Journal of The Faculty of Science and Technology (JFST)
http://journal.oiu.edu.sd/index.php/JFST
https://doi.org/ 10.52981/jfst.vi8.1957
ISSN: 1858 - 6007
مجلة العلوم و التقانة – جامعة أم درمان الإسلامية – وزارة التعليم العالي و البحث العلمي - السودان

Research article **JFST Issue No. 8 (2021) 17 – 23**

Total Proteins, Total phenol and Flavonoid contents and Antioxidant activities of *Morettia phillaeana* aerial parts extract

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Abstract: *Morettia phillaeana* (Del.) DC. (Brassicaceae) is one of the two Morettia species which occur in Sudan. It used in folkloric medicine to cure some diseases. Aqueous extract was used to evaluate the total proteins, total phenol and flavonoids content and in vitro antioxidant activity using DPPH and ABTS models. The result indicated that the aerial parts contain 17.35 + 0.31 mg/100g proteins, 5.636+0.21 mg/g phenol and 2.713+0.11 mg/g flavonoids. High antioxidant activity was shown for both DPPH and ABTS, they were revealed concentrations equal to 78.85 and 88.66% respectively.

Keywords: *Morettia phillaena*, Proteins, Phenols, Flavonoids, Antioxidant activity.

Introduction

Free radicals are highly reactive biological molecules, possessing one or more unpaired electrons that play vital roles in the pathophysiology or ethology of several illnesses (Al-Snafi, 2017). This accounts for the recent interest this group of substances is receiving in drug discovery and research (Faria *et al*., 2019). Oxidative stress arises from the imbalance between the production of reactive oxygen species and the effect of the antioxidants. Medicinal plants can maintain the balance because they contain phenolic and alkaloidal antioxidants mainly from fruits and vegetables (Okoye, 2015; Al-Snafi, 2016). Antioxidants serve as guards against oxidative damage. They hold

sufficient immunity, repair or maintain homeostasis, and are also involved in stimulating the immune system.

Phenolic compounds are commonly found in both edible and nonedible plants, and they have been reported to have multiple biological effects, including antioxidant activity. Free radicals have been implicated in the development of a number of disorders, including cancer, neuro-degeneration and inflammation (Halliwell, 2006a, b, 2007). The presence of antioxidants such as phenolics, flavonoids, tannins and proanthocyanidins in plants may provide protection against a number of diseases; for example, ingestion of natural antioxidants has been inversely associated with morbidity and mortality from degenerative disorders (Gülcin, 2012). Medicinal plants are therefore being investigated for their antioxidant properties, and the demand for natural antioxidants and food preservatives is increasing (Peschel et al., 2006). The importance of the antioxidant constituents of plant materials in the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists, food manufacturers, and consumers as the trend of the future is moving toward functional food with specific health effects (Lo¨liger, 1991). Flavonoids and other phenolics have been suggested to play a preventive role in the development of cancer and heart disease.

The plant *Morettia phillaeana* (Del.) DC, (Brassicaceae) is one of two Morettia species which are occur in Sudan (Andrews, 1950). It grows in the Northern and central regions of the country, where it is known by the popular name of "Gabshah". Its smell is characteristic. Stiff erect hispid herb. Leaves linear to lanceolate, hoary or hispid. Petals pinkish-white, slightly longer than the very hairy sepals. Fruit strongly curved. Phytochemical studies conducted on the methanolic extract of the flowering aerial parts of *M. phillaeana* reported the presence of flavonoids (Burham, 2008), which revealed some in vitro antibacterial activity (El-Kamali and Ahmed, 2006).

Previous phytochemical studies conducted on the flowering aerial parts of M. philaena reported the isolation and identification of six flavonoids (Singab et al., 2000). These were kaempferol, quercetin, kaempferol 3-O-β-glucopyranoside, quercetin 3-O-β-glucopyranoside, quercetin 3-O-[2"-(6"'-pcoumaroyl-O-β-glucopyranosyl)-α-L-arabinopyranosyl]-7-O-glucopyranoside and kaempferol 3-O-[2"-(6"'-pcoumaroyl-O-β-glucopyranosyl)-α-L-arabinopyranosyl]-7-O-glucopyranoside. The qualitative and quantitative analysis of the essential oils were also reported (El-Egami et al., 2011). The present work revealed the isolation and identification of nine flavonoids, five of them were reported for the first time from the species under study. Their antimicrobial activity was also discussed.

