



Chemical Investigation of Flavonoid, Phenolic Acids and Vitamins Compositions of *Forsskaolea viridis* Aerial Parts



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SOME nutritional vitamin, flavonoid and phenolic compounds of *Forsskaolea viridis* aerial parts were extracted and investigated for the first time by using high performance liquid chromatography (HPLC). A comparative analysis of fat-soluble vitamins (A, D, E and K) and water-soluble vitamins (C and B complex) were carried out. Results of this study revealed that, the presence of vitamin A (retinol) as the highest concentrated fat soluble vitamins (332.591 μg/100g), and the presence of water-soluble vitamins, in which vitamin C (ascorbic acid) represented relatively high concentration (234.21 μg/100g), followed by vitamin B12 (cobalamin) (386.06 μg/100g) and vitamin B2 (riboflavin) (112.16 μg/100g) in the aerial parts of *Forsskaolea viridis*. The outlined results of HPLC investigation of methanolic extract showed the presence of 18 phenolic and 26 flavonoid known compounds, which were identified for the first time from the aerial parts of *Forsskaolea viridis*. The high concentration of vitamins, flavonoid compounds and phenolic acids contents in the plant reflected to the medicinal importance of the plant as anticancer, antioxidant and antimicrobial activity which play an important role in human health and plant growth.

Keywords: *Forsskaolea viridis*, HPLC, Flavonoids, Phenolics and Vitamins.

Introduction

Ever since ancient times, humans were looking for drugs to rescue their disease. At this time, there are no medicinal source or any means to help them in the treatment of their diseases other than medicinal plants which provided mankind with herbal remedies for several diseases for many centuries. Therefore, human beings tended to use therapeutic plants to treat many diseases where medical plants proved effective in treating diseases because they contain many active materials, proteins, vitamins, hormones etc. *Forsskaolea viridis*, (Urticaceae, Nettle family) which comprises 54 genera and more than 2000 species of herbs, shrubs, small trees, and a few vines [1]. *Forsskaolea* is a small genus in the nettle family, represented by 6 species, distributed in Canary Isles and southeast Spain eastwards to Pakistan, Africa, and Arabia to Western India [2,3].

Forsskaolea viridis Ehrenb. ex Webb is an annual or short-lived perennial herb distribute in Egypt (Southeast Egypt- wadi Kansisrob), Oman (Dhofar), Saudi Arabia, Yemen (Hadhramaut), Namibia, Sudan, Ethiopia, Eritrea, and Kenya [4].

The aim of this study is to investigate the fat and water soluble vitamins of *F. viridis* aerial parts and identified with HPLC technique and estimation of total active constituents because of the plant hasn't been exposed to this type of the studies on it.

Material and Methods

Plant Material

Aerial parts of *F. viridis* Ehrenb. ex Webb (Family: Urticaceae) were collected from their wild habitat in wadi Kansisrob, Gebel Elba region, southeast corner of Egypt in January 2016. The plant specimens were identified and authenticated by Dr. Omran Ghaly, Desert Research Center. A

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voucher herbarium specimen was deposited in the herbarium of Desert Research Center (CAIH) with Code Number: CAIH-1000-R

Chemicals and instruments

All chemicals used in this study were purchased from Al-Shark office for chemicals and scientific instruments which obtained from India, the HPLC used for analysis was (Agilent technologies, Germany 1200 series) in Food Technology Research Institute, Agricultural Research Center. The all standards used were purchased from Merck.

Methods

Extraction and Analysis of Fat Vitamins

The fat soluble vitamins (A, D, E and K) of *F.viridis* aerial parts were extracted according to the method described in [5]. Briefly, 10g from the plant powder, 1g of pyrogalllic acid, 70ml ethanol and 30ml (50%) KOH were added, stirred, and refluxed together for 40min using a water bath at (50 ± 2 °C). The extraction was replicated three times using various ether concentrations (50 ml, 30 ml, and 20 ml). Double-distilled water was used to neutralize the extract, which was dehydrated using anhydrous sodium sulfate.

