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Original Research Article

Anti-infective and Anti-inflammatory Properties of *Portulaca oleracea* L.

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***Portulaca oleracea* L. (Portulacaceae) is used traditionally in many parts of Africa, Asia and Australia to treat infections, wounds, animal bites and bleeding. Though there has been some scientific investigations on some of its folkloric uses, not all of these claims or practices have been scientifically proven. This study seeks to investigate the antimicrobial, antioxidant and anti-inflammatory activities of methanol extract of leaf and aerial parts of *P. oleracea*. The extract was screened for antimicrobial activity using the agar well diffusion method. Minimum inhibitory concentration (MIC) was determined by the micro-dilution method against typed strains of *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and clinical strains of *Streptococcus pyogenes* and *Candida albicans.* The antioxidant activity of the extract was determined using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging assay. Anti-inflammatory activity of the extract at doses of 100, 200 and 400 mg/kg body weight, were determined by carrageenan-induced footpad oedema in 7-day-old chicks. The preliminary phytochemical screening of *P. oleracea* revealed the presence of tannins, terpenoids, alkaloids, glycosides, sterols and saponins. The methanol extract showed antimicrobial activity against *S. aureus, S. pyogenes, E. coli, P. aeruginosa* and *C. albicans* with MIC values of 12.5, 12.5, 50.0, 50.0 and 50.0 mg/mL respectively. It also showed significant dose dependent anti-inflammatory activity at 100 (p<0.05), 200 (p<0.01) and 400 (p<0.01) mg/kg body weight. Methanol extract of the leaf and aerial parts of *P. oleracea* showed antimicrobial, antioxidant and anti-inflammatory activities and these pharmacological properties may justify the folkloric uses of the plant.**

Keywords: Antioxidant, Antimicrobial, Footpad oedema, Carrageenan-induced

INTRODUCTION

The use of plants as medicines predates written human history. It is estimated that about 60 to 70% of people living in developing countries still rely on plant medicine, and 80% of the world's population presently uses herbal medicine for some aspects of primary health care (WHO, 2012). There is an estimate that 70 to 80% of the people living in developing countries including Ghana almost exclusively use traditional medicine, especially herbal medicine for the treatment of many diseases because of the high cost of orthodox medicines, inadequate health facilities and healthcare professionals, coupled with lack of training of health workers on skin disorders and diseases (Agyare *et al.*, 2009).

A wide variety of chemical compounds are synthesised from plants and these compounds perform important biological functions. Research has shown that at least 12,000 such compounds have been isolated in recent times (Motaleb, 2010). It is also reported that approximately half of all prescription medicines in most part of Europe are derived from

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natural products and more than a quarter of the prescriptions dispensed annually in the United States were originally obtained from plants (WHO, 2012).

The chemical compounds in plants mediate their effect on the human body through processes identical to those understood in conventional drugs; thus plant medicines are as effective as conventional medicines, but also gives them the same potential to cause side effects. Various parts of the plants such as their roots, leaves, stem bark and seeds possess some active components that are of therapeutic value and hence useful in the treatment of diseases such as cancer, coronary heart disease, diabetes and infectious disease. Many of the herbal medicines that proved to be effective have been incorporated into modern medicine (Motaleb, 2010).

In recent years, several products of plants have been investigated for their therapeutic application due to their effect on inflammatory processes. Many herbs which are sources of phenolic compounds have been reported to show good antioxidant activity (Erclisi, 2008). The anti-inflammatory activity of natural compounds have been frequently associated with their antioxidant activity. Antioxidants inhibit the oxidation of other molecules and hence prevent damage of vital components in a cell.

Portulaca oleracea L. (Family Portulacaceae) commonly known as purslane is an herbaceous weed. It is used for the treatment of joint pains, wounds and other skin infections (Chan *et al*., 2000; Agyare *et al*., 2009). It is known to contain many biologically active compounds and it is also reported to be a source of many nutritional supplements (Ezekwe *et al.,* 1999). Some of the biologically active compounds isolated from the plant include free oxalic acids, alkaloids, omega-3 fatty acids, coumarins, flavonoids, cardiac glycosides, and anthraquinone glycosides (Ezekwe *et al.,* 1999). Crude extracts of *P. oleracea* have found to possess potent wound healing properties (Rashed *et al.,* 2003).