Essential oils were obtained from the aerial parts of Morettia philaeana growing in central Sudan, by GLC analysis. Seven compounds were identified (El-Egami et al., 2011). Burham (2008) reported the presence of flavonoids in the methanolic extract in the flowering parts of M. philaeana. Nine flavonoids were isolated from the whole plant (Kawashy et al., 2012).

The present study directed towards outlining polyphenols, flavonoids, protein as well as the antioxidant activity.

Materials and Methods

Plant materials

The plant was collected from EL-Fiteehab area, University City Campus, Omdurman Islamic University in the Omdurman south, Khartoum State,Central Sudan, And authenticated by Prof Hatil .H Elkamali, Botany Department Herbarium. Voucher specimens of the plant are deposited in the Herbarium of the Omdurman Islamic University, Omdurman.

Determination of total phenolic compounds (TPC)

Total phenolic content was in plant ethanol extracts 80% of fresh and dried spices and herbs were determined by the Folin–Ciocalteau method reported by Gülçin *et al.*, (2006). extract of sample (200µl) were mixed with 2.5 ml of 0.2 M Folin–Ciocalteau reagent for 5 min and 2.0 ml of 75 g/L Na2CO3 were then added. The absorbance was measured at

760 nm after 2 hours of incubation at room temperature. Results were expressed as of Gallic acid equivalents (GAE)/g dw. µg/ml

Determination of total flavonoid content (TFC):

Total flavonoid contents (TFC) in plant extract was determined by using the aluminum chloride colorimetric method as described by (Gülçin *et al.*, 2006), with some modifications. Water extract (0.5 ml), 10% aluminum chloride (0.1 ml), 1M potassium acetate (0.1 ml) and distilled water (4.3 ml) were mixed, and the absorbance was measured at 415 nm wavelength using a spectrophotometer (Shimadzu, Japan). Catechin was used to make the calibration curve. The determination of TFC in plant extract was carried out in triplicate and hence values were reported as mean± SD in mg/ml.

The 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay:

Various concentrations of plant extract was added to 5 ml of the DPPH solution

(0.004% methanol solution) as described by Villaño *et., al* (2007). After 30 min incubation at room temperature, the absorbance was read against pure methanol at 517 nm Wavelength. The radical-scavenging activities of the samples were calculated as percentages of inhibition according to the following equation: I% = (A blank - A sample/ A blank) - 100; where A blank is the absorbance of the control (containing all reagents except the test compound), and A sample is the absorbance of the test compound. The concentration required for 50% inhibition (IC_{50}) was also calculated

Determination of radical ABTS scavenging activity:

The stock solutions of ABTS* reagent was prepared according to Dorman and Hiltunen (2004) by reacting equal quantities of a 7 mM aqueous solution of ABTS* with 2.45 mM potassium persulfate for 16 h at room temperature (25°C) in the dark. The working solution was then prepared by diluting 1 mL ABTS* solution with 60 mL of ethanol: water (50:50, v/v) to obtain an absorbance of 1.0± 0.02 units at 734 nm using the spectrophotometer. Extracts (50 µL) were allowed to react with 4.95 mL of the ABTS* solution for 1 h in a dark condition. Then the absorbance was taken at 734 nm using the spectrophotometer. Percent inhibition of the ABTS* free radical was calculated by the following equation:

Inhibition (%) = $100 \times [(A_{control}-A_{sample})/A_{control}]$

Where:

$A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound).

A_{sample} is the absorbance with the test compound.

Extract concentration of sample providing 50% inhibition (IC_{50}) was calculated using linear regression analysis.