Further, the extract was concentrated to approximately 5ml by using a water bath (50 ± 2 °C), diluted to 10 ml by using methanol, filtered using a $0.45\mu\text{m}$ membrane and finally subjected to HPLC (Agilent technologies, Germany 1200 series) analysis equipped with a variable wave length detector ($\lambda 330$ nm for vit. A, 295 for vit E, 266 nm for vit. D and 280 nm for vit. K) with a water series 2695 quaternary solvent delivery system with a cooled autosampler at 4°C and heated column compartment at 30°C. The vitamins were separated on a $10\mu\text{m}$ Bondclone 3.9 x 300 mm C18 column (phenomenex, Sydney, Australia) fitted with a C18 guard column. The mobile phase consisted of water: methanol (5: 95), at a flow rate of 1 ml/min.

Extraction and Analysis of Water Vitamins

The water soluble vitamins (C) of *F.viridis* aerial parts extracted according to the method reported in [6]. Briefly, (10g) of the plant was blended and homogenized with an extracting solution containing metaphosphoric acid (0.3M) and acetic acid (1.4M). The mixture was placed in a conical flask and agitated at 10,000 rpm for 15min. Then filtered through a Whatman No.4 filter paper. Determination of vitamin C content of the plant was accomplished by comparison

to vitamin C standard, using high performance liquid chromatography system (Agilent technologies, Germany 1200). Chromatographic separation was achieved on an RP-HPLC column through isocratic delivery of a mobile phase (A/B 33/67; A: 0.1M potassium acetate, pH = 4.9, B: acetonitrile: water [50:50]) at a flow rate of 1 ml/min. UV absorbance was recorded at 254nm at room temperature.

The water soluble vitamin (B) of *F.viridis* aerial parts extracted according to the method described in [7]. In briefly, *F.viridis* powder (2g) was placed in 25ml of H₂SO₄ (0.1 N) solution and incubated for 30min at 121°C. Then, the contents were cooled and adjusted to pH 4.5 with 2.5M sodium acetate, and 50mg Taka-diastrase enzyme was added. The preparation was stored at 35°C overnight. The mixture was then filtered through a Whatman No.4 filter, then the filtrate was diluted with 50ml of dist. water and filtered again through a microspore filter ($0.45\mu\text{m}$). Twenty microliters of the filtrate was injected into the HPLC system. Quantification of vitamin B content was accomplished by comparison to vitamin B standards. Using HPLC system (Agilent technologies, Germany 1200 series). Chromatographic separation was achieved on a reversed phase (RP-) HPLC column (Agilent ZORBAX Eclipse Plus C18; 250×4.6 mm i.d., $5\mu\text{m}$) through the isocratic delivery mobile phase (A/B 33/67; A: MeOH, B: 0.023M H₃PO₄, pH=3.54) at a flow rate of 0.5 ml/min. Ultraviolet (UV) absorbance was recorded at $\lambda 270$ nm at room temperature. Determination of vitamin B content was accomplished by comparison to vitamin B standards.

Extraction and Analysis of phenolic and Flavonoid contents

The phenolic acids and flavonoid contents in the *F.viridis* aerial parts were extracted by methods reported in [8]. Briefly, 200mg of plant aerial parts fresh powder were extracted with 5ml methanol in ultrasonic bath for 45 minutes. Then the samples were centrifuged for 7 minutes at 4200 rpm. The supernatant was filtered through polyamide filter Chromafil AO-45/25, transferred into vial prior analyses. Using HPLC chromatography system equipped with a variable wave length detector (Agilent, Germany 1100), autosampler, quaternary pump degasser and column compartment. Analyses were performed on a C18 reverse phase (BDS $5\mu\text{m}$, Labio, Czech Republic) packed stainless-

steel column (4×250mm), the chromatographic conditions (mobile phase, gradient program, temperature of column) were similar to those described by [9]. All chromatograms were plotted at λ 280 nm to estimated phenolic acids and at λ 330 nm for flavonoids. All components were identified and quantified by comparison of peak areas with external standards.

