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in vitro Antioxidant Activity, Total Phenolic Content and Antimicrobial Activity of *Coleus forskohlii* Grown in Al-Baha, Saudi Arabia

ABDELFATAH A. FADLELMULA[1,*], ABDULAZIZ Y. AL-GHAMDI[2] and MOHAMED O.M. ABDALLA[3]

[1]Department of Chemistry, Faculty of Science and Arts, Al-Baha University, Al-Makhwah 65931, Saudi Arabia
[2]Department of Biology, Faculty of Science, Al-Baha University, Al-Baha 61008, Saudi Arabia
[3]Department of Biology, Faculty of Science and Arts, Al-Baha University, Al-Makhwah 65931, Saudi Arabia

*Corresponding author: E-mail: abdalfatah55@yahoo.com

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In this study, the active ingredients from *Coleus forskohlii* were detected and evaluated their antioxidant and antimicrobial activities. *Coleus forskohlii* leaves were collected, air dried, powdered and extracted first with aqueous ethanol and secondly with petroleum ether, chloroform, ethyl acetate and *n*-butanol successively. The chemical analysis of the extracts showed the existence of (flavonoids, alkaloids, tannins, terpenoids, steroids, saponins and sugars). Furthermore, the extracts were subjected to analysis of total phenolic contents. Where the *n*-butanol exhibited highest TPC value (384.23±15.86 mg GAE/g), followed by ethyl acetate (199.73±29.35 mg GAE/g) then petroleum ether (96.97±61.29 mg GAE/g), ethanol (90.80±17.51 mg GAE/g), and eventually chloroform extract exhibited the lowest (TPC) value. In addition to that the antioxidant status of the different extracts of *Coleus forskohlii* was detected. Butanolic extract showed the highest radical scavenging (96.33±1.53 %, lowest IC_{50} value 0.03) followed by ethyl acetate extract (46.67±3.50 %, IC_{50} value 0.07). Eventually the antimicrobial activity tests were carried out for all the plant extracts. Where *n*-butanol showed the maximum inhibition (14±0.70 mm). All the pathogens except *C. albicans* exhibited moderate response towards petroleum ether extract. *C. albicans* was sensitive towards ethanol extract only (14.00±1.41), whereas the other Gram-positive and Gram-negative bacteria exhibited high impedance to the ethanol, chloroform, and ethyl acetate extracts. This study scientifically props the usage of whole plant as a medicine for several surface bacterial and fungal strike in folk medicine, in addition to this the plant may serve as a exporter for additional development of indigenous antioxidant and antitumor agents.

Keywords: *Coleus forskohlii*, Antioxidant activity, Total phenolic content, Antimicrobial activity.

INTRODUCTION

Plants and their derived output are portion of the healthcare regulation since old human culture. Herbaceous remedies have been utilized for numerous years by healers in different areas in the world. Generally, they use plants for nourishment and medical purposes [1]. The active ingredients are synthesized by plants as a defense system against diverse threats such as insects and different pathogens especially fungi as well as plant-eating animals, particularly cattle [2]. These compounds show anti-inflammatory, antioxidant, antidiabetic, antimicrobial and anticancer properties and accordingly have power for herbal drug development against specific diseases as well as promoting normal health [2].

Coleus species have 3000 years old history in Ayurvedic medicines which are in practice to cure heart and lung diseases. As having the capacity to break the adipose tissue, it is also useful in weight reduction programs. The rate of metabolism is induced to burn fat tissue by stimulation of adenylyl cyclase enzyme, resulting in cyclic AMP synthesis [3]. It is also seemed to be useful in eye care products to reduce pressure in the eyes. In the small intestine, it helps in adsorption of pre-digested foods [3]. It is also used against allergens (*i.e.*) as an antihistamine drug in diseases like asthma.

Medicinal plants are important exporters of natural products which vary completely in terms of their features, biological characteristics and methods of action. Different plant active components, particularly polyphenols like tannins, flavonoids,

phenyl propanoids and phenolic acids are known to possess antioxidant and free radical scavenging effectiveness. The antioxidant defense mechanisms can lead to the stopping of many diseases such as cancer, arteriosclerosis, diabetes and cardiovascular as well as quicken ageing [4].

