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Full Length Research Paper

Phytochemicals, antioxidant and antibacterial properties of a lichen species *Cladonia digitata*

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Antioxidant activity assessment was performed using the free radical scavenging activity of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and reducing power assay. Phytochemical screening was done using chemical tests. Antibacterial activities of *Cladonia digitata* were determined by a disk diffusion method at concentrations of 0, 10, 50 and 100% (v/v) against pathogenic bacteria, *Eschericia coli*, *Clostridium perfringens* and *Staphylococcus aureus.* Standard cotrimoxazole (10 µg/ml) was used as a positive control. *C. digitata* revealed the presence of alkaloids, tannins, saponins, cardiac glycosides, flavonoids, anthraquinones and steroidal terpenes. The results of antioxidant activity indicate a concentration dependent activity. *C. digitata* extract showed better free radical scavenging activity than ascorbic acid and quercetin. There was no significant difference (T-test, p = 0.05) in the antioxidant activity between the extract and those of ascorbic acid and quercetin as assessed by the reducing power assay. The highest antibacterial activity was 16.1 mm diameter of zone inhibition observed against *Clostridium perfringens* followed by 15.5 mm against *Staphylococcus aureus* at the concentration of 100 and 50%, respectively. The results reveal that the tested extract consisted of several bioactive compounds, significant antioxidant and antimicrobial activity. The present results scientifically authenticate the use of *C. digitata* in folk medicine.

Key words: Phytochemicals, antioxidant, antibacterial, *Cladonia digitata.*

INTRODUCTION

Lichens are well known symbiotic species consisting of one alga or a cyanobacteria and a fungi partner. The belief is that they consist of medicinal properties against various diseases and are often used in folk medicines (Rankovic et al., 2007). In Zimbabwe, traditional healers use them as alternative medicine for various diseases including treatment of severe burns. Lichens synthesize many useful secondary metabolites which are antiviral, anti-microbacterial, antitumor, anti-allergic and have an inhibitory effect on the growth of plants (Halama and van Haluwin, 2004; Huneck, 1999; Rankovic et al., 2007). This makes them an interesting object of research. Previously a lot of attention has been paid to lichens as a source of natural antioxidants (Behera et al., 2006; Gulcin et al., 2002). This is because the use of synthetic antioxidants in food to prevent rancidity and diets to reduce occurrence of diseases due to reactive oxygen and nitrogen species is diminishing rapidly as these are said to be mutagenic and genotoxic (Bergfeld et al., 2005; Maestri et al., 2006). Natural antioxidants consist of antifungal, antibacterial, antiviral, anti-inflammatory and anti allergic properties (François Muanda et al., 2010). They are also believed to lower chances of developing diseases such as cancers, coronary diseases, cerebral malaria and anthritis (Aliyu et al., 2008).

Growing resistance of pathogenic micro-organisms on current existing antibiotics is a major public concern and several researches have focused on lichens as a source of antimicrobial therapeutics or lead compounds to synthesis of new drugs (Gulluce et al., 2006; Halama and van Haluwin, 2004; Rankovic et al., 2008). In Zimbabwe, there are no researches focusing on antioxidant activity and antibacterial activity of lichens species despite their wide use in traditional medicine. Therefore, in the present study we investigated phytochemical composition,

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antioxidant and antibacterial activity of a lichen species growing in the Mavhuradonha range bordering the Zambezi escarpment.

MATERIALS AND METHODS

Samples of the lichens *Cladonia digitata* were collected from Mavhuradonha Mountains near St Alberts, Mashonaland central, Zimbabwe in May 2011 and validated by a taxonomist at Harare botanical garden. Voucher specimen (No. 2010/5) was deposited at Bindura university in the chemistry department (natural product section), for reference. Ethanol, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), quercetin, ascorbic acid, potassium fericyanide, trichloroacetic acid, ferric chloride, sulphuric acid, sodium hydroxide, agar nutrient (Mueller- Hinton - agar), cotrimoxazole, paper discs were obtained from Skylabs South Africa. Bacterial strains used in this research were *Escherichia coli, Clostridium perfringens* and *Staphylococcus aureus.* All other chemicals were of analytical grade.

