugs to treat human diseases, with high efficacy against pathogens and negligible side effects (Liu *et al*., 2017).

The present study was aimed at the *in vitro* screening of the extracts from *Petroselinum crispum* for its antioxidant and antimicrobial activities.

MATERIALS AND METHODS

Sample Collection

Fresh leaves of *Petroselinum crispum* were purchased from Kofar Wambai market, Kano Municipal Local Government Area, Kano, Nigeria. The plant was authenticated at the Herbarium in the Department of Plant Biology, Faculty of Life Sciences, Bayero University, Kano.

Extraction Process

Approximately 500 g of the powdered plant material (whole plant) were percolated in 1000 cm^3 of ethanol for one week. This were then filtered and concentrated using a rotary evaporator at 40ºC in order to obtain the crude ethanol extract. Other fractions were obtained from the crude ethanol extract using polarity gradient maceration, starting with low polar petroleum ether and chloroform before finalizing with polar ethyl acetate.

Determination of Free Radical Scavenging Activity by DPPH Assay

Free radical scavenging activity of the fractions of *Petroselinum crispum* was determined using DPPH assay as described by Baig *et al*., (2011) with slight modification. Various concentrations (1000, 500, 250, 125, 62.5, 31.25, 15.6 and 7.8 µg/cm^3) of each fraction were prepared. Approximately 60 µL of each test sample was aliquoted into a micro plate, in triplicate and absorbance readings were taken at 517 nm using a spectrophotometer. About 140 µL of 50 µM DPPH solution was added and incubated for 30 minutes in a dark room and absorbance readings were taken. A mixture of DPPH solution and methanol was used as blank while ascorbic acid was used as the standard. Percentage antioxidant activity was calculated using the expression in equation 1;

$$\% \text{ inhibition} = \frac{Ao - As}{Ao} \times 100 \quad (1)$$

Where;

A_O is the absorbance of blank DPPH (absorbance of DPPH+ methanol) and

A_S is the absorbance of sample (absorbance of sample –absorbance of blank).

Determination of Total Antioxidant Content by Phosphomolybdate Assay

The total antioxidant content of the fractions was determined using the procedure described by Kannan *et al*., (2010) with slight modification. 100 µg/cm^3 (0.1 mg/ml) concentrations of various fractions of *Petroselinum crispum* were prepared from the 1000 µg/cm^3 stock solution. Approximately 0.3 cm^3 of each sample solution was then mixed with 3 cm^3 of the reagent solution (0.6 M sulphuric acid, 28 mM potassium phosphate, 4 mM ammonium molybdate). The mixture was incubated at 95°C for 90 minutes in a water bath and absorbance was measured at 695 nm. Concentrations (1000, 500, 250,125, 62.5, 31.25, 15.6 and 7.8 µg/ml) of ascorbic acid were used as calibration standard. Total antioxidant activity was expressed as the number of equivalence of ascorbic acid and is expressed as mg ascorbic acid equivalents per gram of sample on a dry weight (DW) basis.

Test Microorganisms

All reference organisms were obtained from the Department of Microbiology, Bayero University, Kano. Three strains of bacteria *Salmonella typhi*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and three strains of fungi *Mucor specie*, *Aspergillus flavus* and *Candida albicans* were used.

Antimicrobial Assay

The extracts of *Petroselinum crispum* were screened for antimicrobial activity against six standard human pathogens. The broth dilution method was used to determine the Minimum Inhibitory Concentration (MIC) as described by (A.A. Aamer *et al.*, 2015) with slight modification. 1 cm^3 of the Mueller Hinton broth was added to sterilized each vials final dilutions of 1000, 500, 250, 125, 62.5 and 31.25 µg/ml. Exactly 0.1 cm^3 of the standard bacterial inoculums of the chosen micro organisms was innoculated into the 6 dilutions and incubated for 24 hours at 37°C. Minimum bactericidal/fungicidal concentration was determined from the test samples that showed no visible signs of growth or turbidity, Loopfuls were inoculated onto sterile Mueller Hinton agar plates by streak plate method. The plates were then incubated for 24 hours at 37°C. the results are then expressed as bactericidal or bacteristatic.

RESULTS AND DISCUSSION

The antioxidant activity of the fractions of *Petroselinum crispum* was evaluated by the radical scavenging activity of 1, 1-diphenyl-1,2-picryhydrazyl (DPPH) where ascorbic acid was used as the standard and the phosphomolybdate antioxidant assay which is based on the reduction of Mo (VI) to Mo (V) by the antioxidant sample. The former is based on potential of the antioxidants to scavenge the DPPH free radicals, while the latter is based on the fact that Mo (VI) is reduced to Mo (V) by the antioxidants which resulted in the formation of a green molybdenum (V) complex at an acidic pH. Figure 1 shows the IC_{50} values of all the tested fractions. It should be noted that a higher

radical scavenging activity is associated with a low IC_{50} value. Thus, ascorbic acid appeared to have a low IC_{50} value of 9.20, while the tested fractions have shown average antioxidant activity. Apparently, the ethyl acetate extract showed the highest radical scavenging activity with an IC_{50} value of 49.7, closely followed by the ethanol fraction and petroleum ether extract which had the least antioxidant activity. Similar trend of activity was observed from the total antioxidant assay. Antioxidant activity of the plant extracts ranged from 34.4 - 59.9 µg/mL of Ascorbic Acid Equivalence (as shown in Figure 1). The ethyl acetate fraction has the highest total antioxidant activity of 59.9 and the petroleum ether fraction has the lowest antioxidant activity of all the tested fractions. Similar results were obtained by Tang et al. (2015), where the ethyl acetate extract showed the highest DPPH radical scavenging activity while the dichloromethane extract of *P.crispum* showed the lowest IC_{50} value.

