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Antioxidant Activity of Yemeni Plants, *Myrtus communis* L. and *Flemingia grahamiana* Wight & Arn.

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Latifa Nasser A. Abdulqawi[*1], Syed Atheruddin Quadri[2]

1 Department of Zoology, Maulana Azad Collage, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, India.

2 Department of Zoology, Maulana Azad Collage Dr. Rafiq Zakaria Campus, Aurangabad, India

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ABSTRACT

The present study was aimed to investigate the antioxidant activity of methanol and aqueous extracts of leaves of *Myrtus communis* and pods (Warrus) of *Flemingia grahamiana* Wight & Arn. Antioxidant activity of extracts was determined by1, 1-Diphenyl-2-picryl hydrazyl (DPPH) method. Both extracts showed antioxidant activity in a concentration-dependent manner. The methanolic and aqueous extract of *Myrtus communis* leaves showed a scavenging ability with an IC_{50} value of 2.79 and 2.65 µg/ml, respectively. And IC_{50} of *Flemingia grahamiana* Warrus extracts were 3.95 and 0.71 µg/ml, respectively. IC_{50} value for the standard ascorbic acid was 3.35 µg/ml.

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INTRODUCTION

The distinguished geographical location of Republic of Yemen and the diversity of landscapes and climate coupled with the Socotra Archipelago gives the Yemeni plants a great degree of diversity. In spite of the introduction of the modern medicinal system during the twentieth century, folk herbal medicine keeping to play an important role in many areas of Yemen [1, 2].

Many of Yemeni medicinal plants have shown good biological activities, but there are Yemeni plants to need future studies which will supply new insights about pharmacological activities [2]. Medicinal and Aromatic plants are the source of natural antioxidants due to their main secondary metabolites [3]. Nowadays, the interest in naturally happening antioxidants has much increased for use in cosmetic, food and pharmaceutical products to exchange synthetic antioxidants that are being limited due to their carcinogenicity [4]. Furthermore, growing evidence related to the event of cancer to the oxidative damage to proteins, lipids, and DNA in the body caused by free radicals and other carcinogens [5]. And due to many plants are hugely free from adverse effects and have good pharmacological actions, Plants are a rich source of bioactive chemicals [6]. *Myrtus communis* L. is one of the important medicinal and aromatic species from the Myrtaceae family [3]. Which it has several antioxidants like tannins, flavonoids, and α-tocopherol [7].

Flemingia grahamiana Wight & Arn. Belongs to the Fabaceae family, the genus Flemingia has about 42 species. It is a perennial shrub [8]. The pods of *F. grahamiana* covered with red powder it's called Warrus. In Arabia, warrus is applied as a cosmetic and in the treatment of skin disease, anthelmintic, cough and chills [9]. There are a few studies have assured the pharmacological activity of Flemingia genus [9, 10, 11].

The main goal of this study is to evaluate the antioxidant activity of methanolic and aqueous extracts of medicinal Yemeni plants: *Myrtus communis* L. and *Flemingia grahamiana* Wight & Arn.

MATERIALS AND METHODS

Plant Materials and Extracts

The leaves of *Myrtus communis* and pods of *Flemingia grahamiana* Wight & Arn were collected from Yaffe Mountains of Yemen. The plant material was authenticated by Dr. Rafiuddin Nasser, Assistant professor, Department of Botany, Maulana Azad College, Aurangabad. The leaves and pods were dried in the shade. The leaves were crushed, and pods of *F. grahamiana* were rubbed and gently shake, to denude them of their outer hairy covering and get powder 'Warrus' (Figure 1). Leaves and powder 'Warrus' (50 g) were extracted with 500 ml methanol separately in a Soxhlet extractor, then the solvents were filtered through a filter paper Whatman No. 1 and evaporated by a rotary evaporator, then transferred to an incubator at 40°C for 24 h to get a dry mass. The extracts were put in sterile labeled bottles and kept in a refrigerator. Also, 50 g of leaves and powder 'Warrus' were taken separately, in a beaker, and 500 ml of distilled water was added. The mixture was stirred by a magnetic stirrer for 24 h. The mixture was clarified by filtration and then subjected to evaporation and kept at -20°C till used [12].