Results and Discussion

Fat-soluble vitamins

The results outlined in Table 1 of fat and water soluble vitamins of *F.viridis* aerial parts showed the following data, presence of three types of vitamin K (vitamins K1, K2 and K3), vitamin A, vitamin D and vitamin E with different concentration where, these vitamins performed by action of sufficient metabolism process inside the plant cells and tissues due to relative high percent of triterpenoids and steroids which plays an important role in manufacturing and synthesized the vitamins and steroidal hormones [10]. The results showed that, vitamin A recorded the high relatively concentration (332.591 μ g/100g) followed by vitamin K1 (114.140 μ g/100g) and vitamin D (104.194 μ g/100g). The high concentration of fat-soluble vitamins was vitamin A which contributed to the pharmaceutical importance of the plant in synthetic drugs used in helping the eyes regulation of light changes, bone growth, tooth development, cell division, gene expression, regulation of the immune system, also an important antioxidant that may play a role in the prevention of certain cancers and reproduction and embryonic development [11,12].

The findings showed the relatively high concentration of vitamin K which referred to its vital role in the body by accumulation of blood clotting which, necessary to prevent a person from bleeding out from small cuts, aids in bone health especially for postmenopausal women, reducing the buildup of calcium in the blood [13] and reducing risk of heart disease [14].

Water-soluble vitamins

The results outlined in Table 2 revealed that, presence of two water soluble vitamins (vitamin B and C) where, six types of vitamin B [niacin (vitamin B3), thiamin (vitamin B1), pyridoxine (vitamin B6), folic acid (vitamin B9), cobalamin (vitamin B12)] were detected using HPLC with different concentration where vitamin B12 (cobalamin) represented the high concentration (386.06 μ g/100g) followed by vitamin B2 (riboflavin) (112.16 μ g/100g). The

presence of two types of vitamin B12 and B2 with high concentration were logically where the two vitamins are related to each other which required for body growth, brain health and keeping the red blood cells levels in the body.

The high concentration of vitamin B12 in the plant increases the medical uses of it where, vitamin B12 supports the plant with effective structure needed for nerve tissue health, healthy brain function and the production of red blood cells, energy levels good eyesight, good digestion, healthy appetite, hormones and cholesterol production, cardiovascular health, muscle tone and treatment of anemia [15,16]. The relatively concentration of vitamin B2 may be plays an effective role for the plant to raise its nutritive values where, vitamin B2 helps break down proteins, fats, and carbohydrates and plays a vital role for keeping the body's energy supply through converting carbohydrates into adenosine triphosphate (ATP) where, ATP produces energy as the body requires it and storing energy in muscles [17]. Also it helps in preventing cataracts and migraine headaches, reduce the risk of high blood pressure during pregnancy, prevent muscle cramps and preserving healthy skin and hair [18].

The findings of *F.viridis* aerial parts showed presence of vitamin C with relatively high concentration (234.21 μ g/100g). The relatively high concentration of vitamin C enhancing the activity of the plant as antioxidant activity and then inhibit growth of some cancer cells where, it considered as a potent antioxidant activity, may reduce the risk of chronic diseases like diabetes and heart disease and slow down the growth of tumor cells [19].