Ethanol aerial parts extract has demonstrated good anti-inflammatory and analgesic properties (*Chan et al., 2000*). *P. oleracea* is also known to exhibit muscle relaxant effect (Parry *et al.,* 1993). Methanol extracts of six different cultivars of *P. oleracea* have been found to possess good antioxidant activity (Lim and Quah, 2007). Phytochemical studies on the plant has revealed the presence of a wide range of alkaloids and flavonoids, including kaempferol, apigenin, myricetin, quercetin and luteolin (Xiang *et al.,* 2000; Xu *et al.,* 2006) and alkaloid oleraceins (A, B, C, D and E) (Xiang *et al.,* 2000). We investigated the antimicrobial and anti-inflammatory effects of methanol leaf and aerial parts extract of *P. oleracea.*

MATERIALS AND METHODS

Collection and preparation of the plant materials

Leaf and aerial of parts of *P. oleracea* were collected in July, 2013 from Jachie in the Bosomtwe District, Ashanti region of Ghana and authenticated by Dr. Alex Asaase of Department of Botany, University of Ghana, Legon, Ghana.

Extraction of plant material

The collected leaf and aerial parts of *P. oleracea* were cleaned and dried at room temperature (25 to 28 ºC) for 14 days. The dried parts were then milled into powdered form using a lab mill machine (Christy and Norris, United Kingdom). A quantity of 200 g of the powdered leaf and aerial parts of *P. oleracea* was weighed into a clean plastic container. To this quantity, 500 mL of 70% v/v methanol (BDH, England, UK) was then added. The extract was obtained by the use of an ultra-turrax (homogenizer) which mixed the solvent and powdered plant thoroughly under a speed of 24000 rpm for 5 mins The resultant mixture was then filtered using No. 5 sintered glass filter and high vacuum pump to obtain a supernatant of extract. This was done until enough extract was collected. The extract obtained was then concentrated using a rotary evaporator at 40ºC. The extract was then transferred into evaporating dishes and dried at 60ºC to solidify. The dried solid extract was then weighed and percentage yield determined. It was then kept in a desiccator until needed.

Phytochemical screening

Phytochemical test on the methanol extract was performed for the presence of alkaloids, glycosides (general test as well as saponins and anthracene), sterols, terpenoids and tannins (Evans, 2002).

Test organisms

The test organisms used for the antimicrobial determination included; *Escherichia coli* ATCC 25922*, Staphylococcus aureus* ATCC 25923*, Pseudomonas aeruginosa* ATCC 4853 and clinical stains of *Streptococcus pyogenes* and *Candida albicans.*

Determination of antimicrobial activity

Agar well diffusion method

The antimicrobial activity of the extract was screened using the agar-well diffusion method (Agyare *et al.,* 2014). Extract solutions of concentrations 100, 50, 25 and 12.5 mg/mL were prepared using distilled water as a solvent. Twenty millilitres of nutrient agar (Oxoid, UK) was melted, stabilized at 45ºC and seeded with 100 µL containing 10^6 colony forming unit (cfu)/mL of a 24 h broth culture of test organisms. The seeded nutrient agar was then poured aseptically into sterile petri dishes and allowed to set. In each petri dish, 5 wells were punched out equidistant from one another using a sterile cork-borer No. 4 (8mm internal diameter) and labeled appropriately. The cups were filled with 100 µL of different concentration of methanol leaf extract of *P. oleracea,* and 100 µg/mL of ciprofloxacin and 10 mg/mL ketoconazole as reference antibacterial and antifungal agents respectively. The plates were allowed to stand on the bench to ensure adequate diffusion of the extract and the reference drug. The plates were then incubated at 37℃ for 24 h, after which the zones of growth inhibition were measured and recorded. The experiment was carried out in triplicates.

MIC determination by micro-dilution method

The minimum inhibitory concentrations (MIC) of the extract was determined using the 96 well microtitre plate (Ellof, 1998). The plates were initially filled with 100 µL double strength nutrient broth (Oxoid, UK) and 10 µL (1 x 10^6 cfu/mL) of 24 h organisms suspension. Calculated volumes of the test solutions (plant extract, ciprofloxacin and ketoconazole) were filled into labelled wells to obtain a final volume of 200 µL with sample concentrations of 50, 25, 12.5, 6.25, 3.125, 1.5625 and 0.78125 mg/mL. The plates were then incubated at 37ºC for 24 h. The MIC was determined as the lowest concentration of test sample that inhibited growth which was indicated by the absence of purple colouration upon the addition of 10 µL of

125 mg/mL MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide). The experiment was carried out in triplicates.