Solvent extraction

Dried lichens were ground twice into powder using a wooden mortar and pestle, followed by sieving through a laboratory king test sieve 75 microns. The fine material which passed through the sieve was collected. The remaining residues were then discarded. Ground material, 10 g was extracted with cold ethanol (absolute) on a shaker at room temperature followed by filtration through Whatman no. 1 filter paper. The residues were re-extracted under the same treatment and the filtrates combined. The filtrates were then evaporated in a rotary evaporator at 40°C and stored in a deep freeze in the dark before use.

Phytochemical screening

Phytochemical screening of the extract was carried out to identify the secondary metabolites, resins, alkaloids (Mayer's and Draggendorff's test), tannins (ferric chloride test), saponins (Frothing test), flavonoids (Shinoda test), anthraquinones (Borntrager's test), cardiac glycosides (Keller-Killiani and Kedde tests), steroids and terpenes(Salkowski test), basing standard methods as reported by Sofowora (1994).

Antioxidant activity assay

Determination of DPPH radical scavenging activity

Free radical scavenging activity of *C. digitata* extract was assessed following a slightly modified previous reported procedure using the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH●) (Masuda et al., 2007). One milliter (1 ml) of 100 µM DPPH solution in ethanol was mixed with 1 ml of ethanolic extract/ascorbic acid/quercetin. The reaction mixture was incubated in the dark for 30 min and thereafter the absorbance was recorded at 517 nm against the blank. The solutions were prepared daily before measurements.

Reducing power assay

The total reducing power of lichen extract was determined according to the method of Oyaizu (1986). One milliter (1 ml) of the ethanolic lichen extract/quercetin/ascorbic acid were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium fericyanide [$K_3Fe(CN)_6$] (1%). The mixture was then kept in a 50°C water bath for 20 min. The resulting solution was then cooled rapidly, spiked with 2.5 mL of 10% trichloroacetic acid and centrifuged at 3000 rpm for 10 min. The supernatant (5 mL) was then mixed with 5 mL of distilled water and 1 mL of 0.1% ferric chloride ($FeCl_3$). The absorbance at 700 nm was then detected after reaction for 10 min. The higher the absorbance the stronger the reducing power. The assays were carried out in triplicate.

Antibacterial screening

Antibacterial screening is often performed by disc diffusion method (Khan et al., 2007; Wei et al., 2006). Twenty millimeter quantities of nutrient agar (Mueller Hinton Agar) were placed in Petri dishes with 0.1 ml of diluted bacterial culture. Filter paper discs (Whatman) (6 mm in diameter) impregnated with various concentrations of *C. digitata* extracts were placed on the organism seeded plates. Blank disc impregnated with ethanol was used as negative control and cotrimoxazole (10 µg/ml) was used as a positive control. The activity was determined after 18 h of incubation at 37°C. The diameters of zone of inhibition produced by the extract were then compared with the standard antibiotic cotrimoxazole. Each assay was done in triplicate for the determination of antibacterial activity.

Statistical analysis

Statistic analysis to compare antioxidant reducing power of extract and standard antioxidants were determined at the significance level of $p = 0.05$, student t test.

RESULTS

Phytochemical screening

The phytochemicals present in the dried powdered extract of *C. digitata* include alkaloids, tannins, saponins, cardiac glycosides, flavonoids, anthraquinones and steroidal terpenes (Table 1).