Coleus forskohlii (wild.) Briq belongs to the family Lamiaceae, which is the extremely important species of genus Coleus. It is distributed in sub-tropical areas of the world, and also grows widely in Saudi Arabia. Traditionally, the roots have been used for preparation as condiments in pickles and preparation of pickles and also for medicinal purposes by the Ayurvedic Schools of medicines. Root juice is given to children suffering from constipation [5]. The objectives of this investigation are to find out the contents of total polyphenols, flavanoids and tannins in *Coleus forskohlii* (wild) Briq extracs in addition to evaluating the free radical scavenging inhibition activities and antimicrobial activity.

EXPERIMENTAL

Sampling: *Coleus forskohlii* dried leaves were detected for their antioxidant potency. The sample leaves were collected, perfectly washed in spout water and then swilled in distilled water. They were break into small segments and air-dried at room temperature for two weeks. After entire drying, the leaves were ground to fine powder by using mortar and pestle and stored at 4°C in air tight vessel until used.

Ethanolic extract of the sample leaves: The fine ground leaves (500 g) were soaking in 80% ethanol and left for three days. The specimens were subsequently filtered and the filtrates were left to dry in air at room temperature for 10 days. Lastly the solid extracts were preserved in coloured glass vessels at 4 ºC until analysis.

Fractionation of ethanolic extract through liquid-liquid extraction: The ethanolic extract was resolved in 250 mL of distilled water and then subjected to successive extraction using separatory funnel with the organic solvents in matter of rising polarity (petroleum ether, chloroform, ethyl acetate and *n*-butanol, respectively). Then evaporate the solvents, the solid extracts were collected and kept in cleaned dried vials at room temperature.

Phytochemical analysis: The phytochemical analyses was carried out for the petroleum ether, chloroform, ethyl acetate and *n*-butanol extracts so as to reveal the existence or the non-attendance of the important chemical classes (alkaloids, tannins, flavonoids, terpenoids, steroids, saponins and sugars) according to the standard methods of determination of chemical constituents of the plant [6,7].

Estimations of total phenolic content (TPC): The amount of phenolics in the plant leaves was quantified using spectrophotometric method [8] with some modifications. Sample solutions of the methanolic extracts in the amount of 1 mg/mL were utilized in the analysis. The reaction blend was set up by blending 0.5 mL of the sample solutions of fractions 2.5 mL of 10% Folin-Ciocalteu′s reagent resolved in water and 2.5 mL 7.5% $NaHCO_3$. Blank was consequently prepared including 0.5 mL methanol, 2.5 mL 10% Folin-Ciocalteu′s reagent resolved in water and 2.5 mL of 7.5% of $NaHCO_3$. The samples were then incubated in a thermostat at 30 ºC for 90 min. The absorbance was found utilizing spectrophotometer at λ_{max} = 765 nm. The specimens were attended in triplicate for each analysis and the average value of absorbance was acquired. The similar method was reduplicate for the standard solution of gallic acid and the calibration graph was constructed. Depending on the recorded absorbance, the concentration of phenolics was determined as (mg/mL) from the calibration graph. The amount of phenolics in extracts was cleared up in terms of gallic acid equivalent (mg of GAE/g of extract) [8].

Antioxidant activity

Free radical scavenging assay: The antioxidant test used was depend on the scavenging power of antioxidant(s) in plant extracts with respect to the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH), which is dark magenta in colour, to form the conformable hydrazine with joining colour change to light magenta.

This procedure was performed consistent to that designated by Shyur *et al*. [9] with some modulations. A stock solution was made by resolving 1mg of the sample in 1 mL of absolute ethanol (98%). Stock solution was diluted to ultimate concentrations of 100, 50, 25, 12.5, 6.25, 3.125, 1.5625 µg/mL in ethanol. About 0.9 mL tris-HCl and 1 mL of 0.1 mM DPPH in methanol solution were added to each concentration and incubated at room temperature in the dark for 30 min. The absorbance of the resulting blend was measured at 517 nm and translated to percentage antioxidant activity using the relation below:

$$\text{Scavenging activity } (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

A blank solution was prepared using 0.9 mL tris-HCl + 0.1 mL absolute ethanol + 1 mL absolute ethanol, while a solution of 0.9 mL tris-HCl + 0.1 mL absolute ethanol + 1 mL DPPH was used as a control. Newly equip DPPH solution shows a dark magenta with a maximum absorbance at 517 nm. The magenta colour vanishs when an antioxidant is existent in the medium. Thus, the change in the absorbance of reduced DPPH was used to estimate the potential of test compound to behave as free radical scavenger [9].

