INTRODUCTION

Ever since ancient times, humans were looking for drugs to rescue their disease. At this time, there is no medicinal source help them in the treatment of their diseases other than medicinal plants which provided humankind with herbal remedies for several diseases for many centuries. Therefore, human beings tended to use therapeutic plants to treat many diseases because they contain many active materials, proteins, vitamins, hormones, …etc. Family Urticaceae 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 Urticaceae family, represented by six species, distributed in Canary Isles and Southeast Spain eastwards to Pakistan, Africa, and Arabia to West India [2]. Reported activities for the Forsskaolea genus are diuretic, calcuolitic, antiflu [3], and antiseptic [4]. Forsskaolea viridis Ehrenb. ex Webb is an annual or short-lived perennial herb distributed in Egypt (Southeast Egypt – Wadi Kansisrob), Oman (Dhofar), Saudi Arabia, Yemen (Hadramaut), Namibia, Sudan, Ethiopia, Eritrea, and Kenya [5]. The survey on the previous studies on the F. viridis plant showed no chemical and biological studies performed on it so; we aimed to investigate the active chemical constituents of its different extracts in addition to their biological activity [6]. In this study, we concerned to focus our study on the methanolic extract of F. viridis aerial parts and its biological activity of F. viridis aerial parts.

METHODS

Plant material

Aerial parts of F. viridis were collected from their natural habitat in Wadi Kansisrob, Gebel ElBa region, Southeast corner of Egypt in January 2016. The plant specimens were identified, authenticated, and deposited in the herbarium of Desert Research Center (CAIH) with Code Number: CAIH-1000- R.

Chemicals

All chemicals used were of high quality and analytical grade purchased from Sigma-Aldrich.

Chemical studies

Extraction

About 1.5 kg of the air-dried powder of F. viridis aerial parts extracted by successive extraction by Soxlet apparatus starting with petroleum ether to remove the lipoidal matters with increasing polarity then, filtered off, the marc lifted was re-extracted by the same way (this process repeated several times). The same combined extracts for each solvent were concentrated separately under reduced pressure at a temperature not exceeding 50°C till dryness. The petroleum ether, chloroform, ethyl acetate, 100% methanol, 50% methanol, and water were used [6]. The same combined extracts for each solvent were concentrated separately under reduced pressure at a temperature not exceeding 50°C till dryness.

Isolation

The aqueous methanolic extract (130 g) was dissolved in a little amount of water, treated with an excess of methanol and filtered to remove inorganic salts and non-phenolic compounds. The residual extract after removal of inorganic salts was extracted with increasing polarity according to scheme (Fig. 1) as 100% MeOH, 50% MeOH, and finally 100% water, then subjected to paper chromatography using butanol:acetic acid:water (BAW) upper layer (4:1:5) and 15% acetic acid:water (15% AcOH) which revealed similarity of two fractions 100% MeOH and 50% MeOH, so they were collected together and subjected to polyamide column starting with water with decreasing polarity to 100% MeOH afforded 22 fractions (M1-M22), each fraction exceeding 50°C till dryness.
column, preparative paper, and thin-layer chromatography, then applied to Sephadex LH-20 column for final purification of isolated compounds.

Methanolic extract (55 g dissolved in 50 ml aqueous methanol 3:1) over polyamide crude (250 g) column (150 cm x 5 cm) and elution with methanol/b-distilled water mixture of decreasing polarities for gradient elution led to the separation of five individual fractions (I-V) which were dried individually, in vacuum, and then subjected to two-dimensional paper chromatographic investigation.

Biological studies

Antimicrobial activity

Antimicrobial activity was determined by the diffusion agar technique in Regional Center for Mycology and Biotechnology Al-Azhar University, Cairo, Egypt (RCMB), according to CLSI [7,8]. Strains were obtained from the bacteria stock present at RCMB. Petri plates containing 20 ml of nutrient (for bacteria) or malt extract (for fungi), agar medium were inoculated with inoculums 1–2 × 10⁶ (for bacteria) or 1–2 × 10⁹ (for fungi) for bacteria strains was replaced with 100 µl of MTT solution prepared fresh as positive control.

Antioxidant activity (2,2-diphenyl-1-picrylhydrazyl [DPPH] assay)

The free radical scavenging effect of plant extract was assessed by the DPPH reaction. Reaction rates were determined at 517 nm in a ultraviolet (UV3) Unicam spectrophotometer. Reaction rates were determined at 517 nm in a ultraviolet (UV3) Unicam spectrophotometer.

