

# Phytochemical composition and antioxidant activity of leaves extracts of *Coleus forskohlii* L. collected from Al-Leith Area, Saudi Arabia

Manal Y. Sameeh

Chemistry Department, Faculty of Applied Sciences, Al-Leith University College, Umm Al-Qura University, Makkah 24831, Saudi Arabia

\*Corresponding authors: e-mail: Myassemih@uqu.edu.sa

**Background:** wild *Coleus forskohlii* L. is a well-known traditional medicine for the treatment of many diseases because of its high forskolin content and several diterpenes. **Objective:** this study aimed phytochemical screening, finding of total phenolic content (TPC), total flavonoid content (TFC) and antioxidation activity of *Coleus forskohlii* L. leaves extracts in Al-Leith area, Saudi Arabia. **Materials and Methods:** dry leaves of wild *C. forskohlii* L. were used. Four solvents from diverse polarity groups were tested on these leaves, which are ethanol, ethyl acetate, chloroform, and hexane. Moreover, obtained extracts were used in phytochemical analyzing, finding of total phenols, and antioxidation activity. **Results:** showed the presence of phenols, flavonoids, tannins, alkaloids, Proteins, carbohydrates, saponins, and glycosides in *Coleus forskohlii* L. leaves. The highest value of total phenolic content (TPC) was significantly ( $P < 0.001$ ) in ethanol extract ( $280.5 \pm 2.33$  mg GAE/gm. Also, the highest value of total flavonoid content (TFC) was in ethanol extract ( $141.4 \pm 1.30$  mg QE /g). The antioxidation activity was significantly ( $P < 0.001$ ) higher in ethanol extract ( $78.55 \pm 2.23\%$ ), followed by ethyl acetate extract ( $60.18 \pm 1.21\%$ ), chloroform extract ( $36.11 \pm 2.54\%$ ), and lowest value in hexane extract ( $20.71 \pm 0.59\%$ ). The study clearly indicated that the leaves extract of *C. forskohlii* L. collected from Al-Leith region- Saudi Arabia has properties to be useful in pharmacological and biological industries.

**Keywords:** *Coleus forskohlii* L., Leaves Extracts, Phytochemical composition, Antioxidant activity.

## INTRODUCTION

Oxidation processes are essential for administering energy in living organisms, so, there is a balance between the production of reactive oxygen species (ROS) and oxidants, otherwise, numerous diseases will appear such as diabetes, cancer, aging, arteriosclerosis, and cardiovascular diseases<sup>1</sup>. Antioxidants are protective substances, that decrease oxidation damage in human cells when the enzymatic mechanisms are inefficient<sup>2</sup>. Despite the modern development of pharmaceutical chemicals to treat diseases, medicinal plants are still an important way of treating diseases<sup>3</sup>. Medicinal plants are considered as very valuable way to obtain natural compounds that are highly different in their properties, structures, and mechanisms of reaction. They have several kinds of polyphenols, such as phenyl propanoids, phenolic acids, flavonoids, and tannins<sup>4</sup>. They are known to have antioxidation activity and free radical scavenging efficacy. Besides, Polyphenols have several biological properties, such as metal chelation and inhibition of lipid peroxidation. *Coleus forskohlii* (Lamiaceae family) is one of the famous medicinal plants in Indian indigenous system of treatment, especially, for heart ailments, insomnia, asthma, bronchitis, anaemia, epilepsy, and inflammation<sup>5-6</sup>. It is highly spread in India, Thailand, and South-East Asia. It has been found also in Egypt, Arabian Peninsula, Ethiopia, and Brazil<sup>7</sup>. the leaves of *C. forskohlii* L. are used as an expectorant, diuretic and emmenagogue, stomach aid<sup>8</sup>, and in the treatment of intestinal disorders<sup>9</sup>. And *C. forskohlii* L. roots are used in increasing the power of myocardial contraction and increased insulin secretion<sup>10</sup>. The roots are also used to treat worms and relieve burning in festering boils. Moreover, it is applied to treat skin infections and eczema<sup>11</sup>. The roots are well-known as the only natural source of forskolin (Figure 1), which is a highly clinical compo-

nent of this plant. It is used traditionally in medicine to treat heart failure and is commonly applied to activate adenylate cyclase which has key regulatory roles in all cells<sup>12-13</sup>. Also, it has antiplatelet aggregation, antithrombotic, antidiuretic, and antidepressant activities<sup>14-15</sup>. More over, other constituents were isolated from roots of *Coleus forskohlii* L. and identified as 14- deoxycoleon U, demethylcryptojaponol, alpha-amyrin, betulinic acid, alpha-cedrol and beta-sitosterol. In other studies, they found several compounds in root extracts which are diterpenoids viz., deactylforskolin, 9 – deoxyforskolin, 1- deoxyforskolin, 1, 9 – dideoxy – 7 – deactylforskolin in addition to forskolin ( $7\beta$  – acetoxy – 8, 13-epoxy-  $1\alpha$ , 6  $\beta$ , 9 – trihydroxylabd-14-en-11-one)<sup>16-18</sup>. In addition, rosmarinic acid (Fig. 2), was isolated from *C. forskohlii* L. leaves. It has several biological effects such as antimicrobial, anti-inflammatory, antioxidative, and antiviral activities. its presence in herbs, medicinal plants, and spices gives them health-promoting effects on the human body<sup>19-20</sup>.