Antimicrobial activity test

Microbial organisms: Bacterial strains of *B. subtilis* (NCTC 8236), *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853), in addition to *Candida albicans* (ATCC 7596) were used in this study for antimicrobial activity test.

Preparation of bacterial suspensions: A portion (1 mL) of 24 h broth culture of the bacteria were aseptically dispensed onto nutrient agar slopes and incubated at 37 ºC for 24 h. The bacterial growth was produced and rinsed off with 100 mL sterile normal saline to output a suspension including about 10^8-10^9 cfu/mL, which was storage in the ice-box at 4 ºC till used. The medium number of fertile organisms per ml of the stock suspension was estimated by means of the surface viable counting method [10]. Sequent dilutions of the stock suspension were performed in sterile normal saline solution and 0.02

mL volumes of the suitable dilution were transmitted by micro pipette onto the face of dried nutrient agar dishes, which were let to stand for 2 h at room temperature for the drizzles to dry and then incubated at 37 ºC for 24 h. After incubation, the number of progressed colonies in each drop was calculated. The mean number of colonies per drop (0.02 mL) was multiplied by 50 and by the dilution factor to get the viable count of the stock suspension, evident as cfu/mL suspension. Every time a fresh stock suspension was equip. All the above experimental situations were made constant so that suspensions with close viable counts would be gained.

Preparation of fungal suspension: The fungal cultures were preserved on Sabouraud dextrose agar, incubated at 25 ºC for 4 days. The fungal development was collected and washed with sterile normal saline and finally suspended in 100 mL sterile normal saline and storage in ice-box until used.

Agar disc diffusion method: The disc diffusion method was used to test the antibacterial efficacy of plant extracts and carried out by using Mueller Hinton agar (MHA) and Sabouraud dextrose agar. The procedure was designated according to Boudjema *et al*. [11]. Bacterial and fungal suspensions were diluted with sterile physiological solution to 10^8 cfu/mL (turbidity = McFarland standard 0.5). A 100 µL of bacterial and fungal suspensions were wiped regularly on surface of MHA and Sabouraud dextrose agar and the inoculum was left to dry for 5 min. Sterile filter paper discs (Whatman No.1, 6 mm in dia.) were locatede on the face of the MHA and Sabouraud dextrose agar and macerated with 20 µL of a solution of each plant extracts. The inoculated plates were incubated at 37 ºC for 24 h in the in reverse place. After incubation term, the antimicrobial activity was found by recording the diameter of the inhibition zone around each disc. The antibacterial activity results were indicated in terms of the diameter of zone of inhibition as follows: < 9 mm zone (resistant strain); 9-12 mm (partially sensitive strain);13-18 mm (sensitive strain); > 18 mm (very sensitive strain).

Statistical analysis: The statistical analyses was achieved with Statistical Analysis Systems (SAS, Ver. 9, SAS Institute Inc., Cary, NC, USA) and the results were presented as the means ± standard deviation (SD) of three replicates repeated estimations. All the data were statistically evaluated using General Linear model (GLM) with completely randomized design and the significant difference was carried out using Duncan multiple range test at $p \leq 0.05$.