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The isolation and structure elucidation of the purified phenolic and flavonoid compounds were done by comparison, R<sub>0</sub>, values, UV; H-nuclear magnetic resonance (NMR), ¹³C-NMR spectral data with the reported data in the literature.

Compound (1) appeared as a dark purple spot under UV light turning orange when filmed with ammonia, which exhibited a Mr of 610 in electrospray ionization mass spectrometry (ESI-MS) analysis ([M+H]+ at m/z=611). R<sub>0</sub> 0.43 (BAW). 0.57% (6% AcOH). UV<sub>λ</sub> max (nm): MeOH: 258, 264, 295, 357; (NaOAc) 271, 325, 408; (NaOAc) 270, 324, 392; (NaOAc/H<sub>2</sub>BO<sub>3</sub>) 260, 296, 385; (AICl) 275, 303, 433; (AICl/H<sub>2</sub>BO<sub>3</sub>) 271, 299, 364, 402. H-NMR (DMSO-d<sub>6</sub>): Quercetin moiety: δ 7.75 (1H, d, J=2.5 Hz, H-2'), 7.58 (1H, dd, J=2.5 and 8 Hz, H-6), 6.93 (1H, d, J=8 Hz, H-5'), 6.85 (1H, d, J=8 Hz, H-5), 6.43 (1H, d, J=2.5 Hz, H-6), 6.22 (1H, d, J=2.5 Hz, H-6). Glucose moiety: δ 5.14 (1H, d, J=8.5 Hz, H-1), 3.15–3.35 (8H, 6 glucose moieties). Rhamnose moiety: δ 4.51 (1H, d, J=8.5 Hz, H-1′), 3.2–3.85 (8H, 6 glucose moieties). Compounds (1) and (2) were identified as rutin. The spectroscopic data of compound (1) were compared with similar compounds of compound (2).

Compound (2) was isolated as a colorless needle crystals, appeared on PC as blue spot under UV light turning to intense blue when filmed with ammonia, it was found to exhibit Mr of 354 in ESI-MS analysis

| Solvent system | Pooled fractions | Weight (g) |
|----------------|-----------------|------------|
| I H<sub>2</sub>O | 1–2             | 5.10       |
| II MeOH:H<sub>2</sub>O (20:100) | 3–10           | 4.3        |
| III MeOH:H<sub>2</sub>O (40:100) | 11–12          | 0.8        |
| IV MeOH:H<sub>2</sub>O (50:100) | 13–18          | 4.0        |
| V MeOH:H<sub>2</sub>O (90:100) | 19–22          | 1.7        |
Figure 1: Isolation scheme of isolated compounds from *Forsskaolea viridis* methanolic extract

![Isolation scheme diagram](image)

Figure 2: Chemical structures of the isolated compounds from *Forsskaolea viridis* methanolic extract

![Chemical structures diagram](image)

Compounds (1) and (2) were identified as chlorogenic acid and lucenin-2, respectively. Compound (3) was identified as isovitexin. Compound (4) was identified as rutin. Compound (5) was identified as hesperidin. Compound (6) was identified as hesperetin. Compound (7) was identified as ellagic acid. Compound (8) was identified as rutinose. Compound (9) was identified as rhamnose. Compound (10) was identified as glucose. Compound (11) was identified as glucuronic acid. Compound (12) was identified as quinic acid. Compound (13) was identified as caffeic acid. Compound (14) was identified as rutinose. Compound (15) was identified as rhamnose. Compound (16) was identified as glucose.