Moreover, the effective importance of vitamin C with this concentration in the plant, promotes the role of the plant to enter the pharmaceutical industries of the drugs used in treatment many diseases such as stress, colds, strokes, skin aging, improve macular degeneration, reduce inflammation, reduce the risk of cancer and cardiovascular disease [20]. Also can reduce blood uric acid levels and prevent gout attacks [21] and can prevent Iron deficiencies by improving iron absorption where, assists in converting iron that is poorly absorbed into a form that is easier to absorb in the body [22]. Vitamin C aids to enhancing the immunity by helping white blood cells function better where, it encourages the production of white blood cells known as lymphocytes and phagocytes, which help protect the body against infections and made white blood cells function more effectively

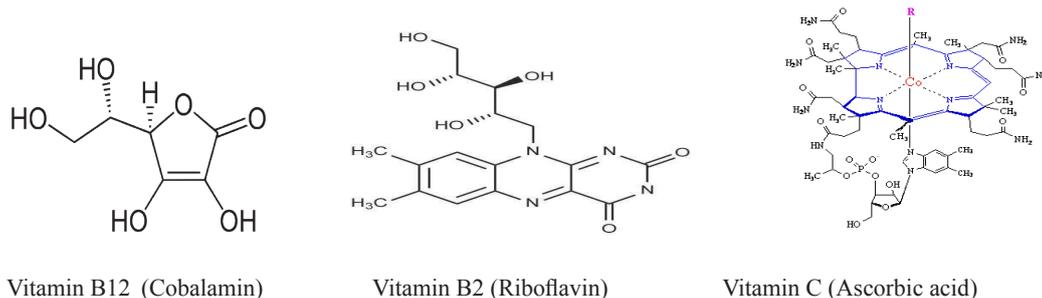


Fig. 2. Structure of the highest concentration of water soluble vitamins.

TABLE 3. Phenolic acids determined of *F. viridis* aerial parts using HPLC.

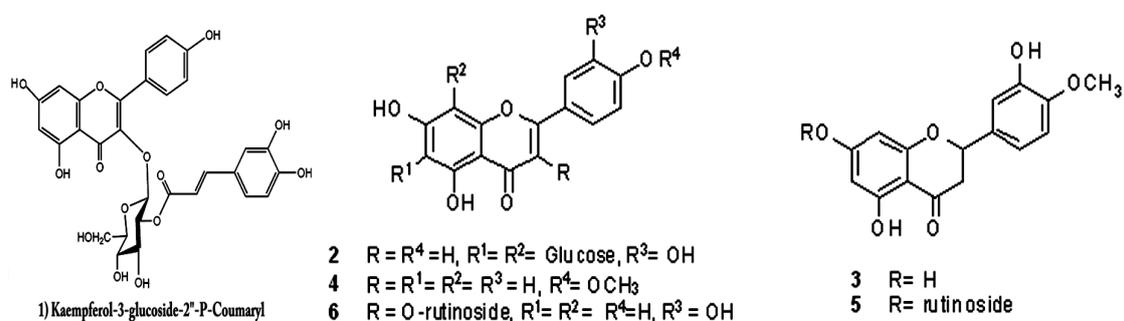
Phenolic Acids	RT (min.)	Conc (µg/100g)
1. Pyrogallol	6.943	1832.56
2. Gallic acid	7.062	126.23
3. 4- Amino benzoic acid	7.541	34.71
4. Protocatechic acid	8.427	650.16
5. Phloroglucinol acetate	9.088	120.24
6. Catechole	9.437	1476.75
7. <i>p</i> - Hydroxy benzoic acid	9.734	197.11
8. Chlorogenic acid	10.180	743.49
9. Caffeic acid	10.337	258.25
10. <i>p</i> - Coumaric acid	11.616	163.72
11. Ferulic acid	11.900	219.21
12. Iso-ferulic acid	12.518	57.17
13. α - coumaric acid	13.220	126.99
14. Ellagic acid	13.473	6063.28
15. 3, 4, 5-Trimethoxy cinnamic acid	14.225	1532.83
16. Coumarin	14.395	609.25
17. Cinnamic acid	15.323	266.71
18. Salicylic acid	16.348	2386.06



Fig. 3. Structure of the highest concentration of phenolic acids.

TABLE 4. Flavonoid compounds determined of *F.viridis* aerial parts using HPLC.

