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Original Article

Appraisal of Radical Scavenging Activities and Phytochemical Constituents of the Aerial Part Extracts of *Guizotia scabra* (Vis.) Chiov

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ABSTRACT

Guizotia scabra (family Asteraceae) is a wild edible and medicinal plant native to Africa. It is widely used for treatment of myriads of diseases associated with oxidative stress, and this study aimed at evaluating the 1, 1-diphenyl-2-picrylhyorazyl (DPPH) radical scavenging activity and detecting major phytochemical constituents of extracts from the aerial part of *G. scabra.* The aqueous and 80% methanolic crude extracts prepared by decoction and by maceration, respectively as well as the different solvent fractions prepared from each of them showed considerable DPPH scavenging activity with IC_{50} ranging from 11.66-55.60 µg/ml. The n-butanol fraction prepared from the aqueous crude extract of the aerial part of *G. scabra* showed the most potent *DPPH scavenging activity* (IC_{50}=11.66 µg/ml) next to ascorbic acid (IC_{50}=1.23 µg/ml). Phytochemical screening on both the aqueous decoction and 80% methanol crude extracts showed the presence of polyphenols, flavonoids, saponins, and phytosteroids. In conclusion, the aerial part of *G. scabra* contains phytochemicals with marked radical scavenging activity that could at least partly justify the traditional use of the plant for treatment of different diseases associated with oxidative stress and its potential as source potent antioxidants.

Key words: Free radicals, radical scavenging, DPPH, phytochemical screening, *Guizotia scabra*, aerial part

INTRODUCTION

Free radicals are very reactive atoms or molecules with lone pair of electrons in their outer most shell and they are known for playing a dual role as both detrimental and valuable species. Excessive free radicals can cause oxidative damage to biomolecules, such as lipids, proteins and deoxyribonucleic acid (DNA) eventually leading to numerous chronic diseases [1-5]. The questionable safety of synthetic antioxidants that are mainly used in food industries and the growing public desire in preventive medicine have increased the interest in the search for more effective, safer and cheaper antioxidants from natural sources that can be used as nutraceuticals in the prevention of chronic diseases and as preservatives in foods, cosmetic and pharmaceutical industries [6-8]. *Guizotia scabra* (Vis.) Chiov., family Asteraceae is a native plant of Africa which is found in Nigeria, Cameroon, Democratic Republic of Congo, Rwanda, Burundi, Sudan, Eritrea, Ethiopia, Kenya, Uganda, Tanzania, Malawi, Zambia, Zimbabwe and Mozambique [9]. Locally it is known as *Meche* (Amharic), *Hadaa-udda* (Oromic), and *Nehuka* (Tigrigna) languages. In Ethiopia and other parts of Africa the leaf and seed parts are edible and the different parts of the plant are used for treatment of myriads of diseases associated with oxidative stress [10-12]. In Harar, eastern parts of Ethiopia, the aqueous decoction of the dried leaf and aerial parts of the plant are used for treatment of gastrointestinal disorders, inflammation, diabetes, malaria and cancer while the flower is used for treatment of eczema. Previous pharmacological studies revealed the antiviral, antioxidant, anticomplement, and antibacterial activities of the plant [12-14]. Sesquiterpene lactone and flavonoid was isolated from the leaf part of the plant [15]. Though *G. scabra* is widely used for treatment of

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different diseases associated with oxidative stress, there was no prior scientific study on the antioxidant activity of the aerial part of the plant. Therefore, the main objectives of this study were to determine the radical scavenging activities and phytochemical constituents of the aerial part of the plant.

MATERIAL AND METHODS

Plant material: The aerial part of *G. scabra* at its flowering stage were collected from Harar in the Harari Region, 525 km East of Addis Ababa, Ethiopia in October 2013 and authenticated by Mr. Melaku Wondafrash at the National Herbarium, Addis Ababa University, Biology Department and a voucher specimen was deposited at the laboratory of Pharmacognosy Course and Research Unit, College of Health Sciences, Mekelle University for future reference.

