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Antioxidant activity of *Clematis brachiata* Thunb. leaf

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Abstract

The antioxidant activity, total phenolic and flavonoid contents of different extracts of the *Clematis brachiata* Thunb leaves were determined. The antioxidant activity was evaluated using spectroscopic methods against 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) and 2,2′-azinobis [3-ethylbenzothiazoline-6-sulphonic acid] diammonium salt radical cation (ABTS). Folin Ciocalteu method was used to determine the total phenolics and Aluminium Chloride Colorimetric method was used to determine the flavonoids contents in these extracts. The results showed that the methanol extract of the leaf exhibited the highest antioxidant activity with the value of 180.45 ±2.4 µg mL^{-1} in DPPH and 60 ±0.80 µg mL^{-1} in ABTS assay among the extracts. The methanol extract contains more phenolic compounds (178±2.20 mg/g as galic acid equivalent per g dry matter) and the acetone extract contains more flavonoids (135.11±1.20 mg/g as quercitin equivalent per g dry matter) among the extracts. This study provides the evidence that the leaves of the *Clematis brachiata* Thunb could be a good source of natural antioxidant.

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Introduction

Medicinal plants are becoming more popular to the world of research as natural antioxidant sources because of their ability to detoxify free radicals from the biological system. Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function. Cell damage caused by free radicals appears to be a major contributor to aging and to degenerative diseases such as cancer, cardiovascular disease, cataracts, immune system decline and brain dysfunction (Cadenas, 2000). Fortunately, free radical formation is controlled naturally by various beneficial compounds known as antioxidants. Free radicals are electrically changed molecules i.e they have an unpaired electron, which causes them to seek out and capture electrons from other substances in order to neutralize themselves (Muthiah, 2012).

Antioxidants are compounds with the ability to protect a biological system against potentials harmful effects of processes or reactions involving reactive oxygen and nitrogen species (ROS and RNS) (Mishra *et al*., 2006) and they are natural disease preventing, health promoting and anti-ageing substances (Ozyurt *et al,* 2004). Phenolics are an important class of secondary plant metabolites possessing an impressive array of pharmacological activity. One of the more prominent properties of the phenolics is their excellent radical scavenging ability. Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes (Frankel, 1995). Some evidence suggests that the biological actions of these compounds are related to their antioxidant activity (Gryglewski *et al*., 1987).

Clematis brachiata Thunb, commonly known as Traveller's joy, is a deciduous climber that grows up to 5 m high belonging to Ranunculaceae family. It tends to clamber to the tops of trees and shrubs, sprawling over the crowns. Leaves are compound with from 1 to 7 leaflets.

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Attractive fragrant flowers appear in summer. Achenes are covered in fine silky hair. It is widely distributed in South Africa, Swaziland, Namibia and Botswana. Several species of the genus *Clamatis* have been widely used for folk medicine in many countries of the world. The decoction of the fruits and leaves of *Clematis vitalba* L. for the treatment of mouth inflammation and rheumatic pain in Italy (Pieroni *et al*., 2004; Loi *et al*., 2004) and the leaves of *Clematis drummondi* T & G used as a disinfectant and antibiotic in Mexico (Cantrell *et al*., 1998). The infusion of the leaves and stem bark is used for treating schistomiasis in South Africa (Spang *et al.*, 2000). Ethnomedicinal information from the indigenous people of the Eastern Cape Province revealed that the leaf extract is also used as a remedy for eye infection, skin disorder and wounds (Pendota *et al*., 2008). The antimicrobial activity of *C. hirsuta* Perr & Guill. leaves and *C. vitalba* L. (Cos *et al*., 2002; Khan and Kihara, 2001), antifungal activity of the aerial part of *C. drummondu* T & G. (Alanis-Garza *et al*., 2007) as well as antibacterial activity of the aerial part of *C. cirrhosa* L. (Tosun *et al*., 2004) have been reported. We had earlier been reported the antimicrobial activity, toxicity as well as anti-inflammatory, analgesic and anti-pyretic properties of *C. brachiata* leaves (Mostafa *et al*., 2013, 2009, 2010). Literature survey showed, there is no information on the antioxidant activities as well as total phenolic and flavonoids contents of *Clamatis brachiata* leaves. Therefore, the present study is an attempt to evaluate the antioxidant activity, total phenolics and total flavonoids contents of the different extracts of *C. brachiata* leaves.