RESULTS AND DISCUSSION

Phytochemical analysis: The outcomes of phytochemical screening of leaf extracts of *Coleus forskohlii* (ethanol, ethyl acetate, chloroform, *n*-butanol and petroleum ether) showed the presence of flavonoids, alkaloids, tannins, terpenoids, steroids, saponins and sugars (Table-1). The results confirmed the existence of flavonoid, alkaloid, tannin, saponin and sugar in all extracts in considerable amount, whereas terpenoids and steroids were few in ethanol, chloroform and petroleum ether extracts. These results are in coincidence with the results of Rajkumar and Malathi [12] and Singh *et al.* [13]. Shanmugam *et al*. [2] ensured that rhizome extract of *C. forskohlii* was free from saponin, favonoid, glycoside, terpinoid and steroid. Flavonoids serve the plants in their own protection *versus* microbial agents. Terpenoid efficacy is might be attributed to their power to deactivate membranes, while tannins doing by meddlesome with protein formation through fastening to proline rich areas [14]. On the other hand, *C. forskohlii* extracts showed positive tests for phenolic compounds. The phenolic compounds are aromatic secondary metabolites that add colour, flavour and correlating with health advantages such as minimized risk of heart and cardiovascular diseases [15,16]. According to Aliyu *et al*. [17], phenolic compounds counting for most of the antioxidant activities in plants. Alkaloids have been notified to hold analgesic, antispasmodic and bactericidal, antimalarial and analgesic activities [18].

Total phenolic content: The results in Table-2 shows that the total phenolic content (TPC) of *Coleus forskohlii* directly affected by the nature of solvent used in liquid-liquid extraction. Where *n*-butanol recorded highest TPC value (384.23±15.86 mg GAE/g), followed by ethyl acetate (199.73±29.35 mg GAE/g) then petroleum ether (96.97±61.29 mg GAE/g), and eventually ethanol extract exhibited the lowest TPC value (90.80±17.51 mg GAE/g). This consequence confirmed that the extraction of TPC related to the polarity of the organic solvent used in the extraction, the more polar solvent extract more TPC. These outcomes are in accordance with that obtained by Rasineni *et al.* [19]. Nugraheni *et al*. [20] found low concentration of TPC in *C. tuberosus* leaves extract (7.73±0.08, 6.20±0.11 and 1.78± 0.03 GAE/g) for methanol, ethyl acetate and chloroform extracts, respectively). It has been reported by Soni and Bodakhe [21] that plant extracts of *C. forskohlii* were rich in polyphenols and major phytoconstituents have been offered to spend potent antioxidant and free radical scavenging activities in difference antioxidant types [21]. The high content of phenol and flavonoids of *C. forskohlii* extracts were reported by Shanmugam *et al*. [2] (38.82 ±0.22 mg GAE/g 21.34±0.32 mg GAE/g), whose using different solvents: ethanol showed the maximum antioxidant activity followed by acetone, aqueous (water), chloroform and petroleum ether [1], these findings correspond with present results.

TABLE-1
PHYTOCHEMICAL SCREENING OF *Coleus forskohlii*

Extract	Flavonoids	Alkaloids	Tannins	Terpenoids	Steroids	Saponins	Sugars
Ethanol	+	–	++	+	+	++	+
Chloroform	++	++	+	+	+	+	++
Ethyl acetate	+	+++	++	–	–	+	++
n-Butanol	+++	++	++	–	–	+	++
Petroleum ether	–	+	–	+	+	+	+

+ = Positive, ++ = Good present, +++ = Strongly present, – = Not detected

TABLE-2
TOTAL PHENOLIC CONTENT OF *Coleus forskohlii*

Extract	mg GAE/g ± SD
Ethanol	90.80 ± 17.51c
Petroleum ether	96.97 ± 61.29c
Chloroform	71.73 ± 23.57c
Ethyl acetate	199.73 ± 29.35b
n-Butanol	384.23 ± 15.86a
$p = < 0.0001$	