**References**

Ahmed and El-Bassossy. *Asian J Pharm Clin Res*, Vol 13, Issue 3, 2020, 40-46.
Compound (5) was purely isolated as a pale yellow amorphous powder from Fraction IV, appeared as a dark purple spot on PC under UV light turning to blue when fumed with NH₃ vapor, it was found to exhibit Mr of 610 in ESI-MS analysis ([M+H]⁺ at m/z=611.1). R, 0.54 (BAW), 0.77 (15% AcOH). UV λₘₚ(max) (nm): MeOH: 287, 335; (NaOMe): 240, 284, 350; (NaOAc): 285, 336; (NaOAc/H₂O): 284, 327; (AlCl₃): 308, 384; (AlCl₃/HCl): 300, 340. 1H-NMR (DMso-d₆): 6.70 (1H, d, J=2.3 Hz, H-2), 6.05 (1H, d, J=8.5 Hz, H-3), 6.10 (1H, s, H-6), 6.70 (1H, d, J=8.5 Hz, H-5), 6.02 (1H, s, H-8), 5.82 (1H, H-9, 5.53 (1H, dd, J=12.0, 2.4 Hz, H-2), 3.89 (3H, s, 4-OC₆H₄), 3.17 (1H, dd, J=13.0, 5.0 Hz, H-3 eq), 2.78 (1H, dd, J=13.0, 5.0 Hz, H-3 ax). Sugar moiety: δ 5.12 (1H, d, J=7.2 Hz, H-1) and 4.51 (1H, d, J=9.2 Hz, H-1‴). 13C-NMR (DMso-d₆): Hesperetin moiety: δ 182.4 (C-4), 166.5 (C-7), 164.4 (C-5), 161.3 (C-9), 114.6 (C-2‴), 114.7 (C-3‴), 132.4 (C-4‴), 122.0 (C-6‴), 111.6 (C-2‴), 111.2 (C-5″), 103.4 (C-10), 96.5 (C-6), 94.5 (C-8), 77.7 (C-2), 51.3 (OCH₃), 43.2 (C-3) Glucose moiety: δ 102.4 (C-1‴), 79.3 (C-3‴), 77.5 (C-5‴), 7.39 (C-2‴), 724 (C-4‴), 69.6 (C-6‴). Rhamnose moiety: δ 98.7 (C-1‴), 73.3 (C-4‴), 71.0 (C-3‴), 70.5 (C-2‴), 681 (C-5‴), 19.1 (C-6‴) rhamnose. Compound (5) was identified as hesperidin. The spectroscopic data of compound (5) were similar to the reported data outlined by Mabry et al. and Han et al.[17,19].

Compound (6) was purely isolated as a pale yellow powder from Fraction IV, appeared as a dark purple spot on PC under UV light turning to blue when fumed with NH₃ vapor, it was found to exhibit Mr of 302 in ESI-MS analysis ([M+H]⁺ at m/z=303). R, 0.54 (BAW), 0.77 (15% AcOH). UV λₘₚ(max) (nm): MeOH: 287, 335; (NaOMe): 240, 284, 350; (NaOAc): 285, 336; (NaOAc/H₂O): 284, 327; (AlCl₃): 308, 384; (AlCl₃/HCl): 300, 340. 1H-NMR (DMso-d₆): 6.70 (1H, d, J=2.3 Hz, H-2), 6.05 (1H, d, J=8.5 Hz, H-3), 6.10 (1H, s, H-6), 6.70 (1H, d, J=8.5 Hz, H-5), 6.02 (1H, s, H-8), 5.82 (1H, H-9, 5.53 (1H, dd, J=12.0, 2.4 Hz, H-2), 3.89 (3H, s, 4-OC₆H₄), 3.17 (1H, dd, J=13.0, 5.0 Hz, H-3 eq), 2.78 (1H, dd, J=13.0, 5.0 Hz, H-3 ax). 114.6 (C-2‴), 114.7 (C-3‴), 132.4 (C-4‴), 122.0 (C-6‴), 111.6 (C-2‴), 111.2 (C-5″), 103.4 (C-10), 96.5 (C-6), 94.5 (C-8), 77.7 (C-2), 51.3 (OCH₃), 43.2 (C-3) Compound (6) was identified as hesperetin. The spectroscopic data of compound (6) were similar to the reported data outlined by Mabry et al. and Han et al.[17,19].

Compound (7) was purely isolated as a faint yellow needles from Fraction V, appeared as a blue spot on PC under UV light turning to intense blue when fumed with NH₃ vapor, it was found to exhibit Mr of 302 in its negative ESI-MS analysis ([M-H]⁻ at m/z=301). R, 48 (BAW) 09 (15% HOAc). UV λₘₚ(max) (nm): MeOH: 255, 362. 1H-NMR (DMso-d₆): 7.48 (3H, s, H-5 and, H-5'). Compound (7) was identified as ellagic acid. The spectroscopic data of compound (7) were typical to the reported data outlined by Nawwar et al.[20].