Materials and methods

Plant material

Clematis brachiata was collected from a natural population within the premises of the University of Fort Hare, Alice, South Africa. The plant was identified by Prof DS Grierson of the Department of Botany, University of Fort Hare. A voucher specimen (M. Mostafa med. 2008/1) was prepared and deposited at the Giffen Herbarium of the University.

Preparation of extract

The dried leaves of the plant were pulverized and portions of 50 g each were separately extracted in hexane, acetone, methanol and water for 24 h on an orbital shaker (Stuart Scientific Orbital Shaker, UK). The extracts were filtered using a Buchner funnel and Whatman no. 1 filter paper. The acetone and methanol extracts were evaporated to dryness under reduced pressure at 40°C using a vacuum rotary evaporator (Laborot 4000-efficient, Heldolph, Germany), while the water extract was freeze-dried with Savant Refrigerated Vapor Trap (RVT4104, USA).

Antioxidant activity

DPPH free radical scavenging assay

The radical scavenging activities of the extracts on DPPH radical was determined by using the method described by Liyana-Pathirana and Shahidi (2005), with slightly modification. Briefly, one ml of DPPH solution (0.135 mM) in methanol was mixed with one ml of varying concentrations of the extracts. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was read at 517 nm using butylated hydroxytoluene (BHT) as standards. A blank solution was prepared containing the same amount of methanol and DPPH. The ability to scavenge DPPH free radical was calculated from the expression: (%) DPPH radical scavenging activity = $[(Abs_{control} - Abs_{sample})/ (Abs_{control})] \times 100$, where $Abs_{control}$ was the absorbance of DPPH radical + methanol and Abs_{sample} was the absorbance of DPPH radical + sample extract/ standard. The IC_{50} (the concentration of sample required to decrease the absorption at 515 nm by 50%) was calculated to quantify the antioxidant activity (Table I).

ABTS radical scavenging assay

The method described by Re *et al*. (1999) was adopted for the ABTS radical scavenging assay. The stock solution (equal volumes of 7 mM ABTS salt and 2.4 mM potassium persulfate) was allowed to stand in the dark for 14 hrs at room temperature. The resultant $ABTS^+$ solution was diluted with methanol until the absorbance of 0.70 ± 0.01 at 734 nm was attained. Varying concentrations of the plant extracts (1 mL) was reacted with 1 ml of the $ABTS^+$ solution and the absorbance read at 734 nm within 1-3 min using the spectrophotometer (Beckman DU-7000, USA) using butylated hydroxytoluene (BHT) as standards. The percentage inhibition was calculated from the expression: (%) ABTS radical scavenging activity = $[(Abs_{control} - Abs_{sample})/ (Abs_{control})] \times 100$, where $Abs_{control}$ was the absorbance of ABTS radical + methanol and Abs_{sample} was the absorbance of ABTS radical + sample extract/ standard. The IC_{50} (the concentration of sample required to decrease the absorption at 734 nm by 50%) was calculated to quantify the antioxidant activity (Table I).