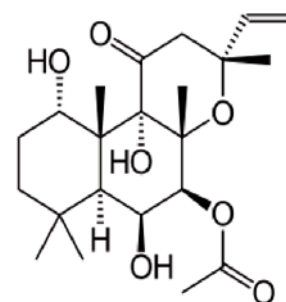


**Figure 1.** Chemical structure of Forskolin

Some studies carried out on the essential oil *C. forskohlii* L. leaves, The highly abundant components of the essential oil are a sesquiterpene hydrocarbon (7.5%),  $\gamma$ -eudesmol (12.5%),  $\beta$ -sesquiphellandrene (13.15%), 3-decanone (7.0%), and bornyl acetate (15%)<sup>21</sup>. Another

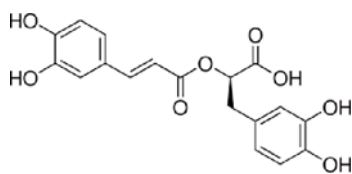


Figure 2. Chemical structure of Rosmarinic acid

study on essential oil identified twenty-five compounds, whereas thymol (52.02%),  $\gamma$ -terpinene (18.70%) 2-Undecanone (2.57%), (+) -4-carene (1.47%), o- cymene (12.73%), 6-methyl-2-heptanol acetate (1.50%), and caryophyllene oxide (1.36%) as the major components<sup>22</sup>. Based on scientific surveys, there is no systematic report in the literature on *C. forskohlii* L. collected from Al-Leith region - Saudi Arabia (located around 250 km south of Jeddah City). Because it is rich of remarkable concentrations of Mn, Co, Fe, Al, Ni, Zn, Cr, and Cu elements<sup>23</sup>. Therefore, the aim of this study is to calculate the potential antioxidation of leaves extracts of *C. forskohlii* L. collected from Al-Leith region – Saudi Arabia, using various extracts. Also, total phenolic content, total flavonoid content, and phytochemical analysis.

## MATERIAL AND METHODS.

### Chemical reagents and solvents:

Quercetin, gallic acid, folin-Ciocalteu reagent, aluminum chloride, and sodium carbonate from Merck Company (Darmstadt, Germany). All other solvents and reagents were of analytical grade.

### Processing of plant samples:

*C. forskohlii* L. leaves (Fig. 3) were collected from the Al-Leith area, Kingdom of Saudi Arabia. Authentication of the plant was performed by Dr. Haidar Abd Algadir, Department of Biology, Faculty of Science at Al-Baha University. The leaves of this plant were correctly washed in water tap and then in distilled water. The leaves were dried in an oven at a temperature of 35 to 40°C for 3 days. Then by using an electric blender, the dried leaves of this plant were fine crushed.



### Preparation of sample extracts:

The fine powder of dry leaves was put into the Soxhlet apparatus for the continuous filtration extraction process. Applying standard methods given by Harbone et al., and Szada et al.,<sup>24–26</sup> with each of ethanol (95%), ethyl acetate (95%), chloroform (95%), and hexane (95%), which were used as a solvent for extraction in 1:4 (raw materials to solvent) ratio. Then filtration of these four mixtures using filter papers (Whatman no. 4) (Fig. 4). This protocol was repeated two times to make sure that the extraction is well done with each solvent. After that, by using the rotary evaporator at room temperature the filtrates were dried, and the extracts were stored in dark bottles at 4–6°C for analysis.

### Phytochemical screening:

Chemical analysis was achieved in ethanol, ethyl acetate, chloroform, and ethanol extracts of dry leaves of *C. forskohlii* L. using standard protocols to analyze constituents<sup>24–28</sup>.

### Determination of total phenolic content (TPC):

The quantitative analysis of polyphenols in leaves extracts was analyzed by applying a procedure<sup>29</sup>. Each extract (1 ml) was added to Folin Ciocalteu reagent (1 ml). After 3 min, saturated sodium carbonate solution (30%) (1ml) was mixed with them and completed to 10 ml with distilled H<sub>2</sub>O. Then this mixture was saved in a dry and shade place for one hour with little shaking. Total phenolic contents (TPC) were calculated, and the absorbance was taken at 725 nm using a spectrophotometer (UNICAM UV300) based on the standard curve for gallic acid (GAE). The results were presented as mg of gallic acid equivalent per g of dry extract.

### Determination of total flavonoid contents (TFC):

The total flavonoid contents (TFC) were calculated by applying a modified aluminum chloride assay procedure<sup>30</sup>. Summarily, each extract (1 ml) was mixed with water (4 ml) in a 10 ml volumetric flask. In the beginning, 5% NaNO<sub>2</sub> solution (0.3 ml) was added to each volumetric flask, 10% AlCl<sub>3</sub> (0.3 ml) was added after 5 min, then 1.0 M NaOH (2 ml) was added after 6 min. Finally, water (2.4 ml) was added and mixed very well. The absorbance



Figure 3. *Coleus forskohlii* L. leaves



Figure 4. Preparation of the extracts

of the reaction mixture was taken at 510 nm. TFC was calculated based on the standard curve of Quercetin equivalents (mg QE /g of dry weight).