Determination of antioxidant activity

The free radical scavenging activity of the extract was determined according to the method described by Braca *et al*. (2001) using 1,1-diphenyl-2-picryl-hydrazyl (DPPH). Extracts and reference antioxidant (α-tocopherol) solutions of concentration 0.10, 0.30, 1.0, 3.0, 10.0, 30.0 and 100.0 µg/mL were prepared in methanol. DPPH solution of concentration 5.0×10^{-6} M was prepared in methanol in a dark room. Three millilitres of this solution was added to 1.0 mL of the methanol extract and reference antioxidant.

The tubes were kept in the dark for 30 mins after which absorbance (A_1) of excess DPPH in the extracts and standard solutions were measured at 517 nm using a UV spectrophotometer. The absorbance (A_o) reading for a blank solution containing equal volume of methanol and DPPH served as a control (Hataro, 1988). The percentage of free radical scavenged was calculated from the equation [% inhibition = $((A_o\text{-}A_I)/A_o \times 100]$. Inhibition concentration IC_{50} was determined as the concentration of samples which scavenged 50% of free DPPH radicals.

Ethical approval for animal studies

The *in vivo* anti-inflammatory studies were approved by the Animal Ethical Committee, Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. The procedure was performed in accordance with the guide for care and use of laboratory animals.

Determination of anti-inflammatory activity

Carrageenan induced foot-pad demo model in chicks (Roach *et al.*, 2003) was used to evaluate the anti-inflammatory properties of the methanol leaf and aerial parts extract of *P. oleracea*. Chicks were randomly divided into five groups with each group containing five chicks per cage. Foot volumes were measured by water displacement (Fereidoni *et al.,* 2000). Test groups 1 to 3 were orally administered with 100, 200 and 400 mg/kg of the methanol extract, respectively whiles test group 4 was given aspirin (100mg/ kg) orally.

Test group 5 which was the control group received distilled water which was used as solvent in preparing doses. Inflammation or oedema was induced by a subplantar injection of carrageenan (0.1 mL of a 1% v/w solution in normal saline) into the right footpad of the chicks 1 h post treatment. The footpad volumes of the chicks were taken on the principle of volume displacement immediately before the experiment (zero time) and at 1, 2, 3, 4, 5 and 6 h post-carrageenan injection. Average oedema at every interval was assessed in terms of difference in volume displacement after injecting the philogistic agent and zero time volume displacement of the injected paw. Percent inhibition of oedema was also calculated for each dose from the AUC using the equation below:

$$\% \text{ Inhibition} = \frac{\text{AUC Control} - \text{AUC Treatment}}{\text{AUC Control}} \text{x} 100\%$$

Statistical analysis

All results were plotted and analysed with GraphPad Prism 5.0 for windows (GraphPad software, San Diego, CA, USA) and analysed by two-way ANOVA followed by Bonferroni post-test analysis which recognises **p<0.05, **p<0.01, ***p<0.001* as statistically significant.

RESULTS

Phytochemical screening

Phytochemical screening of the extract revealed the presence of tannins, terpenoids, alkaloids, glycosides, sterols and saponins.

Antimicrobial activity

The methanol leaf and aerial parts extract of *P. oleracea* demonstrated both antibacterial and antifungal activity against all the test organisms. Its antibacterial activity was evident on both Gram-positive and Gram-negative bacteria (Table 1).

Minimum inhibitory concentration (MIC) determination

The extract demonstrated some relatively good antimicrobial activity with MIC ranging from 12.50 to 50.00 mg/mL (Table 2).

Antioxidant activity

Methanol elaf and aerial parts extract of *P. oleracea exhibited high* free radical scavenging activity comparable to the reference standard (α-tocopherol) (Table 3).

Anti-Inflammatory activity

The extract showed a dose dependent reduction in oedema. The extract showed significant reduction in paw volume at 100 ($p< 0.05$), 200 ($p< 0.01$) and 400 mg/kg ($p< 0.01$) (Figure 2).

DISCUSSION

The continual and increasing use of plant extracts in the pharmaceutical, food and cosmetic industries suggests that a systematic study of medicinal plants is very important (Nostro *et al*., 2000). In this study on *P. oleracea*, phytochemical screening of methanol extract and the powdered plant revealed the presence of tannins, glycosides (saponins and anthracenes), terpenoids and sterols.

Antioxidants are agents that protect cells against damage caused by free radicals. The antioxidant activity of a plant is principally due to the presence of tannins, flavonoids and polyphenols (Esimone *et al.,* 2009). Antioxidant activity capacity, is widely used as a parameter for medicinal bioactive components. Preliminary phytochemical screening on *P. oleracea* showed the presence of tannins, which may play a role in preventing and protecting cells from oxidative damage, thereby augmenting the body's natural resistance to oxidative damage.