Determination of total phenolic

The total phenolic content of the extracts was determined using the modified Folin-Ciocaltu method (Wolfe *et al*., 2003). Briefly, 1 mL extract (1mg/mL) was mixed with 5 ml

Table I. Total phenolic, flavonoid and IC_{50} value of the leaf extracts of *Clematis brachiata* against DPPH and ABTS model. (n = 3, x ± SD)

Extract	Total phenolic (mg g^{-1})	Total flavonoids (mg g^{-1})	DPPH IC_{50} (µg mL^{-1})	ABTS IC_{50} (µg mL^{-1})
Acetone	135.11± 1.20	65.58 ± 0.60	330.40 ± 2.12	130 ± 1.4
Methanol	178.00 ± 2.20	36.37 ± 0.36	180.45 ± 2.4	60 ± 0.80
Water	64.88 ± 0.80	10.03 ± 0.40	480.25 ± 3.6	170 ± 0.75
BHT	-	-	80.60 ± 0.60	60 ± 0.45

Folin-Ciocalteu reagent (1:10 v/v distilled water) and 4 ml (75 g/L) of sodium carbonate. The mixture was vortexed for 15 s and allowed to stand for 30 min at 40°C for colour development. The absorbance was read at 765 nm with a spectrophotometer (Beckman DU 700, USA). Total phenolic content was determined as mg of gallic acid equivalent per g of dry extract using the equation obtained from a standard gallic acid calibration curve: y = 6.993 x + 0.0379, R^2 = 0.9995. where × is the absorbance and y is the gallic acid equivalent (GAE). All the tests were performed in triplicate.

Determination of total flavonoid

Aluminium chloride colourimetric assay was used to determine the total flavonoid contents in the extracts as previously reported method described by Ordonez *et al*. (2006). Briefly 0.5 mL of 2% $AlCl_3$ was prepared in ethanol and was then added in 0.5 ml of the extracts. The mixture was allowed to stand for 60 min at room temperature and the absorbance was read at 420 nm with a spectrophotometer (Beckman DU 700, USA). The extracts were evaluated at a final concentration of 0.1 mg/mL. Total flavonoid content was calculated and expressed as mg of quercetin equivalent per g of dry extract using the equation obtained from a standard quercetin calibration curve: y = 43.862 x - 0.1757, R^2 = 0.9931. where × is the absorbance and y is the quercetin equivalent (QE). All the tests were performed in triplicate.

Results and discussion

There are many different experimental methods by which the free radical scavenging activity can be estimated. One such method, by which total free radical scavenging can be evaluated, is by determining their efficiency to scavenge DPPH radicals. The DPPH, (2, 2 diphenyl-1-picryhydrazyl) is a stable free radical, which is widely used as a tool for estimating free radical-scavenging activities of antioxidants (Fenglin *et al.,* 2004; Kim *et al*., 2002; Leong and Shui, 2002). The percentage of DPPH radical scavenging activity of the acetone, methanol and water extracts of *C. brachiata* leaves at different concentrations are shown in Fig **1**. The concentration of each extracts required to inhibit 50% DPPH free radical at 515 nm was calculated to quantify the antioxidant activities (IC_{50} value) and was shown in Table **I**. IC_{50} values were found to be 300.04 µg/mL, 180.45 µg/mL and 480.25 µg/mL for the acetone, methanol and water extract respectively. Among the studied extracts, the methanol showed the higher scavenging activity.

The ABTS radical cation scavenging assay is another widely used method to evaluate antioxidant activities. The percentage of inhibition of ABTS cation radical of the acetone, methanol and water extract at different concentrations are shown in Fig **2**. The concentration of each extracts required to inhibit 50% ABTS cation radical was calculated to quantify the antioxidant activities (IC_{50}) and was shown in Table **I**. IC_{50} values were found to be 130 µg/mL, 60 µg/mL and 170 µg/mL for the acetone, methanol and water extract respectively. Among the studied extracts, the methanol extract showed the higher scavenging activity at 60 µg/mL. The methanol extract displayed antioxidant activity that is