Chemicals and instruments: DPPH (SIGMA), methanol (Natason, India), n-butanol (Unichem, India), ethyl acetate (CarloErba, India) and dichloromethane (CarloErba, India) were used. All other chemicals and solvents were analytical and laboratory grade. JENWAY 6405 UV/Vis, UK was used for DPPH analysis.

Preparation of crude extracts: The shade dried and powdered aerial part of *G. scabra* (500 gm) was transferred to a 5 L conical flask and boiled for 90 min in 2 L of distilled water with occasional string. The extract was cooled and filtered through muslin cloth and then with Whatman No.1 filter paper in a suction filtration. The filtrates were then combined and dried in an oven at 45° C. For the preparation of the 80% methanol extract, 400 gm of the dried and powdered aerial part of *G. scabra* was extracted by maceration using 2 L of 80% methanol for 72 h with occasional shaking. The extraction was repeated three times. The extract was filtered first through muslin cloth and then by Whatman no.1 filter paper. The filtrates were combined together and concentrated under reduced pressure <50 °C using a rotary evaporator and further dried in an oven at temperature of 45°C. The dried aqueous and 80% methanol crude extracts were packed in glass containers and stored in a refrigerator until used for the experiment.

Preparation of fractions: For preparations of fractions from the aqueous crude extract 15 gm was suspended in 150 ml of distilled water and successively partitioned in 100 ml of dichloromethane, ethyl acetate and n-butanol, each three times in a separatory funnel then similar fractions were combined and the solvent removed under reduced pressure at 40° C in a rotary evaporator and further dried in an oven at 45° C. The 80% methanol crude extract was fractionated in the same way to the aqueous crude extract except that it was suspended in 70% ethanol instead of distilled water.

Radical scavenging activity: The method described elsewhere [16] was used with slight modification. 3 ml of 0.004% DPPH (SIGMA) in methanol was mixed with 1 ml of various concentrations (100, 50, 25, 12.5, 6.75, µg/ml) of the crude aqueous extract and fractions of the aerial part of *G. scabra* and ascorbic acid (a reference compound) separately. Then after 30 min incubation at room temperature in the dark, the absorbance of the mixture in the samples was measured using a spectrophotometer (JENWAY 6405) at 517 nm against methanol as blank. The percentage radical scavenging activities of the samples were evaluated by comparing with a control (3 ml DPPH solution and 1ml methanol). Each sample was measured in triplicate and averaged.

The percentage radical scavenging activity (RSA) was calculated using the following formula:

$$\% \text{ RSA} = [(A0 - A1)/A0] \times 100$$

Where $A0$ is the absorbance of the control, and $A1$ is the absorbance of samples after 30 min.

The free radical scavenging activity of the crude extract, fractions and ascorbic acid is expressed as IC_{50}. The IC_{50} value is defined as concentration (in µg/ml) of sample that scavenges 50% of the DPPH radical.

Preliminary phytochemical screening: The phytochemical screening of the aqueous and 80% methanolic crude extracts of the aerial part of *G. scabra* was carried out using standard procedures as described by Debela with slight modification [17].

Test for alkaloids: Five hundred milligrams of the extract was treated in a test tube with 10 ml of 1% HCl for 30 minutes in a water bath and then filtered through cotton. Small portion of the extract was transferred into two test tubes and to one of the test tubes, five drops of Mayer's reagent and to the second five drops of Wagner's reagent was added and the formation of whitish opalescence (Mayer's reagent) or reddish brown precipitate (Wagner's reagent) was inspected.

Test for anthraquinones

Free anthraquinones: Hundred milligrams of the extract was vigorously shaken with 10 ml of benzene and the extract was filtered. The filtrate

was treated with 5 ml 10% ammonia solution and shaken. The formation of pink, violet or red colour in the ammonia phase was considered positive for free anthraquinones.

Anthraquinone-O-glycoside: To about 1 g of the extract, 10 ml of 2 N HCl was added and boiled for 1 hr. It was then cooled, filtered, and the filtrate was extracted with 10 ml of benzene. To 5 ml of benzene extract, equal volume of 10% ammonia solution was added and shaken. The formation of pink, red or violate colour in the aqueous (ammonia) phase was taken as positive for anthraquinones-o-glycoside. The aqueous layer was kept to test for the presence of anthraquinone-c-glycoside.