The process of inflammation leads to the release of chemical mediators which attracts neutrophils, leucocytes and monocytes, to an inflamed or wounded area and these attack foreign debris and micro-organisms through phagocytosis (Guo and DiPietro, 2010).

Table 1: Antimicrobial activity of leaf and aerial parts extarct of *P. oleracea*

Organisms	Mean zone of inhibition (mm±SEM)					
	Extract (mg/mL)				Ciprofloxacin (mg/mL)	Ketoconazole (mg/mL)
	100	50	25	12.5	0.1	10.0
S. aureus	16.00 ±0.17	15.00 ±0.57	17.23 ±0.67	14.00 ±0.58	22.50 ±0.289	nd
P. aeruginosa	na	na	na	Na	13.70 ±0.14	nd
S. pyogenes	17.67 ±0.33	17.50 ±0.29	15.90 ±0.10	14.00 ±0.12	22.67 ±0.33	nd
E. coli	na	na	na	Na	16.00 ±0.18	nd
C. albicans	15.90 ±0.21	13.33 ±0.33	10.97 ±0.033	10.07 ±0.07	nd	22.33 ±0.67

diameter of well: 8 mm; na: no activity; nd- not determined; SEM: standard error mean

Table 2: MIC of methanol leaf and aerial parts extract of *P. oleracea*

Organism	Minimum inhibitory concentration (MIC)		
	Extract (mg/mL)	Ciprofloxacin (µg/mL)	Ketoconazole (µg/mL)
S. aureus	12.50	0.16	nd
S. pyogenes	12.50	0.16	nd
P. aeruginosa	50.00	2.50	nd
E. coli	50.00	0.16	nd
C. albicans	50.00	nd	25.00

nd – not determined

Table 3: IC_{50} of leaf and aerial parts extract of *P. oleracea*

Sample	IC_{50}
P. oleracea extract	2.7
α-tocopherol	1.2

IC_{50} - Concentration that gives 50% reduction in DPPH

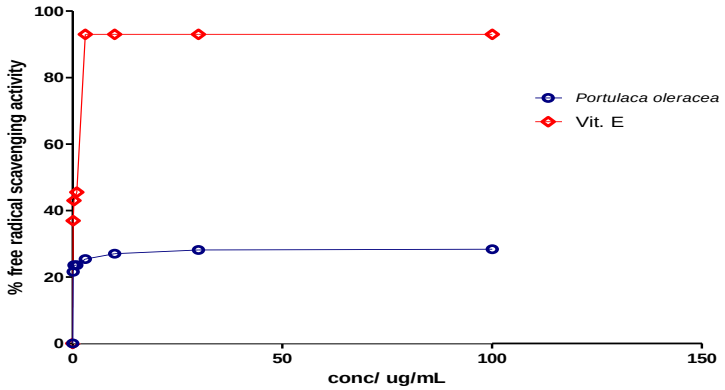
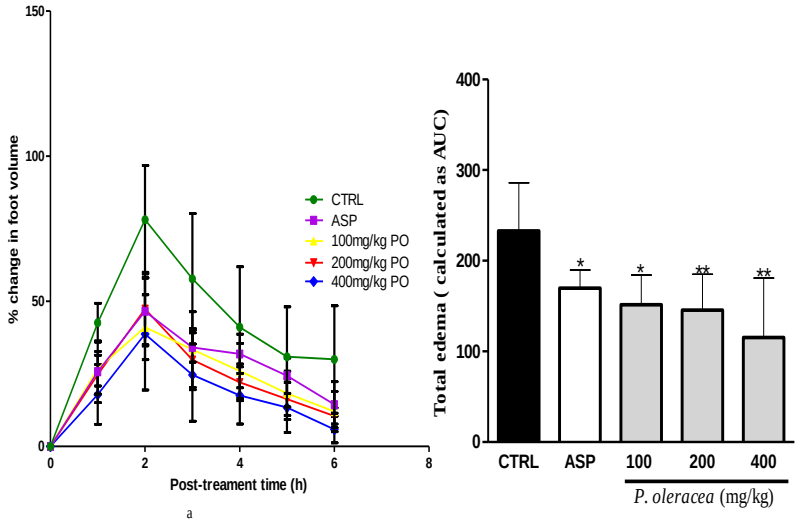


Figure 1: Antioxidant activity of leaf and aerial parts extract of *P. oleracea* **.** Vit E: α-tocopherol.



a

Figure 2: Influence of methanol extract of the leaf and aerial parts of *P. oleracea* on carrageenan- induced inflammation in chicks.