#### Determination of antioxidation activity:

Determination of antioxidation activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) Free radical scavenging procedure. The used method was applied according to standard method<sup>31</sup> with little modifications. First, a solution of 1 ml of DPPH (0.1 mM) and 0.9 ml of Tris-HCl in methanol was prepared. Then 1 ml of the prepared solution was mixed with 3 ml of each extract at all concentrations (100, 200 and 300 µg/ml) and incubated in the dark for 30 minutes at room temperature. Butylated hydroxytoluene (BHT) was applied as a positive control. Discoloration of the resulting mixture was measured at 517 nm. The below formula was used for the calculation of activity to scavenge the DPPH radical.

$$\text{DPPH scavenging activity (\%)} = [\text{ADPPH-AS} / \text{ADPPH}] \times 100$$

Where, AS is the absorbance of the solution when the sample extract was used and ADPPH is the absorbance of the DPPH solution.

#### Statistical analysis:

The statistical analysis was carried out by statistical analysis systems (SAS, Ver. 9, SAS Institute Inc., Cary, NC, USA) then the results were presented of three replicates as the mean  $\pm$  standard deviation (SD). Thus, the data were statistically evaluated by using the general linear model (GLM) and variance was calculated by using Duncan's multiple range test at  $P \leq 0.05$ .

## RESULTS AND DISCUSSION

#### Phytochemical analysis of *C. Forskohlii* L. leaves

Phytochemical-analyzing results (Table 1) of the leaves powder sample extracted in ethanol, ethyl acetate, chloroform, and hexane showed the presence of all the components in ethanol extract, followed by ethyl acetate extract that is positive for all components except tannins and carbohydrates. Chloroform extract was positive for phenols, flavonoids, carbohydrates, and proteins. Whereas hexane extract was negative for all components,

Table 1. Phytochemical constituents of *C. forskohlii* L. leaves extracts

| Chemical components | Extracts |               |            |        |
|---------------------|----------|---------------|------------|--------|
|                     | Ethanol  | Ethyl acetate | Chloroform | Hexane |
| Phenols             | +        | +             | +          | –      |
| Flavonoids          | +        | +             | +          | –      |
| Alkaloids           | +        | +             | –          | –      |
| Tannins             | +        | –             | –          | –      |
| Proteins            | +        | +             | +          | –      |
| Saponins            | +        | +             | –          | –      |
| Carbohydrates       | +        | –             | +          | –      |
| Glycosides          | +        | +             | –          | –      |

+: present, –: absent

this indicates that ethanol can extract all the available phytochemicals.

The presence of phenols, flavonoids, alkaloids, tannins, protein, saponins, carbohydrates, and glycosides in the ethanol extract of leaves, of *C. forskohlii* L., showed that this solvent is efficient to isolate reactive biological compounds due to its high polarity<sup>32–33</sup>. So, it is suggested that the phytochemical extract from this plant gives approval for use in allopathic medicine because they are potential sources of antitumor antiviral, and antifungals<sup>34</sup>.

#### Total phenolic contents (TPC) and Total flavonoids contents (TFC) of *C. forskohlii* leaves

TPC of *C. forskohlii* L. leaves extracts in Table 2 were shown significantly ( $P < 0.05$ ) highest TPC in ethanol extract ( $280.5 \pm 0.21$  mg GAE/gm), followed by ethyl acetate extract ( $199.18 \pm 1.21$  mg GAE/gm), then chloroform extract ( $162.94 \pm 1.45$  mg GAE/gm), and the lowest content was in hexane extract ( $51.72 \pm 2.16$  mg GAE/gm). The TPC values of *C. forskohlii* leaves were significantly different in all the four-extract used indicating that ethanol extract is the preeminent among them. Phenols commonly contain high antioxidant, anticancer and antimutagenic activities, so, the highest content of total phenol for leaves extracts is responsible for better antioxidant<sup>34</sup>. Phenolic compounds appeared from nonpolar to very polar compounds, so selecting the highest suitable solvent is significant in maximizing the extraction process. Ethanol is always preferable for the extraction of antioxidants from plants mostly because of its good extractability.

**Table 2.** TPC and TFC of *C. forskohlii* L. leaves

| Extract       | TPC (mg GAE/gm)          | TFC (mg QE /g)           |
|---------------|--------------------------|--------------------------|
| Ethanol       | 280.5±2.33 <sup>A</sup>  | 141.4±1.30 <sup>A</sup>  |
| Ethyl acetate | 199.18±1.21 <sup>B</sup> | 111.65±1.81 <sup>B</sup> |
| Chloroform    | 162.94±1.45 <sup>C</sup> | 88.4±2.10 <sup>C</sup>   |
| Hexane        | 51.72±2.16 <sup>D</sup>  | 41.7±0.60 <sup>D</sup>   |

Different letters superscripted represents statistical differences ( $p < 0.05$ ) within a column