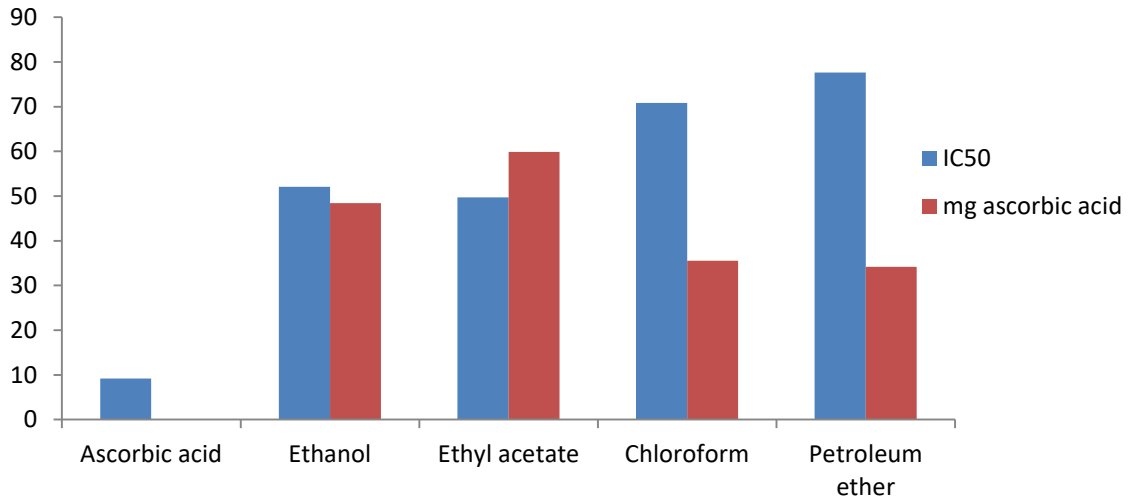

Fig 1: Antioxidant activity (IC_{50} and mg ascorbic acid) of the extracts of *Petroselinum crispum* and the standard used

The fractions showed significant activity against the microorganisms tested as they all furnished MIC values in the range of 62.5 – 250 µg/ml. Petrolini *et al*. (2013) described that the crude extract of a plant can only be considered promising when MIC < 100 µg/ml is achieved. In this work, the chloroform fraction is the most active as the MIC against all the tested microbes was found to be 62.5µg/ml, while the ethanol and ethyl acetate fractions showed poor antimicrobial activity.

Furthermore, the minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) confirmed the chloroform fraction to be the most active as it was able to inhibit the growth of micro-organisms at low concentrations compared to the other extracts. *Escherichia coli* was found to be the most susceptible strain and *Klebsiella pneumonae* was found to be the most resistant of the bacteria tested. The result obtained is similar to that reported by El Astal *et al*. (2005), where *Escherichia coli* was found to be more affected by the ethanol extract of parsley. Similarly, Dostalova *et al*. (2014) showed that the parsley aqueous extract inhibited all of bacteria with the most susceptible being *Hafnia alvei* and *Klebsiella oxytoca*. On the other hand, *Raoultella terrigena* and *Klebsiella pneumoniae* were the least susceptible.

The fungicidal effects were observed at the highest concentrations for all the fungi tested. The effects were found to be dose-dependent. Non polar solvent fraction, that is petroleum ether was susceptible to *Aspergillus flavus* where the highest concentration was the MFC and fungistatic effects was observed at 500 µg/ml. Similarly, Shaza and Aisha (2016) reported the non polar petroleum ether fraction to have shown significant activity against *Candida albicans.* They concluded that the non-polar fraction was the most active fraction.

Table 1: Results of minimum inhibitory concentration and minimum bactericidal/fungicidal concentration of the extracts of *Petroselinum crispum*

Bacteria/Fungi	Ethanol (µg/ml)			Ethyl acetate (µg/ml)			Chloroform (µg/ml)			Petroleum ether (µg/ml)		
	MIC	MBC/MFC BC/FC	BS/FS	MIC	MBC/MFC BC/ FC	BS/FS	MIC	MBC/MFC BC/FC	BS/FS	MIC	MBC/MFC BC/ FC	BS/FS
Escherichia coli	250	250	125	125	250	125	62.5	250	62.5	62.5	500	62.5
Salmonella typhi	62.5	1000	125	62.5	250	31.25	62.5	250	62.5	62.5	250	62.5
Klebsiella pneumonia	62.5	500	125	62.5	250	62.5	62.5	125	62.5	125	250	62.5
Aspergillus flavus	125	500	125	62.5	124	-	62.5	500	62.5	-	1000	62.5
Candida albicans	125	250	62.5	62.5	250	125	62.5	250	62.5	125	125	-
Mucor species	62.5	250	125	125	125	62.5	125	125	62.5	62.5	250	62.5

BC-Bactericidal; FC-Fungicidal
BS-Bacteristatic ; FS-Fungistatic

CONCLUSIONS

Various fractions of *Petroselinum crispum* were investigated for antioxidant and antimicrobial activities. The results revealed significant antioxidant activities for the fractions with ethylacetate being the most active. The antimicrobial activities against all the tested microorganisms indicated that the chloroform extract possessed the most promising activity. The result obtained from this research support some of the ethno-medicinal uses of parsley. However, further studies are needed to isolate and characterize the bioactive components of parsley which would enable more work on understanding their mechanism of action.

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