Anthraquinone-C-glycosides: The aqueous layer remained from the determination of anthraquinone-o-glycoside above was washed with benzene until it gives negative for any free anthraquinone derived from it, and the remaining aqueous layer was treated with ferric chloride and heated for 30 minutes. It was then partitioned with benzene and the benzene layer was tested for the presence of free anthraquinone derived from the hydrolysis of anthraquinone-c-glycosides.

Test for polyphenols: To 100 mg of the extract dissolved in methanol, three drops of a mixture (prepared immediately before the reaction of one ml 1% $FeCl_3$ and one ml 1% $KFe(CN)_6$) were added and the formation of green blue colour was inspected.

Test for flavonoids (Shinoda reduction test): Hundred milligrams of the extract dissolved in 5 ml of 50% methanol was divided into two test tubes and to one of the test tube metallic magnesium and to the other zinc was added then five drops of concentrated HCl were added to each test tubes and the formation of an orange or red colour was taken as positive for the presence of flavonoids.

Test for coumarins: To 200 mg of the extract dissolved in 5 ml of ethanol, 2 ml of 10% ammonia was added and the occurrence of an intensive fluorescence under UV light was inspected. Comparison was made by taking another 5 ml of the extract in ethanol without 10% ammonia as a reference.

Test for saponins (Formation of honeycomb froth): Five hundred milligrams of the extract in 10 ml of distilled water was shaken in a test tube and the formation of honeycomb froth that persists for half an hour was considered as positive for saponins.

Test for tannins: One gram of the extract was heated in a test tube with 10 ml of distilled water for five minutes. After cooling, the solution was filtered through filter paper and 5 ml of 2% NaCl was added to the filtrate and filtered again. To the clear filtrate 5 ml of 1% gelatin was added and the formation of a precipitate was inspected.

Test for phytosteroids (Salkwoski reaction): One gram of the extract was macerated with 20 ml of petroleum ether and filtered. The filtrate was concentrated. The residue was then dissolved in chloroform and to it 5 drops of concentrated H_2SO_4 were carefully added and the production of a red or violet colour was regarded as positive for the presence of steroidal compounds.

RESULTS AND DISCUSSION

The antioxidant activity of the aqueous and 80% methanol crude extracts as well as the fractions prepared from the dried aerial part of *G. scabra* were determined by measuring their DPPH radical scavenging activity. As shown in Table 1, both the aqueous and 80% methanolic crude extracts showed marked DPPH radical scavenging activity. DPPH is a purple coloured, stable nitrogenous radical that absorbs at 517 nm and it is one of the most commonly used *in vitro* antioxidant testing methods. In the presence of free radical scavengers that donate a hydrogen atom or an electron, the purple colour changes to yellow and the absorbance decreases.

The potency of radical scavenging substances is usually compared by determining the concentration that cause 50% reduction in DPPH absorbance (IC_{50} value) and it is determined from the graph of percentage radical scavenging versus concentration. Lower IC_{50} indicates more potency [18-20]. As shown in Table 1, the 80% methanol extract showed better radical scavenging activity, IC_{50} three times lower (IC_{50} = 16.21 µg/ml) than the aqueous extract (IC_{50} = 55.60 µg/ml**).** This difference in DPPH scavenging activity between the aqueous and 80% methanol extracts could be due to variation in the nature and/or amount of phytochemicals extracted by them.

The 80% methanol extract is considered a total extract as it extracts polar, medium polar and nonpolar compounds while the aqueous extract is a polar extract containing mainly of polar and medium polar phytochemicals and this difference in phytochemical constituents may contribute for the observed higher DPPH scavenging activity by the methanol extract. Furthermore, the application of heat in the preparation of the aqueous extract may have damaged the active constituents.

Although locally the aqueous decoction or maceration of the plant is used for the management of myriads of diseases related with oxidative stress, in the present investigation the 80% methanol extract was tested in order to see the effect of other nonpolar phytochemicals that may not be extracted by aqueous decoction on the radical scavenging activity of the plant.