DPPH radical-scavenging activity: The free radical DPPH has a distinct absorption at 517 nm (magenta in colour), which diminish significantly on subject to radical-scavengers by supplying hydrogen atoms or by electron grant A lower absorbance at 517 nm represent a higher radical-scavenging activity of the extract [1]. Table-3 represents the potential of different *C. forskohlii* leaves extracts inhibit the DPPH free radicals activity, *n*-butanol extract showed the highest radical scavenging (96.33 ±1.53%, lowest IC_{50} value 0.03) followed by ethyl acetate extract (46.67±3.50 % , IC_{50} value 0.07). It was also found the different extents of DPPH scavenging activity for different solvents used, *i.e.* for ethanol extract, 40.40±3.50% and for petroleum ether extract, it was 30.90±2.07% whereas for chloroform it was 22.07 ±3.22 %. These findings differ from that obtained by Shanmugam and Pradeep [3], who reported that the highest DPPH radical scaven-ging activity (87.6%) was observed in ethanol extract of rhizome collected from Thiruvannamalai, India. Adam *et al.* [22] confirmed that the radical scavenging ability of *C. barbatus* determined by DPPH test showed highly large diversity in their capacity to inhibit DPPH where the highest radical scavenging effect was seen with the methanol extracts (56.00±0.03%), followed by dichloromethane (28.98± 0.09%) and the lowest one was by ethyl acetate (23.12± 0.06%) [22]. Bajpai *et al.* [23] recorded that ethanolic root extract of *Coleus forskohlii* showed antioxidant capacity with the inhibition of DPPH radical by 71.21%, which differ of the present findings, this may be attributed to the use of different part of the plant and also the different tool of extraction.

TABLE-3
ANTIOXIDANT ACTIVITY IN % AND IC_{50} OF *Coleus forskohlii*

Extract	Antioxidant activity (%)	IC_{50}
Ethanol	40.40 ± 3.50c	–
Petroleum ether	30.90 ± 2.07d	–
Chloroform	22.07 ± 3.22e	–
Ethyl acetate	46.67 ± 3.50b	0.07
n-Butanol	96.33 ± 1.53a	0.03
$p = < 0.0001$		

Antimicrobial activity: The results of antimicrobial activity of *Coleus forskohlii* L. (wild) carried out by disc diffusion method is given in Table-4. When the solvents were considered, *n*-butanol showed the maximum inhibition (14 ± 0.70 mm). All the pathogens except *C. albicans* exhibited moderate response towards petroleum ether extract, this finding agreed with that reported by Singh *et al*. [5]. *C. albicans* was sensitive towards ethanol extract only (14.00 ± 1.41), whereas the other bacteria showed high resistance to the ethanol, chloroform, and ethyl acetate extracts. Among the organisms, *B. cereus* and *C. albicans* were found to be more susceptible pathogens showing more inhibition. Singh & Singh [13] informed that *C. albicans* was sensitive towards ethanol , chloroform and petroleum ether extracts from *Coleus forskohlii* L. roots, these finding were agreed with what we found using leaves extracts. They also established that *E. coli*, *B. cereus* and *S. aureus* exhibited high response towards the root extracts of ethanol, chloroform, and petroleum ether, whereas ethyl acetate showed less response, these results were not corresponded with present findings since the outcome showed less response [13]. It is concluded that the extracts of *Coleus forskohlii* possess antimicrobial activity against tested organisms. The zone of inhibition difference proposing the varying grade of activity and different phyto-constituents of herb on the object organism. The antimicrobial activity of the plant may be referred to the existence of different active ingredients in the leaves.

Conclusion

The ethanol, petroleum ether, chloroform, ethyl acetate, and *n*-butanol extract of leaves of *Coleus forskohlii* were found to be a rich in phytoconstituents (flavonoids, alkaloids, tannins, terpenoids, steroids, saponins and sugars). The leaves extract possessed important power of antioxidants that could make apart of defense against oxidant and free radical injuries, furthermore they have their medicinal characteristics. Thus, the effective exporter of *Coleus forskohlii* could be utilized in all medicinal synthesis to resist plentiful diseases joined with oxidative stress, include cancer and related disturbance. The selected plant characterized by powerful antimicrobial components that may be of prime important for the evolution of pharmaceutical industries as a medication against variety remedies. The extracts possess considerable inhibitory influence against tested pathogens.

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TABLE-4
INHIBITION ZONE OF *Coleus forskohlii*

Extract	Inhibition zone (mm)				
	E. coli	*P. aeruginosa*	*S. aureus*	*B. cereus*	*C. albicans*
Ethanol	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	14.00 ± 1.41a
Petroleum ether	10.00 ± 0.00a	11.00 ± 1.41a	12.00 ± 0.00a	12.5 ± 0.71a	0.00 ± 0.00b
Chloroform	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b
Ethyl acetate	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b
n-Butanol	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	14.00 ± 0.70a	13.00 ± 0.00a
$p = < 0.0001$					

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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