Flavonoid compounds	RT (min.)	Conc (µg/100g)
1. Quercetin-3, 7, 3', 4'-tetramethoxy	8.065	343.21
2. Catechin	8.520	596.29
3. Epicatechin	9.169	620.14
4. Luteolin-6,8-C-di-glucose	9.481	14552.96
5. Luteolin-7-O-glucoside	9.984	453.75
6. Luteolin-6-glucose-8- arabinose	10.800	73.07
7. Apigenin-6-rhamnose-8-glucose	11.143	440.77
8. Apigenin-6-arabinose-8-glucose	11.638	705.03
9. Apigenin-6-glucose-8- rhamnose	12.133	575.79
10. Naringin	12.347	272.74
11. Hesperidin	12.467	2343.71
12. Quercetin-3-O-glucoside	12.520	169.14
13. Rutin	12.732	2180.49
14. Apigenin-7-O-neohespiroside	13.129	229.04
15. Kaempferol-3, 7- dirhamnside	13.327	296.25
16. Quercitrin	13.435	353.18
17. Quercetin	14.987	259.12
18. Narengenin	15.037	264.23
19. Kaempferol-3-O-glucoside-2''-p-coumaroyl	15.347	14579.09
20. Hesperitin	16.207	10660.77
21. Kaempferol	16.316	349.47
22. Rhamnetin	16.532	342.49
23. Apigenin-7-glucose	17.359	339.82
24. Apigenin	17.574	370.38
25. Luteolin 6-glucose-8-arabinose	17.742	312.42
26. Acacetin	18.767	4373.82

**Fig. 4. Structure of the highest concentration of flavonoid compounds respectively.**

and the highest contents of vitamin A and K1. The relatively high concentration for both fat and water soluble vitamins may be due to presence of sufficient concentration of steroids and triterpenoids inside the plant cells and tissues which plays an important role in manufacturing and synthesized the vitamins and steroidal hormones, which is under studying. The high amount of flavonoids and phenolic compounds in the plant increased its medicinal importance as antioxidant, antiulcer, antimicrobial and anti-cancers where, flavonoids inhibit the proliferation of various cancer cells and tumor growth in animal models.

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التحقق الكيميائي للمركبات الفلافونويدات والأحماض الفينولية والفيتامينات للأجزاء الهوائية لنبات فورسكوليا فيريديس

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تم استخراج بعض الفيتامينات والفلافونويدات والمركبات الفينولية الغذائية للأجزاء الهوائية لنبات فورسكوليا فيريديس والتحقق منها لأول مرة باستخدام تحليل كروماتوجرافي سائل عالي الأداء. تم إجراء تحليل مقارنة للفيتامينات القابلة للذوبان في الدهون K ، E ، D ، A والفيتامينات القابلة للذوبان في الماء C و B complex . كشفت نتائج هذه الدراسة أن وجود فيتامين (A) (الريتينول) (332.591 ميكروغرام / 100 جم) كأعلى تركيزاً للفيتامينات القابلة للذوبان في الدهون ، أما الفيتامينات القابلة للذوبان في الماء ، فيمثل فيتامين C (حمض الأسكوربيك) (234.21 ميكروغرام / 100 جم) كأعلى تركيز نسبياً ، يليه فيتامين B12 (الكوبالامين) (386.06 ميكروغرام / 100 جم) وفيتامين B2 (ريبوفلافين) (112.16 ميكروغرام / 100 جم) في الأجزاء الهوائية من نبات الفورسكوليا فيريديس.

أظهرت النتائج المحددة لبحث HPLC لمستخلص الميثانول وجود 18 مركباً معروفاً من الفينول و 26 من المركبات الفلافونويدية المعروفة ، والتي تم تحديدها لأول مرة من الأجزاء الهوائية من نبات الفورسكوليا فيريديس.

ينعكس التركيز العالي للفيتامينات والمركبات الفلافونويدية ومحتويات الأحماض الفينولية في النبات على الأهمية الطبية لها من حيث تأثير نشاطها على الخلايا السرطانية الميكروبية وكمضادات للأكسدة.