Determination of protein

Nitrogen/Crude protein determination of proteins by "Macro kjeldahl distillation method"Reagents: 32% NaOH, Conc. H2SO4, 4% Boric Acid, K2SO4, CuSO4 and 0.1 N standard HCl solution. Mixed indicator: Dissolve 0.016g of methyl red and 0.03g of bromocresol green in 100 ml of alcohol. Apparatus: Kjeldahl flask, apparatus of digestion and distillation, burette etc. One gm of wet plant sample add in concentrated selenium sulphuric acid and hydrogen peroxide was added to each digestion tube for digestion. On heating blocks these digestion tubes with sample was heated. In order to remove the color digestion was continued at 350°C. Then these prepared solutions were diluted with distilled water and stored in tubes. Macro kjeldahl method Protein (% Nx6.25) was determined. One gram of dry ground plants samples were taken in digestion flask repectively. Digestion mixture (Cu SO4, K2SO4 and ferrous sulphate in

the ratio of 5, 94 and 1 respectively) then added 25 ml of conc Sulphuric acid to the flask and digested in digestion flask (kjeldatherm) for 6 hours. Then the flask was removed, cooled and then transferred to 250 ml flask. Distilled water was added in order to make the volume level to 50 ml of the above solution. Strong alkali 10 ml was added to make it alkaline and then added 50ml of 4% Boric Acid solution. Then transferred it to the distillation flask and mixed 3-5 drops of indicator. Then 50 ml water and 60 ml of 32% NaOH solution were added to it. After distillation, for titration it was then collected in flask. Add 0.1 N HCl in burette to the content of the flask. Noted the reading and the percentage of protein was determined using the following formula:
15(N%)=(V1−V2)×14.01×0.5×100(sampleinmg) V1= Reading of sample after titration,V2= Reading of blank after titration, 14.01= Nitrogen Atomic weight (N). Contents of crude protein (%) were calculated for plant sample by multiplying the nitrogen content of the sample by 6.25Protein (%) = Percent of Nitrogen × 6.25.

Result and Discussion

Table 1 showed that the protein contents exhibited value equal to 17.35±0.31, it same to be important hence the plant used by Sudanese local people to nourish the sheep and chicken (El-Kamali and Ahmed, 2006), that is due to high content of protein which represented by the obtained result. On the other hand, the flavonoid content represented moderate result equal to 2.713±0.1, while phenol content showed high value equal to 5.636 ±0.21, this result is disagreeing with Abu Ziada, et. Al, (2015) who reported that the methanolic shoot extract represented value of 1.09 for flavonoid while phenolic content showed 3.33, Burham (2008) reported the presence of flavonoids in flowering parts of *M. philaeana* and nine flavonoids were identified.

Antioxidant activity (Table2) indicated high inhibition by DBTS equal to 88.66% whereas DPPH showed 78.85%. Antioxidant activity of Morettia shoots methanolic extracts was .105 $EC_{50\%}$ by DPPH (Abu Ziada, et. al, 2015). The rate of antioxidant activity of the plant extract rise with the rising of the phenolic content of the extract, the obtained results are in agree with what was reported by Miser Saliboglu *et al*. (2013). El-Egami *et al* (2010) identified composition of *M. phillaeana* oil, seven compounds representing 93.67% of the oil were identified. Among them 4-isothiocyanato1-Butene (69.35%), isothiocyanatomethyl Benzene (18.88%), cymene (2.65%), C9H14O3 (2.3%) and 2-phenyl acetonitrile (2.18%) were the major constituents of the oil.

Table 1. Total protein and phenol and flavonoid contents.

Total protein%	Total phenol	Total flavonoids
Mg/100g	Mg/g	Mg/g
17.35 ± 0.31	5.636 ± 0.21	2.713 ± 0.11

Table 2. Antioxidant activity by ABTS and DPPH assay.

Antioxidant activity	ABTS	DPPH
IC_{50} mg/m	10.905	19.32
Inhibition%	88.66%	78.85%

Conclusion

We conclude that, the *M. phillaeana* aerial parts is a good natural source as antioxidant and proteins

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