Similarly, for TFC (Table 2), the highest value was shown in ethanol extract (141.4±1.30 mg QE /g) followed by ethyl acetate (88.4±2.10 mg QE /g), chloroform extract (88.4±2.10 mg QE /g), while hexane normally has given low content (41.7±0.60 mg QE /g). The TFC values of *C. forskohlii* leaves were also statistically significantly different ( $P < 0.05$ ) in all the four-spate extracts used indicating that ethanol extract is the outstanding among them. It is recognized that flavonoid compounds with a specific conformation and hydroxyl group in their molecules can be related to health and medical effects, such as inflammation, lowering the problems of aging, heart and blood vessel diseases, and some types of cancers<sup>32</sup>. Another study<sup>34</sup> carried out on the leaves of *C. forskohlii* L. collected from different land, it has given different values of TPC and TFC, this can be explained by the fact that the presence of phenolic compounds is affected by plant tissues and growth conditions. Several plants have already been shown to have antioxidant activity with higher amounts of phenols and flavonoids<sup>32</sup>. TPC was significantly ( $P < 0.05$ ) higher in tubers (27.05 µg catechol equivalents/g dry tissue) than in roots (24.22 µg catechol equivalents/g dry tissue) and stem (21.26 µg catechol equivalents/g dry tissue)<sup>34</sup>. The stems of *C. aromaticus* showed a higher content of total polyphenols (62.12 mg/g LW) compared with *C. forskohlii*. The ethanolic extract of *C. forskohlii* tubers had a maximum phenolic content of 38.82 ± 0.22 mg GAE/g<sup>35</sup>.

#### Antioxidant activity of *C. forskohlii* leaves extract

The antioxidation activity of *C. forskohlii* L. leaves is detected using methanol solution of DPPH reagent. It is proved that when the concentration of polyphenolic compounds increases, the antioxidation activity of a plant extract and DPPH radical scavenging activity increases as well. Antioxidants are compounds that delay or inhibit the oxidations in the cells by preventing the initialization of oxidizing reactions<sup>35</sup>. the antioxidant activity of leaves extract of *C. forskohlii* (Table 3), was significantly ( $P < 0.05$ ) higher in ethanol (78.55±2.23%), followed by ethyl acetate (60.18±1.21%), chloroform (36.11±2.54%), and hexane (27.76±2.76%). The antioxidant activity values of *C. forskohlii* leaves showed significant differences ( $P < 0.05$ ) in all four extracts used. Also, it has presented values of antioxidation activity close to the control (BHT) (Fig. 5). it can be concluded that the scavenging activity of leaves extracts at all concentrations (100, 200, and 300 µg/ml) on DPPH radicals were highly better, compared to BHT. The scavenging effects of ethanolic extract on the DPPH radical was decreasing in the order of BHT > leaves. So, the leaves extract of *C. forskohlii* L. collected from Al-Leith region of Saudi Arabia exhibited a stronger antioxidant activity than the same plant collected from another region of Saudi Arabia<sup>36</sup> This is attributed to the coastal nature of the land of Al-Leith,

which affects the concentration of compounds in the plant. Our findings demonstrated that the correlation coefficient between the phenolic concentration of leaves, flavonoids concentration and DPPH scavenging activity is 0.907, 0.911 and 0.918, respectively, indicating a strong correlation which are in correlation with other studies reported by researchers<sup>37–44</sup>. Hence the relationship between antioxidant activity and the phenolic& flavonoids presented that these components were the major contributors to the antioxidant properties which have been confirmed by several researchers<sup>45–53</sup>.

**Table 3.** Antioxidant activity (%) of *C. forskohlii* leaves extracts

| Extract       | Antioxidant activity (%) |
|---------------|--------------------------|
| Ethanol       | 78.55±2.23 <sup>A</sup>  |
| Ethyl acetate | 60.18±1.21 <sup>B</sup>  |
| Chloroform    | 36.11±2.54 <sup>C</sup>  |
| Hexane        | 27.76±2.76 <sup>D</sup>  |

Different letters superscripted represents statistical differences ( $p < 0.05$ ) within a column

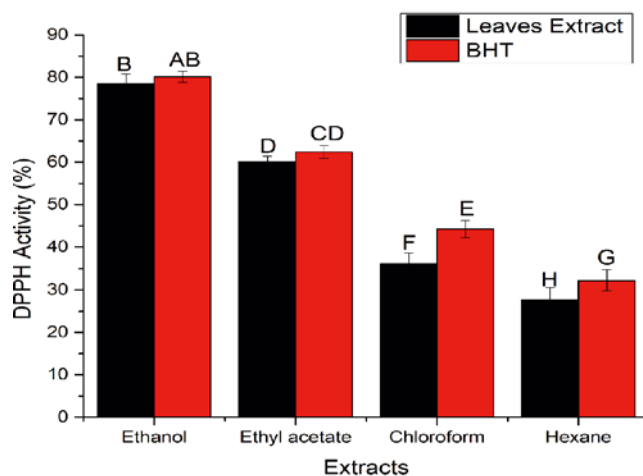


**Figure 5.** Antioxidant activity (%) of *C. forskohlii* L. leaves extracts compared with BHT. Each value is as mean ± SD, n = 3

#### CONCLUSION:

This study gives a comprehensive profile, phytochemical components, phenolic and flavonoid contents, and DPPH antioxidant activity of a wild plant *C. forskohlii* L. collected from Al-Leith region in Saudi Arabia. This plant presents a high value of antioxidant activity compared to the same plant grown in other areas so, it can be used in the biological, pharmaceutical, and food industries. Moreover, phytochemicals (phenols, flavonoids, tannins, alkaloids, proteins, carbohydrates, steroids, saponins, and glycosides) have been identified as all in ethanol extract of the leaves. Phenolic and flavonoid contents of leaves extracts are varying concentrations depends on the solvent in extraction.