Biological studies
Antimicrobial activity
The inefficacy of currently available antibiotics urges the searching for new type antibacterial agents against the drug-resistant bacteria. Since the pharmaceutical development has historically relied on natural products to provide biological active compounds, screening for natural antibacterial agents have been broadly studied, and even become new leads for antibacterial drug discovery [21,22]. The antibacterial and antifungal activities of the methanolic extract of F. viridis were carried out by diffusion agar technique. Mean zone of inhibition in millimeter produced on a range of pathogenic microorganisms was measured and the results are recorded in Table 2. The maximum inhibitory responses were indicated after the treatment of all tested bacteria and fungi strain with the concentration of 5 mg/ml of the methanol extract, where the methanolic extract showed potent antimicrobial activity about (62-86%) for the most of tested organisms compared to gentamicin and ketoconazole. The maximum response of Gram-positive bacteria was methicillin-resistant Staphylococcus aureus with inhibition zone 13 mm with activity 86.6% near to the gentamycin control and Micrococcus sp. with inhibition zone 18 mm with activity 81.8% when compared to inhibition zone of gentamicin as a reference drug. This refers to the methanolic phytochemical constituents of the methanolic extract recommended to treat some of the respiratory tract infections such as coughing, sneezing and lungs, throat inflammatory or through contact with infected wounds or sores, or during vaginal delivery (from mother to child) [23]. The maximum response of Gram-negative bacteria was both Escherichia coli and Klebsiella pneumoniae with inhibition zone 26 mm and 14 mm with activity 86.6 and 66.6% respectively, according to gentamicin control. On the other hand, the maximum inhibitory zone of yeasts and filamentous fungi was Cryptococcus neoformans with inhibition zone 19 mm with activity about 76% for yeast fungi while the filamentous fungi were Aspergillus fumigatus with inhibition zone 12 mm with activity 70.5% when compared of inhibition zone of ketoconazole as a reference drug.

The relatively high inhibition and the activity of the methanol extract against most of the bacteria and fungi strains may be due to their contents from phenolic and flavonoid compounds and other bioactive metabolites [24], which causes damage to the cell membrane of bacteria and fungi leading to the inhibition of macromolecular synthesis, depolarization of membrane, and inhibition of DNA, RNA, and proteins synthesis of microbe which effect on its growth or its death [25]. The reason for the antimicrobial activity of isolated phenolic and flavonoid compounds may be due to their skeleton structures that possess hydroxy groups at special sites on the aromatic ring that increase its activity. Furthermore, the hydrophobic substituents such as prenyl groups, allyl chains, and oxygen-containing heterocyclic moieties usually enhance the activity for all the flavonoids. Hence, the antibacterial mechanisms of isolated compounds are as follows: Inhibition of nucleic acid synthesis, inhibition of cytoplasmic membrane

| Tested organism | Inhibition zone diameter (mm) |
|-----------------|------------------------------|
| Gram-positive bacteria | Gentamicin (4 mg/ml) |
| Micrococcus sp. (RCMB 029) | 22±0.12 |
| Streptococcus mutans (RCMB 017) | 21±0.14 |
| (ATCC 25175) | 13±0.19 |
| Methicillin-resistant Staphylococcus aureus | 15±0.13 |
| (ATCC 25955) | 13±0.11 |
| Gram-negative bacteria | Gentamicin (4 mg/ml) |
| Salmonella typhimurium (RCMB 006) | 17±0.09 |
| (ATCC 14028) | 13±0.11 |
| Escherichia coli (RCMB 010052) | 30±0.11 |
| (ATCC 25955) | 26±0.21 |
| Klebsiella pneumoniae (RCMB 003) | 21±0.23 |
| (ATCC 13883) | 14±0.06 |
| Filamentous fungi | Ketoconazole (100 mg/ml) |
| Aspergillus fumigatus | 17±0.23 |
| (RCMB 002008) | 12±0.11 |
| Penicillium expansum | 17±0.23 |
| (RCMB 001001) | 11±0.11 |
| Yeasts | Ketoconazole (100 mg/ml) |
| Candida albicans (RCMB 05003) | 20±0.02 |
| (ATCC 10231) | 12±0.18 |
| Cryptococcus neoformans | 25±0.05 |
| (RCMB 049001) | 19±0.13 |