Antioxidant activity assay

DPPH assay

Free radicals are implicated in the pathology of diseases such as coronary heart diseases and cancer. DPPH• is considered to be a model of a stable lipophilic radical. Antioxidants react with DPPH• by either addition of an electron or a hydrogen atom. This reduces the number of DPPH• free radicals. Therefore, the absorption at 517 nm is proportional to the amount of residual DPPH•. It is observed by a discolouration from purple to yellow. The results of the free radical scavenging activity of the 1,1-diphenyl-2-picryl-hydrazyl radical (DPPH) assay showed that absorbance decreased with increasing concentration of extract (10 µg/ml to 200 µg/ml) (Figure 1). *C. digitata* extract proved to be a better free radical scavenger than quercetin and ascorbic acid. *C. digitata* line is below that of quercetin and ascorbic acid (Figure 1).

Table 1. Phytochemical screenings of *Cladonia digitata* extract (chemical tests results).

Phytochemical	Result
Resins	-
Alkaloids	++
Tannins	+
Saponins	++
Glycosides	+
Flavonoids	++
Anthraquinones	++
Cardiac glycosides	-
steroidal terpenes	++

+, Trace; ++, present in appreciable quantities base on the colour intensity of the chemical test; **-**, absent.

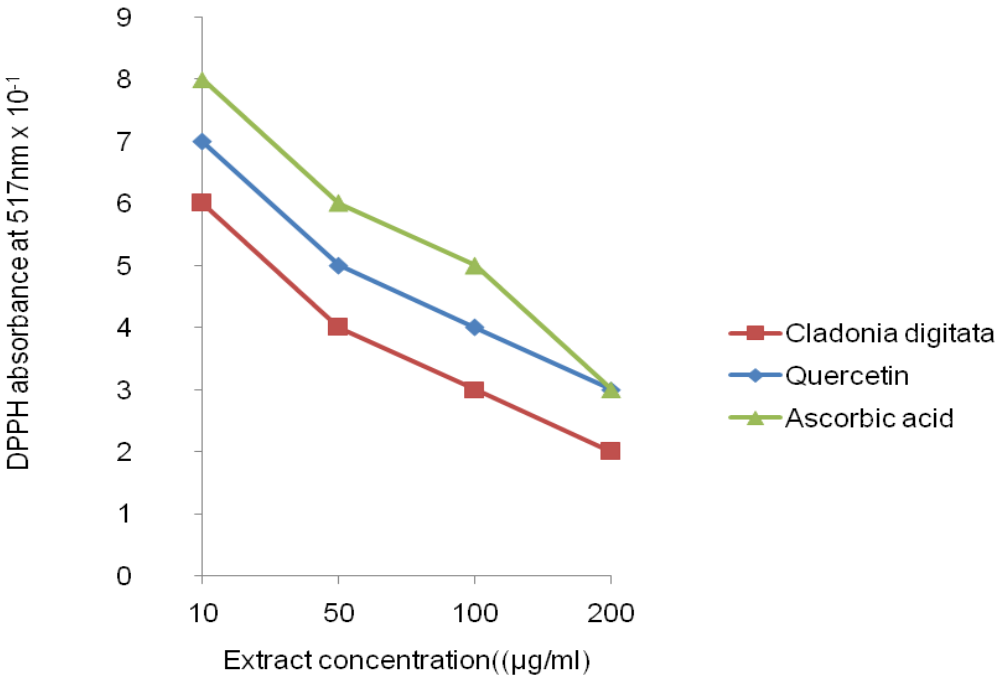

Figure 1. Free radical scavenging activity of *Cladonia digitata*.

Reducing power assay

In the reducing power assay, presence of antioxidants in the extract reduced iron (III) ferricyanide complex to the ferrous form. The reducing power of compounds can serve as an indicator of potent antioxidant properties (Aliyu et al., 2008) and increasing absorbance usually indicate an increase in reducing power. The reducing power of the extract (0.379 ± 0.018 nm) was comparable to those of standards quercetin and ascorbic acid (0.374 ± 0.012 and 0.372 ± 0.012, respectively) showing that there was no marked difference between antioxidant reducing power of extract and that of standards (Table 2). This suggests that the extract is an electron donor and could neutralize free radicals (Aliyu et al., 2008).