#### LITERATURE CITED

- Basoudan, N., Taie, H.A.A., Abu-Gabal, N.S., Al-Ghamdi S.N. & Shalaby N.M.M. (2019). Phytochemical study and antioxidant activity of some flowering plants growing wild in Al-Bahah in Saudi Arabia. *Res. J. Pharm. Biol. Chem. Sci.* 10(3), 498–514. DOI: 10.21474/IJAR01/10660.

2. Soliman, M., Qari, S.H., Abu-Elsaoud, A., El-Esawi, M., Alhaithloul, H. & Elkelish, A. (2020). Rapid Green Synthesis of Silver Nanoparticles from Blue Gum Augment Growth and Performance of Maize, Fenugreek, and Onion by Modulating Plants Cellular Antioxidant Machinery and Genes Expression. *Acta Physiol. Plant.* 42, 148. DOI: 10.1007/s11738-020-03131-y.
3. Singh, A.K., Arun, P., Shukla, J., Kumar, A., Nandi, M.K., Abduljaleel, Z., Al-Ghamdi, Saeed S. Alzahrani, Abdullah, R., Shahzad & Naiyer Shahzad, N. (2023). A Review on Traditional Anti-ulcer Medicinal Plants. *Lett Drug Des Discov.* 20(4), 408–419. DOI: 10.2174/1570180819666220909090512.
4. Khalid, S., Almalki, F. A., Hadda, T.B., Bader, A., Abu-Izneid, T., Berredjem, M., Elsharkawy, E. & Alqah-tani, A.M. (2021). Medicinal applications of cannabinoids extracted from *Cannabis sativa* (L.): a new route in the fight against COVID-19?. *Current pharmaceutical design*, 27(13), 1564–1578. DOI: 10.2174/1381612826666201202125807.
5. Badhepuri, M.K., Manokari, M., Raj, M.C., Jogam, P., Dey, A., Faisal, M. & Shekhawat, M.S. (2023). Meta-Topolin enhanced direct shoot organogenesis and regeneration from leaf explants of *Coleus forskohlii* (Willd.) Briq. *Industrial Crops and Products*, 197, 116584. DOI: 10.1016/j.indcrop.2023.116584.
6. Kulkarni, C., Sharma, S., Porwal, K., Rajput, S., Sadhukhan, S., Singh, V., Akanksha S., Sanjana, B., S-roj, K., Aboli, G., Alka, R.P., Suriya P.S., Koneni V.S., Navin, K., Lal, H. & Naibedya, C. (2023). A standardized extract of *Coleus forskohlii* root protects rats from ovariectomy-induced loss of bone mass and strength, and impaired bone material by osteogenic and anti-resorptive mechanisms. *Frontiers in Endocrinology* 14. DOI: 10.3389/fendo.2023.1130003.
7. Lunz, K. & Stappen, I. (2021). Back to the roots – an overview of the chemical composition and bioactivity of selected root-essential oils. *Molecules*, 26(11), 3155. DOI: 10.3390/molecules26113155.
8. Sofowora, A., Ogunbodede, E. & Onayade, A. (2013). The role and place of medicinal plants in the strategies for disease prevention. *AJTAM*, 10(5), 210–229. DOI: 10.4314/ajtcam.v10i5.2.
9. Peltzer, D., Dreyer, E., & Polle, A. (2002). Differential temperature dependencies of antioxidative enzymes in two contrasting species: *Fagus sylvatica* and *Coleus blumei*. *Plant Physiology and Biochemistry*, 40(2), 141–150. DOI: 10.1016/S0981-9428(01)01352-3.
10. Romero-Martínez, B.S., Sommer, B., Solís-Chagoyán, H., Calixto, E., Aquino-Gálvez, A., Jaimez, R., Juan, C., Gomez-V., Georgina, G., Edgar, F. & Luis, M. (2023). Estrogenic Modulation of Ionic Channels, Pumps and Exchangers in Airway Smooth Muscle. *Int. J. Mol. Sci.* 24(9), 7879. DOI: 10.3390/ijms24097879.
11. Balasubramanian, V., Suresh, J., Srinivasan, R., Prabhu, M., Manikandan, E. & Nandha, V.G. (2020). Evaluation of soil characteristics and yield variation of coleus (*Coleus forskohlii*) in different agro climatic zones of Tamil Nadu. *Int. J. Conserv. Sci.* 8(4), 2841–5. DOI: 10.22271/chemi.2020.v8.i4ah.10076.