*NA: No activity; **Positive control for fungi: Ketoconazole (MIC) 100 mg/ml. Positive control for bacteria: Gentamicin (MIC) 4 mg/ml; RCMB: Regional Center for Mycology and Biotechnology in Cairo, Egypt; ATCC: American Type Culture Collection
function, inhibition of energy metabolism, inhibition of the attachment and biofilm formation, inhibition of the porin on the cell membrane, and inhibition of a number of bacterial virulence factors, including quorum sensing signal receptors, enzymes, and toxins and alteration of the membrane permeability and attenuation of the pathogenicity [26]. From the previous data obtained, we can say that the methanolic extract of the plant has a good antimicrobial activity for both selected strains of bacteria and fungi but lower than reference used drugs.

**Antioxidant activity**

Oxygen is involved in chemical reactions in the body, in which electrons are shifted around it. To generate energy, our cells remove electrons from sugars, fatty acids, and amino acids and add them to other molecules, especially oxygen. This creates highly reactive, unstable particles known as free radicals, which combine quickly with other elements. Free radicals considered highly reactive compounds formed when an atom or a molecule gains or loses an electron and causes harmful for cells, dangerous to the body and damages all major components of cells, including DNA, proteins, and cell membranes. It may lead to aging and chronic illnesses, such as heart disease, cancer, Alzheimer’s, and other health conditions [27,28].

DPPH scavenging method was utilized to evaluate the antioxidant activity of the methanolic extract of *F. viridis* aerial parts using ascorbic acid as a reference standard, and the results are summarized in Table 3. Methanol extract at concentrations (25, 50, 100, 200, 400, 800, 1200, and 3200 µg/ml) exhibited antioxidant capacity (51.52–92.43%) with The IC$_{50}$ 32.24 as compared to ascorbic acid activity with IC$_{50}$ 29.12. The relative activity of the methanol extract may be due to a sufficient amount of flavonoid and phenolic contents besides other chemical constituents present in the methanolic extract as (Vitamin C, polyphenols, terpenoids, and saponins) [6,29]. These phytoconstituents characterized by their powerful antioxidant properties, where they might be responsible for the antioxidant activity of MeOH extract through breakdown the free radical reaction and neutralize the unpaired electron with donating electrons to eliminate the unpaired condition of the radical [30]. The flavonoid and phenolic contents in the methanolic extract may directly react with the reactive radicals and destroy them. Furthermore, they may decrease the cellular level of free radicals either by inhibiting the activities or expressions of free radical generating enzymes [31,32]. The antioxidant activity of isolated flavonoids depends on their chemical configuration, the arrangement of the functional group in the aromatic ring, substitutions, and the total number of hydroxyl group significantly influence several mechanisms of antioxidant activity such as radical scavenging and metal ion chelation ability [33,34]. Furthermore, the B ring hydroxyl configuration is the most significant determinant of scavenging of reactive oxygen species and reactive nitrogen species because it donates hydrogen and an electron to hydroxyl, peroxy, and peroxyxinitrite radicals, stabilizing them and giving rise to a relatively stable flavonoids radical [35]. The presence of unsaturated 2–3 bond in conjugation with a 4-oxo function is more potent antioxidants where the conjugation between the A and B rings allows a resonance effect of the aromatic nucleus that provides stability to the flavonoid radical so it increases its upregulation or protection of antioxidant defenses [36]. The outlined results indicated that most of the isolated compounds from the methanolic extract characterized by flavonoids chemical skeleton which reflected their biological activity as an antioxidant, antimicrobial, and antitumor activity.

**Cytotoxic activity**

In the past decades, natural compounds have achieved remarkable achievements in the treatment of tumors through chemotheraphy. This inspired scientists to continuously explore anticancer agents from natural compounds. Cancer is considered as a genetic illness caused by mutated genes. It is implicated in cell proliferation and cell death. DNA damage may lead to cell death. The most genetic abnormalities in human cancer cells are based on p53-mutated proteins which responsible genes to proliferate the cells. The flavonoid compounds act on the expression of p53 proteins which may lead to arrest cancer cells in the G$_1$ and mobile phases of the cell cycle. Tyrosine kinases are proteins which considered as growth factor signals for the nucleus. The expression of the protein is involved in oncogenesis. The flavonoid compounds can inhibit tyrosine kinase activity, controlled in inhibition of cancer cell growth, arrest cell cycle in proliferating lymphoid cells and inhibit heat shock proteins in several malignant cell lines, comprising leukemia, colon cancer, hepatic, and breast cancer [37].