Table 4: Percentage inhibition of carrageenan induced oedema by leaf and aerial parts of *P. oleracea*

Drug	% Inhibition
Extract of *P. oleracea*	56.6
Aspirin	36.0

This leads to the production of oxygen-free radicals such as hydrogen peroxide, superoxide anion, and hydroxyl anion. The excess of these reactive oxygen species will cause tissue damage in man or animals if they overwhelm their body's natural antioxidants. Therefore the presence of tannins and other biologically active components of *P. oleracea* reduce the activity of the free radicals and thereby prevent the damage to cells and tissues, providing protection to human and animal subjects and burns (Ercisli *et al.,* 2008).

The antioxidant activity of the extract was determined using DPPH free radical scavenging method, which measures the ability of the extract to scavenge free radicals. The IC_{50} values, which is the concentration that gives 50% reduction in DPPH, gives the potency of an agent as an antioxidant. The IC_{50} values for the reference sample (α-tocopherol) and methanol extract of *P. oleracea* were 1.24 µg/mL and 2.77 µg/mL, respectively (Table 3). The lower the IC_{50} value, the better the free radical scavenging activity and vice versa. The demonstration of antioxidant activity by the methanol extract could be a means of potentiating its anti-inflammatory effects as indicated earlier. The presence of tannins as a phytochemical component in the extract could be responsible for the antioxidant effects.

P. oleracea demonstrated antibacterial and antifungal activity. According to Fabry *et al.* (1998), extracts with MICs below 8 mg/mL are classified as possessing potent antimicrobial activity. With reference to this study, the antimicrobial activity of the methanol extract of *P. oleracea* may be classified as low (Tables 1 and 2). The antimicrobial activity of the plant may also be attributed to the presence of some biologically active constituents, particularly tannins; which are known to possess astringent and antimicrobial properties. Different mechanisms have been proposed to explain tannin antimicrobial activity including; inhibition of extracellular microbial enzymes, deprivation of the substrates required for microbial growth, or direct action on microbial metabolism through inhibition of oxidative phosphorylation (Scalbert, 1991). The antimicrobial activity of the extract could also be attributed to the terpenoids which were also present during the phytochemical screening of the plant. Terpenoids are known to have a possible effect on the non-mevalonate pathway which is essential in fungi, protozoans, Gram-negative bacteria and other organisms for the synthesis of their cell membrane components and as a secondary source of carbon (Nayak *et al.,* 2010). Carrageenan-induced acute inflammation is mediated by cytokines (IL-1 and TNF-α), to cause increased vascular permeability, vasodilatation and fluid exudation. Proinflammatory mediators (PGE2 and PGI2) are also released by local tissues, mainly mast cells and leukotriene, in the foot of the chick leading to an increase in foot volume (Cashman, 1996).

The level of anti-inflammatory activity of the extract was dose dependent. Hence the higher the dose the more sensitized the receptors to the anti-inflammatory effect. The extract showed significant antiinflamatory activity at 100 (p< 0.05), 200 (p< 0.01) and 400 mg/kg (p<0.01). The mechanism

of anti-inflammatory activity may be due to the steroids present in the extract. The inhibition of arachidonic acid release may be the mechanism of action of steroids as anti-inflammatory agents. Steroids prevent phospholipase A2 from hydrolyzing arachidonic acid from phospholipids in the cell membrane with a resultant reduction in the production of the prostaglandins and thromboxanes. Steroids at cellular level causes adrenergically mediated vasoconstriction and non-competitive inhibition of vasodilatation due to prostaglandin E and bradykinin. Steroids also cause reallocation of granulocytes, resulting in increased circulating granulocytes and reduced tissue pools. These inhibitory effects parallel the relative anti-inflammatory potencies of these steroids. Aspirin, which was the reference drug used is a non-steroidal anti-inflammatory agent (NSAID) that also decreases prostaglandin and thromboxane production by inhibiting cyclooxygenase enzymes (especially COX-2) (Cashman, 1996).

P. oleracea extract showed better anti-inflammatory activity than the reference drug (aspirin). Since these two drugs work by different mechanisms, this may imply that the arachidonic pathway inhibition is more effective than the cyclooxygenase pathway inhibition.

CONCLUSION

The methanol extract of the leaves and aerial part of *P. oleracea* exhibited broad spectrum antimicrobial, antioxidant and a dose-dependent anti-inflammatory properties.

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CONFLICT OF INTEREST

Authors declare that they have no competing interests.

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