As both the aqueous and 80% methanol crude extracts showed prominent DPPH scavenging activities, and in order to know the nature of phytochemicals responsible for their DPPH scavenging activities, they were fractionated into nonpolar to polar fractions using different solvents of increasing polarities. As shown in Table 1, all the fractions showed good DPPH scavenging activities with IC_{50} ranging from 11.6-55.6 µg/ml. Those fractions prepared from the aqueous crude extract showed IC_{50} ranging from 11.66-33 µg/ml and their DPPH scavenging activities were stronger than the aqueous crude extract they were prepared from while the IC_{50} of the fractions prepared from the 80% methanol crude extract range from12.05-18.79 µg/ml. Among the extracts, the n-buthanol fraction from the aqueous crude extract showed the most potent activity followed by the ethyl acetate fraction prepared from the 80% methanol crude extract. However, the DPPH scavenging activities of the crude extracts and fractions were less potent than the ref standard antioxidant, ascorbic acid (IC_{50} = 1.23µg/ml). As the plant is used for treatment of different diseases, such as cancer, inflammation, diabetes and liver diseases that are associated with oxidative stress [22-25], the strong DPPH radical scavenging activities of both the aqueous and 80% methanol extracts may justify its use in traditional medicine.

Phytochemical screening on the aqueous and 80% methanol crude extracts of the aerial part of *G. scabra* (Table 2) revealed the presence of polyphenols, flavonoids, saponins and phytosteroids. Although both the aqueous and 80% methanol extracts of the plant possess similar classes of phytochemicals, they may not be identical compounds and even if they contain identical compounds, they may vary in quantity that might contribute for the difference in the DPPH scavenging activities between the crude extracts. Polyphenolic compounds like flavonoids and phenolic acids are potent free radical scavengers [26-31] and the presence of these compounds may be responsible for the promising DPPH scavenging activities of the aerial part extracts of *G. scabra*. Further studies involving isolation of the active compounds from the most active fractions may provide novel compounds with potent antioxidant activities that can be used as nutraceutical in the prevention of chronic diseases associated with oxidative stress. *In vivo* antioxidant and toxicity studies are also very important in order to utilize the plant effectively and rationally.

CONCLUSION

The results of the present investigation revealed that the aerial part of *G. scabra* possesses phytochemicals with promising radical scavenging activity that may at least partly justify its traditional use and its potential as a source of potent antioxidants that can be used as nutraceuticals and/or as a preservative in food, pharmaceutical and cosmetic industries.

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Table 1: DPPH scavenging activities of the aqueous and 80% methanolic crude extracts and fractions from the aerial part of *Guizotia scabra* in comparison with *ascorbic acid.*

Extracts & standard antioxidant		IC_{50} (µg/ml)
Aqueous crude extract and fractions prepared from it	Aqueous crude extract	55.60
	Dichloromethane fraction	33.00
	Ethyl acetate fraction	16.73
	N-butanol fraction	11.66
	Water fraction	29.53
80% methanolic crude extract and fractions prepared from it	80% methanol crude extract	16.21
	Ethyl acetate fraction	12.05
	N-butanol fraction	13.37
	70% ethanol fraction	18.79
	Ascorbic acid	1.23

Table 2: Preliminary phytochemical screening on the aqueous and 80% methanolic crude extracts prepared from the aerial part of *Guizotia scabra*.

Phytochemicals	**Aqueous crude extract**	**80% methanolic crude extract**
Alkaloid	Negative	Negative
Anthraquinones (Free)	Negative	Negative
Anthraquinone-o-glycosides	Negative	Negative
Anthraquinone-c-glycosides	Negative	Negative
Coumarins	Negative	Negative
Flavonoids	Positive	Positive
Phytosteroids	Positive	Positive
Polyphenols	Positive	Positive
Saponins	Positive	Positive
Tannins	Negative	Negative

Fig. 1. *Guizotia scabra* (Vis.) Chiov. (Asteraceae). (Picture taken in 2013 at *Saint George Church*, Harar, Eastern Ethiopia).

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