Our study concerned with *in vitro* studies of isolated compounds in the methanolic extract of *F. viridis* aerial parts against Caco-2 and MCF-7 and HEPG-2. The findings recorded in Table 4 revealed that the methanolic extract exhibited potent antitumor activity against (HEPG-2) with IC$_{50}$ 49.72 µg/ml with effective about 80.7%, weak activity against colon

### Table 3: Antioxidant activity of methanolic extract of *Forsskaolaea viridis* compared to ascorbic acid

| Conc. (µg/ml) | Methanol extract | Conc. (µg/ml) | Ascorbic acid |
|--------------|-----------------|--------------|--------------|
| 0            | 0               | 0            | 0            |
| 25           | 41.52±0.08      | 5            | 11.78±0.02   |
| 50           | 57.25±0.09      | 10           | 17.98±0.04   |
| 100          | 63.74±0.14      | 15           | 54.86±0.09   |
| 200          | 72.32±0.16      | 20           | 70.94±0.07   |
| 400          | 81.21±0.07      | 25           | 77.41±0.10   |
| 800          | 89.84±0.23      | 30           | 80.65±0.10   |
| 1600         | 90.89±0.11      | 35           | 87.53±0.08   |
| 3200         | 92.43±0.15      | 40           | 92.48±0.03   |
| IC$_{50}$    | 32.24           | IC$_{50}$    | 14.20        |

DPPH: 2,2-Diphenyl-1-picrylhydrazyl hydrate

### Table 4: Antitumor activity of the methanolic extract of *Forsskaolaea viridis* aerial parts against breast (MCF-7), colon (Caco-2), hepatic (HEPG-2), and normal (Vero) cell lines

| Conc. × 10 µg/ml | Viability % of methanol extract | Vero | Caco-2 | MCF-7 | HEPG-2 |
|------------------|--------------------------------|------|--------|-------|--------|
| 1000             | 4.62±0.07                      | 4.06±0.23 | 4.98±0.29 | 3.37±0.21 |
| 500              | 5.40±0.20                      | 5.64±0.31 | 5.6±0.45  | 4.13±0.23 |
| 250              | 6.02±0.15                      | 7.08±0.36 | 7.02±0.36 | 5.46±0.21 |
| 125              | 10.05±0.15                     | 10.49±0.24 | 10.49±0.24 | 3.69±0.33 |
| 62.50            | 22.61±0.09                     | 18.76±0.13 | 18.76±0.13 | 3.37±0.42 |
| 31.25            | 37.56±0.02                     | 28.36±0.11 | 28.36±0.11 | 4.64±0.20 |
| 15.62            | 72.48±0.11                     | 59.31±0.09 | 8.43±0.14 | 19.62±0.19 |
| 7.81             | 86.27±0.09                     | 97.90±0.06 | 97.90±0.06 | 53.16±0.02 |
| 3.90             | 94.72±0.04                     | 98.75±0.06 | 98.75±0.06 | 49.72    |
| 1.53             | 100.00±0.00                    | 100.00±0.00 | 100.00±0.00 | 100.00±0.00 |
(Caco-2) with IC₅₀ 154.41 µM with effectively about 40.3%, and no activity for breast carcinoma cell lines (MCF-7) with IC₅₀ 224.19 µM with effectively about 13.1% when compared to IC₅₀ 258.07 µg/mL of the normal cell. The antitumor activity of the methanolic extract of both hepatic and colon carcinoma cell lines may be due to its isolated phytochemical constituents from phenolic and flavonoid components where they exert their effects on cytochrome P₄₅₀ to inhibit the activities of certain P₄₅₀ isozymes, inhibit DNA damage, mutagenic signaling, cell proliferation, and proto-oncogenes, inhibit pro-oxidant enzymes which are responsible for the production of number of procarcinogens and help in the production of metabolizing enzymes such as glutathione-S-transferase, quinone reductase, and uridine 5-diphospho-glucuronyl transferase by which carcinogens are detoxified and thus eliminated from the body [38,39].