12. Abd-Allah, H., AbdAlhady, M.M., Abdelall, M.N., Mohamedy, S.N., Alharbi, W.D. & Bahathiq, A.O. (2017). Hormonal and immunological responses to *Coleus forskohlii* treatment in female rats with experimentally polycystic ovaries syndrome. *Zagazig Veter. J.* 45(1), 74–81. DOI: 10.21608/zvzj.2017.7689.
13. Pateraki, I., Andersen-Ranberg, J., Jensen, N.B., Wubshet, S.G., Heskes, A.M., Forman, V., Björn, H., Britta, H., Mohammed, S., Carl, E., Dan, S., Jørgen H., Birger, L. & Björn, H. (2017). Total biosynthesis of the cyclic AMP booster forskolin from *Coleus forskohlii*. *Elife*, 6. DOI: 10.7554/eLife.23001.
14. Krishna, G., Sairam, Reddy, P., Anoop, Nair, N., Ramteke, P.W. & Bhattacharya, P. (2010). In vitro direct shoot regeneration from proximal, middle, and distal segment of *Coleus forskohlii* leaf explants. *Physiol. Mol. Biol. Plants*, 16, 195–200. DOI: 10.1007/s12298-010-0021-y.
15. Kotia, A., Adhikari, B.S., Rawat, G.S. & Pasha, M. (2007). Status and Distribution of *Coleus barbatus* Benth. In Tehri Garhwal District, Uttarakhand. *J. Biodivers Endanger Species*, 2, 127. DOI: 10.4172/2332-2543.1000127.
17. Kharwar, R.N., Sharma, V.K., Mishra, A., Kumar, J., Singh, D.K., Verma, S.K., Surendra, K., Anuj, K., Nutan, K., Bharadwaj, R. & Souvik, K. (2020). Harnessing the phytotherapeutic treasure troves of the ancient medicinal plant *Azadirachta indica* (Neem) and associated endophytic microorganisms. *Planta Medica*, 86(13/14), 906–940. DOI: 10.1055/a-1107-9370.
18. Srivastava, S., Misra, A., Mishra, P., Shukla, P., Kumar, M., Sundaresan, V., Kuldeep, S., Pawan, K. & Ajay, K. (2017). Molecular and chemotypic variability of forskolin in *Coleus forskohlii* Briq., a high value industrial crop collected from Western Himalayas (India). *Rsc. Advances*, 7(15), 8843–8851. DOI: 10.1039/C6RA26190F.
19. Sivakumar, P., Bavithra, V.S., Ashokkumar, K., Deepadharsini, R., Selvaraj, K.V. & Gopal, M.R. (2021). Comprehensive review on phytochemistry and in vitro biotechnology of *Coleus forskohlii*. *J. pharmacogn. phytochem*, 10(1), 448–453. DOI: 10.22271/phyto.2021.v10.i1g.13346.
20. Godard, M.P., Johnson, B.A. & Richmond, S.R. (2005). Body composition and hormonal adaptations associated with forskolin consumption in overweight and obese men. *Obesity research*, 13(8), 1335–1343. DOI: 10.1038/oby.2005.162.
21. Luo, C., Zou, L., Sun, H., Peng, J., Gao, C., Bao, L., Renpeng, J., Yue, J. & Shuangyong S. (2020). A review of the anti-inflammatory effects of rosmarinic acid on inflammatory diseases. *Frontiers in pharmacology*, 11, 153. DOI: 10.3389/fphar.2020.00153.
22. Al-Ghamdi, A.Y., Fadlelmula, A.A., Abdalla, M.O. & Zabin, S.A. (2021). Phytochemical Screening, Chemical Composition, Antimicrobial Activity and in Silico Investigation of the Essential Oil of *Coleus forskohlii* L. Collected from the Southwestern Region of Saudi Arabia. *J. Essent. Oil-Bear. Plants*, 24(1), 120–133. DOI: 10.1080/0972060X.2021.1901613.
23. Kanyal, J., Prakash, O., Kumar, R., Rawat, D.S., Srivastava, R.M., Singh, R.P. & Pant, A.K. (2021). Study on comparative chemical composition and biological activities in the essential oils from different parts of *Coleus barbatus* (Andrews) Bent. ex G. Don. *J. Essent. Oil-Bear. Plants*, 24(4), 808–825. DOI: 10.1080/0972060X.2021.1958701.