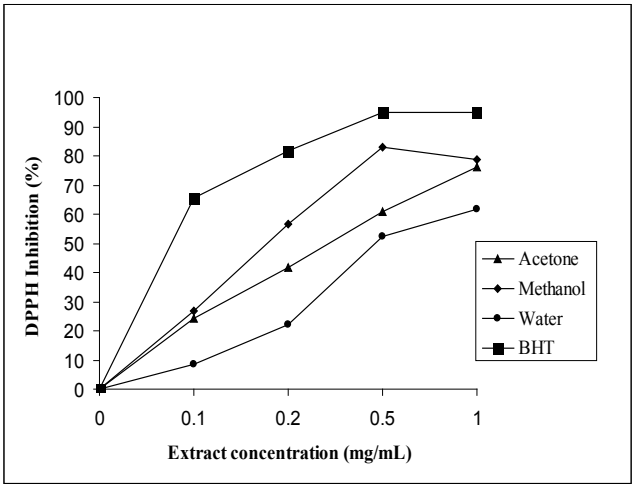

Fig. 1. DPPH free radical scavenging activity of the acetone, methanol and water extracts of the leaves of *Clematis brachiata* compared to Butylated hydroxytoluene (BHT) as standard antioxidant.

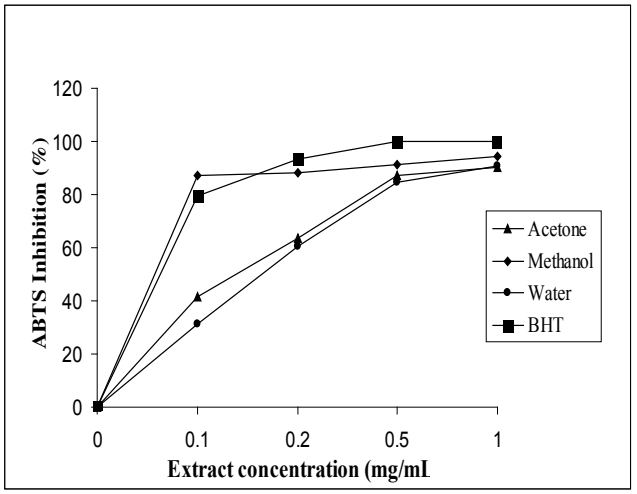

Fig. 2. ABTS scavenging activity of the acetone, methanol and water extracts of the leaves of *Clematis brachiata* compared to Butylated hydroxytoluene (BHT) as standard antioxidant.

similar to the reference compound, Butylated hydroxytoluene (BHT). The antioxidant activities decreased in the order of methanol, acetone and water in both DPPH and ABTS assays.

Total phenolic contents of different extracts of *C. brachiata* leaves were presented in Table **I**. The total Phenolic (TP) content were found to be 135.11 mg/g, 178 mg/g and 64.88 mg/g of gallic acid equivalents per g of dry matter for the acetone, methanol and water extract respectively. The highest TP content was recorded in *methanol extract* while the lowest TP content was found in water extract. Total flavonoids contents of different extracts were presented in Table **I**. The total flavonoid (TF) were found to be 65.58 mg/g, 36.32 mg/g and 10.03 mg/g of quercetin equivalents per g of dry matter for the acetone, methanol and water extract respectively. The highest level of TF was detected acetone extract while the lowest concentration was found in water extract.

Generally, total phenolic and flavonoids are groups of compounds that act as primary antioxidants (Samarth *et al*., 2008). These compounds possess a wide spectrum of chemical and biological activities including radical-scavenging properties. Studies have shown a strong relationship between total phenolic content and antioxidant activity in plants (Dorman *et al*., 2003; Velioglu *et al*., 1998). The phenols contain hydroxyls that are responsible for the radical scavenging effect mainly due to redox properties (Olayinka A, *et al*., 2010). On the other hand, the flavonoids compounds contain hydroxyl groups which are responsible for the radical scavenging activity (Das and Pereira, 1990). Plant phenolics and flavonoids have also been reported to have a lot of biological activities including inflammation, anti-carcinogenic, antioxidant and anti-mutagenic (Biju *et al*., 2014). The high antioxidant activities of methanol and acetone extracts might be due to their flavonoid and phenolic contents. Therefore, the antioxidant activity of these extracts of *C. brachiata* may explain its use to treat inflammations and wounds. The current investigation also confirmed that leaves of *Clematis brachiata* can be considered as potential natural antioxidants that play a major role in human health as free radical scavenger.

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