CONCLUSION

The plant phytochemical constituents act the basics of folkloric medicine, where the medicinal plants are rich sources of a wide variety of chemical compounds and have been used as a major constituent of most indigenous medicines for a variety of diseases. The present study resulted in the characterization of seven pure compounds from the methanolic extract of F. viridis aerial parts that have been characterized as rutin, lucenin-2, chlorogenic acid, isovitexin, hesperidin, hesperetin, and ellagic acid. Flavonoids and phenolic compounds play an important role in diseases treatment where, they have antioxidant, hepatoprotective, anticancer, anti-inflammatory, antiviral, antimicrobial, antipyretic, antispasmodic, antiseptic activity, used as liniment, cough remedy, cold and flu drug, diuretic, treatment of kidney ailments, and urinary tract infections, hematuria, prostatitis, cholera, stomach ailments, rheumatism, gout, bruises, and calculus. In the present study, the methanolic extract was evaluated for the exploration of their antimicrobial activity against certain Gram-negative and Gram-positive bacteria strains and fungi species, evaluation of the antioxidant and antitumor activity of the methanolic extract. The obtained result demonstrates that the isolated compounds possess a significant inhibitory effect against tested pathogens, moderate antioxidant, potent antitumor activity for hepatic carcinoma cell line, and fair antitumor. 

AUTHORS’ CONTRIBUTIONS

Taha El Bassossy author for this publication, research scholar, desert research center, Cairo, Egypt (DRC) under the guidance and contributions of Dr. Fatma Aly Ahmed, professor of phytochemistry, DRC who summarized, edited, and explained the discussion of the obtained data.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHORS FUNDING

Not applicable.

REFERENCES

1. Changkyun K, Tae D, Mark C, Dais-Gui Z, Ze-Long N, Hang S. Generic phylogeny and character evolution in Urticaceae (Urticaceae) inferred from nuclear and plastid DNA regions. Taxon 2013;64:65-78.
2. Alfarhan AH, Al-Turk TA, Basaly AV. Flora of Jizan Region. Final Report. Vol. 1. King Abdulaziz City for Science and Technology, 2005.
3. Darias V, Bravo L, Barquin E, Martin Herrera D, Fraicle C. Contribution to the ethnopharmacological study of the Canary Islands. J Ethnopharmacol 1986;15:169-93.
4. Darias V, Martin-Herrera D, Abdala S, Fuente D. Plants used in urinary pathologies in the Canary Islands. Pharm Biol 2001;39:170-80.
5. GBIF Secretariat: GBIF Backbone Taxonomy. Available from: http://www.gbif.org/species/4101865. [Last accessed on 2016 Jun 09].
6. Ahmed FA, El-Mesallamy AM, El-Bassossy TA. Phytochemical analysis and statistical evaluation of Forskohlia viridis aerial parts.