24. Bantan, R.A., Khawfany, A.A., Basaham, A.S., & Gheith, A.M. (2020). Geochemical Characterization of Al-Lith Coastal Sediments, Red Sea, Saudi Arabia. *Arab. J. Sci. Eng.* 45, 291–306. DOI: 10.1007/s13369-019-04161-6.
25. Adetunji, L.R., Adekunle, A., Orsat, V. & Raghavan, V. (2017). Advances in the pectin production process using novel extraction techniques: A review. *Food Hydrocolloids*, 62, 239–250. DOI: 10.1016/j.foodhyd.2016.08.015.
26. Morsy, N. (2014). Phytochemical analysis of biologically active constituents of medicinal plants. *Main Group Chemistry*, 13(1), 7–21. DOI 10.3233/MGC-130117.
27. Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D.G., & Lightfoot, D.A. (2017). Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants*, 6(4), 42. DOI: 10.3390/plants6040042.
28. Nortjie, E., Basitere, M., Moyo, D., & Nyamukamba, P. (2022). Extraction methods, quantitative and qualitative phytochemical screening of medicinal plants for antimicrobial textiles: a review. *Plants*, 11(15), 2011. DOI: 10.3390/plants11152011.
29. Vaou, N., Stavropoulou, E., Voidarou, C., Tsigalou, C. & Bezirtzoglou, E. (2021). Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives. *Microorganisms*, 9(10), 2041. DOI: 10.3390/microorganisms9102041.
30. Oboh, G. & Akindahunsi, A.A. (2004). Change in the ascorbic acid, total phenol and antioxidant activity of sun-dried commonly consumed green leafy vegetables in Nigeria. *Nutrition and Health*, 18(1), 29–36. DOI: 10.1177/026010600401800103.
31. Akbay, P., Basaran, A.A., Undeger, U. & Basaran, N. (2003). In vitro immunomodulatory activity of flavonoid glycosides from *Urtica dioica* L. *Phytotherapy Research*, 17(1), 34–37. DOI: 10.1002/ptr.1068.
32. Brand-williams W, Cuvelier, M.E. & Berset, C. Use of free radical method to evaluate antioxidant activity. *Lebensmittel Wissenschaft und Technologie*, 1995, 28(1); 25–30. DOI: 10.1016/S0023-6438(95)80008-5. Dai, J., & Mumper, R.J. (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*, 15(10), 7313–7352. DOI: 10.3390/molecules15107313.
33. Zlotek, U., Mikulska, S., Nagajek, M. & Świeca, M. (2016). The effect of different solvents and number of extraction steps on the polyphenol content and antioxidant capacity of basil leaves (*Ocimum basilicum* L.) extracts. *Saudi J Biol. Sci.*, 23(5), 628–633. DOI: 10.1016/j.sjbs.2015.08.002.
34. Khatun, S., Chatterjee, N.C. & Cakilcioglu, U. (2011). Antioxidant activity of the medicinal plant *Coleus forskohlii* Briq. *Afr. J. Biotechnol.* 10(13), 2530–2535. DOI: 10.5897/AJB10.2526.
35. Jamwal, V.L., Gulfam, S., Manhas, R.S., Qayum, A., Kapoor, N., Chouhan, R., Singh, S.K., Chaubey, A. & Gandhi, S.G. (2019). Isolation, identification, and bioactive potential of bacterial endophytes from *Coleus*. *IJBB*, 56(5), 392–398. DOI: 10.56042/ijbb.v56i5.28246.
36. Al-Ghamdi, A.Y. (2021). Phytochemical screening and antimicrobial activity of stem extract of *Coleus forskohlii* L. collected from Al-Baha Area, Saudi Arabia. *GSCBPS*, 16(2), 078–086. DOI: 10.30574/gscbps.2021.16.2.0114.
37. Almulaiky, Y.Q., Kuerban, A., Aqlan, F., Alzahrani, S.A., Baeshen, M.N., Afifi, Ammar A., Al-Shawafi, W. & Alkhaled, M. (2017). In vitro antiglycation, antioxidant properties of *Coleus forskohlii* "Balady" leaves and stem and their antioxidant enzyme activities. *Annu. Res. Rev.*, 1–11. DOI: 10.9734/ARRB/2017/37619.
38. Velázquez, L., Quiñones, J., Díaz, R., Pateiro, M., Lorenzo, J. M. & Sepúlveda, N. (2021). Natural antioxidants from endemic leaves in the elaboration of processed meat products: Current status. *Antioxidants*, 10(9), 1396. DOI: 10.3390/antiox10091396.
39. Naveed, M., Batool, H., Rehman, S.U., Javed, A., Makhdoom, S.I., Aziz, T., & Alhomrani, M. (2022). Characterization and evaluation of the antioxidant, antidiabetic, anti-inflammatory, and cytotoxic activities of silver nanoparticles synthesized using *Brachychiton populneus* leaf extract. *Processes*, 10(8), 1521. DOI:10.3390/pr10081521.
40. Sameeh, M.Y., Mohamed, A.A. & Elazzazy, A.M. (2016). Polyphenolic contents and antimicrobial activity of different extracts of *Padina boryana* Thivy and *Enteromorpha* sp marine algae. *J. Appl. Pharm. Sci.* 6(9), 087–092. DOI: 10.7324/JAPS.2016.60913.
41. Naveed, M., Bukhari, B., Aziz, T., Zaib, S., Mansoor, M.A., Khan, A.A., Shahzad, M., Dablood, A.S., Alruways, M.W., Almalki, A.A., Alamri, A.S. & Alhomrani, M. (2022). Green Synthesis of Silver Nanoparticles Using the Plant Extract of *Acer oblongifolium* and Study of Its Antibacterial and Antiproliferative Activity via Mathematical Approaches. *Molecules*. 30, 27(13), 4226. DOI: 10.3390/molecules27134226.
42. Naveed, M., Batool, H., Rehman, S.u., Javed, A.; Makhdoom, S.I., Aziz, T., Mohamed, A.A., Sameeh, M.Y., Alruways, M.W., Dablood, A.S., Almalki, A.A., Alamri, A.S., Alhomrani, M. Characterization and Evaluation of the Antioxidant, Antidiabetic, Anti-Inflammatory, and Cytotoxic Activities of Silver Nanoparticles Synthesized Using *Brachychiton populneus* Leaf Extract. *Processes*, (2022). 1521. DOI: 10.3390/pr10081521.
43. Hamouda, A.F., Sameeh, M.Y. & Shrourou, R.M. (2016). Effect of avocado (*persea americana*), cabbage (*brassica oleracea*) and ginger (*zingiber officinale*) on rat liver and thyroid injuries induced by CCl<sub>4</sub> (carbon tetrachloride). *J. Pharm. Pharmacol.* 4(3), 108–118. DOI: 10.17265/2328-2150/2016.03.002.
44. Mohamed, A.A., Sameeh, M.Y. & El-Beltagi, H.S. (2022). Preparation of Seaweed Nanopowder Particles Using Planetary Ball Milling and Their Effects on Some Secondary Metabolites in Date Palm (*Phoenix dactylifera* L.) Seedlings. *Life*, 13(1), 39. DOI: 10.3390/life13010039.
45. Hayat, P., Khan, I., Rehman, A., Jamil, T., Hayat, A., Rehman, M.U. & Aziz, T. (2023). Myogenesis and Analysis of Antimicrobial Potential of Silver Nanoparticles (AgNPs) against Pathogenic Bacteria. *Molecules*, 28(2), 637. DOI: 10.3390/molecules28020637.
46. Laaroussi, H., Aouniti, A., Mokhtari, O., Hafez, B., Sheikh, R.A., Sameeh, M.Y., Manal M., K., Suliman, A., Alderhami, A., Elhenawy, Mohamed, E., 1, Ilyesse, R., Chaouki, B., Belkheir, H., Taibi, B. & Hicham, E., (2022). Experimental and Theoretical Investigations of *Argania spinosa*'s Extracts on the Antioxidant Activity and Mild Steel Corrosion's Inhibition in 1 M HCl. *Appl. Sci.*, 12(24), 12641. DOI: 10.3390/app122412641.