Antibacterial screening assay

Antibacterial activities of ethanol extract of *C. digitata* were determined at the concentrations of 0, 10, 50, and 100% against three pathogenic bacteria. The positive control was cotrimoxazole at a concentration of 10 µg/ml (Table 3). The highest activity was 16.1 mm diameter of zone inhibition observed against *Clostridium perfringens* followed by 15.5 mm diameter of zone inhibition against *Staphylococcus aureus* at the concentration of 100 and 50%, respectively. Antibacterial activity was comparable

Table 2. Antioxidant reducing power of *Cladonia digitata*.

Concentration(µg/ml) of extract	Reducing power n = 3 (Abs. 700 nm)
10	0.188 ± 0.022
50	0.285 ± 0.028
100	0.314 ± 0.007
200	0.379 ± 0.018*
200(µg/ml) Quercetin	0.374 ± 0.012*
200(µg/ml) Ascorbic acid	0.372 ± 0.012*

*(P = 0.05) student t test, no significant difference in antioxidant reducing power of extract and that of quercetin and ascorbic acid.

Table 3. Antibacterial activity of *Cladonia digitata* for extract concentration 0, 10, 50, and 100% (v/v).

Test organism	Diameter of zone of inhibition (mm) n = 3				
	0% (- control)	10%	50%	100%	cotrimoxazole (10 µg/ml) (+ control)
Escherichia coli	0	7.0 ± 0.1	11.1± 0.2	15.0± 0.1	16.2± 0.2
Clostridium perfringens	0	14.3± 0.3	15.0± 0.1	16.1± 0.5	17.2± 0.1
Staphylococcus aureus	0	12.1± 0.4	12.5± 0.3	12.7± 0.1	17.9± 0.7

to that of 10 µg/ml cotrimoxazole.

DISCUSSION

Phytochemicals are specialized plant metabolic compounds that act as antioxidants (Oktay et al., 2003; Wangensteen et al., 2004). They participate in redox systems which allow them to act as electron donors, hydrogen donors and singlet oxygen quenchers (Kahkonen et al., 1999). The DPPH model has been widely used as a quick, reliable and reproducible parameter to search for the *in vitro* general antioxidant activity of pure compounds as well as plant extracts (Kähkonen et al., 1999; Maestri et al., 2006). The decrease in absorbance in the DPPH assay with increase in concentration of the extract (Figure 1) which was accompanied with a rapid colour change of the purple DPPH, suggest that the ethanol extract of *C. digitata* has antiradical activity. Antioxidant activity may be due to the presence of terpenes, tannins and flavonoids (El-Massy et al., 2009; Maestri et al., 2006). Base on the present results of phytochemical screening, DPPH antiradical activity, ferric antioxidant reducing ability and antibacterial activity, it is possible to affirm that *C. digitata* can be used as a source of natural antioxidants and alternative method for treatment of diseases caused by bacteria and prevention of diseases due to free radicals. According to Ebana et al. (1991), alkaloids inhibit pathogenic bacteria, and tannins are important in herbal medicine in treating wounds which includes severe burns and to arrests bleeding (Nguyi, 1988). This confirms the use of *C. digitata* for wounds treatment in traditional medicine in Zimbabwe. The reason for different sensitivity of the extracts towards the selected bacteria can be rationalized as due to morphological differences between the organisms, for example differences in the porosity of the cell walls (Rankovic et al., 2008).

Conclusion

The present study showed that *C. digitata* consists of appreciable quantities of phytochemicals, significant antioxidant and antibacterial activity thereby supporting its use in traditional medicines by herbalists in Zimbabwe. It can be used as a source of antioxidant to prevent food rancidity and prevention of diseases. It is suggested that future studies should focus on the use of other models of *in vitro* antioxidant assessment, separation of active components from the extracts, structural elucidation, synthesis and antifungal screening as well as their possible use in the reduction of food rancidity in actual food samples.

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