Acta Pol Pharm 2019;76:815-23.
7. CLSI, Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts, Approved Guideline. CLSI Document M44-A. CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA; 2004.
8. CLSI, Performance Standards for Antimicrobial Disk Susceptibility Tests, Approved Standard, 7th ed., CLSI document M02-A11. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087-1898, USA; 2012.
9. Letelier ME, Molina-Berrios A, Cortes-contronco J, Jara-Sandoval J, Holst M, Palma K, et al. DPPH and oxygen free radicals as pro-oxidant of biomolecules. Toxicol In vitro 2008;22:279-86.
10. Joyce M, Lobein A, Anton R, Mortier F. Comparative antiproliferative, antimicrobic and scavenging properties of terpenes and biflavones from Ginkgo and some flavonoids. Planta Med 1995;61:126-9.
11. Viturro C, Molina A, Schmeda-Hirschmann G. Free radical scavengers from Multia fiesiunia (Asteraceae) and Sancinula graveolens (Asteraceae). Phytother Res 1995;9:222-4.
12. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65:55-63.
13. Wei XH, Yang SY, Liang N, Hu DY, Jin LH, Xue W, et al. Chemical constituents of Causulipina decapetula (Roth) Aiton. Molecules 2013;18:1325-36.
14. Erel SB, Karaalp C, Bedir E, Kaehl H, Glasi S, Khan S, et al. Secondary metabolites of Centaurea calolotis and evaluation of cunca for anti-inflammatory, antioxidative and cytotoxic activities. Pharm Biol 2011:49:840-9.
15. Berregi I, Ignacio JS, Campoa A, Ignacio JM, Azipurma JM. Quantitation determination of chlorogenic acid in cider apple juices by H NMR spectrometry. Anal Chim Acta 2003:486:269-74.
16. Lee JH, Park KH, Lee MH, Kim HT, Seo WD, Kim JY, et al. Identification, characterisation, and quantification of phenolic compounds in the antioxidant activity-containing fraction from the seeds of Korean perilla (Perilla frutescens) cultivars. Food Chem 2013;136:843-52.
17. Mahy TJ, Markham KR, Thomas MB. The Systematic Identification of Flavonoids. Berlin: Springer-Verlag. 1970.
18. Luzzatto T, Golan A, Yishay M, Bilkis I, Ben-Ari J, Yedidia I. Priming of antimicrobial phenolics during induced resistance response towards Pectobacterium carotovorum in the ornamental monocot calla lily. J Agric Food Chem 2007;55:16-20.
19. Han S, Mok Y, Kim H, Lee J, Lee D, Lee Y. Determination of hesperidin in mixed tea by HPLC. CNU J Agric Sci 2011;38:295-9.
20. Nawwar MA, Hussein SA, Merfort I. NMR spectral analysis of polyphenols from Punica granatum. Phytochemistry 1994;36:793-8.
21. Brown DG, Lister T, May-Dracka TL. New natural products as new leads for antibacterial drug discovery. Bioorg Med Chem Lett 2014;24:413-8.
22. Newman DJ, Crag GM. Natural products as sources of new drugs over the 30 years from 1981 to 2010. J Nat Prod 2012;75:311-35.
23. Menichetti F. Current and emerging serious Gram-positive infections. Clin Microbiol Infect 2005;11 Suppl 3:22-8.
24. Savitha T, Arivukkarasu R. Determination of phytocompounds from Terminalia chebula retz by HPTLC densimetric methods. Int J Pharm Pharm Sci 2014;6:516-20.
25. Dzoyem JP, Hamamoto H, Nagneni B, Ndagjui BT, Sekimizu K. Antimicrobial action mechanism of flavonoids from Dorstenia species. Drug Discov Ther 2013;7:66-72.
26. Cushman TP, Lamb AJ. Recent advances in understanding the antibacterial properties of flavonoids. Int J Antimicrob Agents 2011:38:99-107.
27. Diplock AT, Charleux JL, Crozier-Willi G, Kok FJ, Rice-Evans C, Roberfroid M, et al. Functional food science and defence against reactive oxidative species. Br J Nutr 1997;78 Suppl 1:S77-112.
28. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007;39:44-84.
29. El-Mesallamy AM, Ahmed FA, El-Bassossy TA. Chemical Investigation of Flavonoids, phenolic acids and Vitamins compositions of Forskohlia viridis aerial parts. Egypt Chem J 2019;62:1815-22.
30. Viswanathswamy AH, Yadala P. In vitro antioxidant and cytotoxic activity of rutin and piperine and their synergistic effect. Int J Pharm Sci 2016;8:78-82.
31. Li JM, Lin PH, Yao Q, Chen C. Chemical and molecular mechanisms of oxayhydrazides: Experimental approaches and model systems. J Cell
32. Wang X, Ouyang YY, Liu J, Zhao G. Flavonoid intake and risk of CVD: A systematic review and meta-analysis of prospective cohort studies. Br J Nutr 2014;111:1-11.

33. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. J Nutr Biochem 2002;13:572-84.

34. Pandey AK, Mishra AK, Mishra A. Antifungal and antioxidative potential of oil and extracts derived from leaves of Indian spice plant Cinnamomum tamala. Cell Mol Biol (Noisy-le-grand) 2012;58:142-7.

35. Cao G, Sofic E, Prior RL. Antioxidant and prooxidant behavior of flavonoids: Structure-activity relationships. Free Radic Biol Med 1997;22:749-60.

36. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med 1996;20:933-56.

37. Duthie GG, Duthie SJ, Kyle JA. Plant polyphenols in cancer and heart disease: Implications as nutritional antioxidants. Nutr Res Rev 2000;13:79-106.

38. Ren W, Qiao Z, Wang H, Zhu L, Zhang L. Flavonoids: Promising anticancer agents. Med Res Rev 2003;23:519-34.

39. Panche AN, Diwan AD, Chandra SR. Flavonoids: An overview. J Nutr Sci 2016;5:e47.