47. Mohamed, A.A., Ali, S.I., Sameeh, M.Y. & El-Razik, T.M.A. (2016). Effect of solvents extraction on HPLC profile of phenolic compounds, antioxidant, and anticoagulant properties of *Origanum vulgare*. *Research Journal of Pharmacy and Technology*, 9(11), 2009-2016. DOI: 10.5958/0974-360X.2016.00410.8.
48. Sumaira, S., Shazia, K., Jahangir, K., Razia, B., Abid, S., Tariq, A., Majid, A., Abdulhakeem, S.A., Manal, Y.S. & Faten, Z. (2023). Enhancement of shelf-life of food items via immobilized enzyme nanoparticles on varied supports. A sustainable approach towards food safety and sustainability. *Food Res. Int.*, 169, 112940. DOI: 10.1016/j.foodres.2023.112940.
49. Khan, J., Khurshid, S., Sarwar, A., Aziz, T., Naveed, M., Ali, U., Makhdoom, S.I., Nadeem, A.A., Khan, A.A., Sameeh, M.Y., Alharbi, A.A., Filimban, F.Z., Rusu, A.V., Göksen, G. & Trif, M. (2022). Enhancing Bread Quality and Shelf Life via Glucose Oxidase Immobilized on Zinc Oxide Nanoparticles – A Sustainable Approach towards Food Safety. *Sustainability*, 14, 14255. DOI: 10.1016/j.foodres.2023.112940.
50. Saleem, K., Aziz, T., Ali Khan, A., Muhammad, A., Ur Rahman, S., Alharbi, M., Alshammari, A.F. & Alasmari, A. (2023). Evaluating the in-vivo effects of olive oil, soya bean oil, and vitamins against oxidized ghee toxicity. *Acta Biochim. Pol.* 6549.
51. Sameeh, M.Y., Khowdiary, M.M., Nassar, H.S., Abdelall, M.M., Amer, H.H., Hamed, A. & Elhenawy, A.A. (2022). Thiazolidinedione Derivatives: In Silico, In Vitro, In Vivo, Antioxidant and Anti-Diabetic Evaluation. *Molecules*, 27(3), 830. DOI: 10.3390/molecules27030830.
52. Sameeh, M.Y., Khowdiary, M.M., Nassar, H.S., Abdelall, M.M., Alderhami, S.A. & Elhenawy, A.A. (2022). Discovery potent of thiazolidinedione derivatives as antioxidant,  $\alpha$ -amylase inhibitor, and antidiabetic agent. *Biomedicines*, 10(1), 24. DOI: 10.3390/biomedicines10010024.
53. Benish, R., Sobia, A., Naureen, Z., Sohail, A., Abid, S., Tariq, A., Metab, A., Abdulrahman, A. & Abdullah, FA. (2023). Evaluating the influence of *Aloe barbadensis* extracts on edema induced changes in C-reactive protein and interleukin-6 in albino rats through in vivo and in silico approaches. *Acta Biochimica Polonica* 70(2), 425–433. DOI: 10.18388/abp.2020\_6705.