Figure No. 1: The pods of *F. grahamiana* covered by Warrus

DPPH free radical scavenging assay

Antioxidant activity of methanolic and aqueous extracts of *Myrtus communis* (leaves) and *Flemingia grahamiana* (pods) was determined using 1, 1-Diphenyl-2-picryl hydrazyl radical (DPPH) method with minor modification [13]. 0.05 ml of the extracts dissolved in methanol were diluted to 1.0 ml, using ethanol to attain the concentrations of 1-200 µg/ ml, and were added to DPPH (Final concentration 200 µM, in 95% ethanol). The absorbance of the resulting solution was read at 515 nm after 20 min using a spectrophotometer. Ascorbic acid was used as a reference standard. The tests were performed in triplicate and the percentage inhibition was calculated by using the following formula:

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

Where $Abs_{control}$ is the absorbance of DPPH radical solution without extract, Abs_{sample} is the absorbance of DPPH radical solution mixed with the extract. IC_{50} values were calculated using Linear regression by plotting scavenging activity against sample concentrations using Microsoft Excel software.

STATISTICAL ANALYSIS

Experimental data were conducted in triplicates and results for each measured parameter were expressed as means ± Stander error. Statistical analyses were performed by one-way ANOVA. The difference was considered to be statistically significant when $p < 0.05$. Analysis was done using SPSS ver. 20.0 software.

RESULTS AND DISCUSSION

DPPH free radical scavenging assay

Free radical scavenging (DPPH) properties of methanolic and aqueous extracts from *M. communis* and *F. grahamiana* are presented in (Table 1) and (Figure 2 & 3).

All extracts showed highest antioxidant activity on DPPH radicals with IC_{50} value of 0.71, and 2.65 µg/mL for aqueous extracts of *F. grahamiana* and *M. communis*. And IC_{50} value of 2.79 and 3.95 µg/mL for methanolic extracts of *M. communis* and *F. grahamiana*. DPPH scavenging ability of the extracts of *F. grahamiana* and *M. communis* were higher than

synthetic antioxidant (Ascorbic acid with IC_{50} of 3.35 µg/mL) except methanolic extracts of *F. grahamiana*.

There are several studies showed *M. communis* has a strong antioxidant activity on DPPH radical. Amensour, *et al*, [14] reported antioxidant activity of Moroccan *Myrtus communis* leaves and berries in which four solvents were used. In his results both leaves and barriers extracts showed antioxidant activity in the order of methanol > water> ethanol >ethyl acetate. Leaves extracts have more activity in compare to berries extracts.

The aqueous and methanol extracts from Tunisian *M. communis* showed an important free radical DPPH with IC_{50} value 1.9 µg/ml and 6.5 µg/ml, respectively [15]. Babou *et al*, [16] had reported antioxidant of leaves, berries and seed extracts of myrtle at different times and the best IC_{50} was obtained from leaves harvested in September and December (8.29 µg/mL and 9.44 µg/mL), respectively, berries harvested in September (8.42 µg/mL) and seeds harvested in December (3.89µg/mL). Ascorbic acid used as control with IC_{50} of 6.93 µg/mL.

Also, IC_{50}value of three Myrtle extracts; hydroalcoholic, ethyl acetate and aqueous were 0.36, 2.27 and 2.88 µm, respectively [7]. Gumula *et al*., 2014 [17] reported that leaf extract showed an IC_{50} value of 5.9 µg/mL in a DPPH. Also, there are few antioxidant studies of genus Flemingia. According to Madan*et al*, [18], methanolic extract of root and leaf of *F. strobilifera* showed a very good radical scavenging activity (DPPH) with low IC_{50} values of 11.4 µg/ml and 38.0 µg/ml, respectively. Also, Hsieh et al, [6] have studied antioxidant of aqueous extracts of four flemingia species in Taiwan (*Flemingia macrophylla*, *Flemingia prostrata*, *Flemingia lineata* and *Flemingia strobilifera*) in which aqueous extract of *Flemingia macrophylla* had the strongest antioxidant activity with IC_{50} values of 36 µg/ml.

Table No. 1: IC_{50} values of methanolic and aqueous extracts of *M. communis* and *F. grahamiana* in DPPH radical scavenging activity.

Plants & positive controls	Extracts	IC_{50} (µg/mL)
M. communis	- Methanolic	2.79 ±0.02
	- Aqueous	2.65 ±0.03
F. grahamiana	- Methanolic	3.95 ±0.03
	- Aqueous	0.71 ±0.02*
Ascorbic acid		3.35 ±0.01

All values are expressed as Mean ± SE (n=3). *= Difference statistically significant (P<0.05)

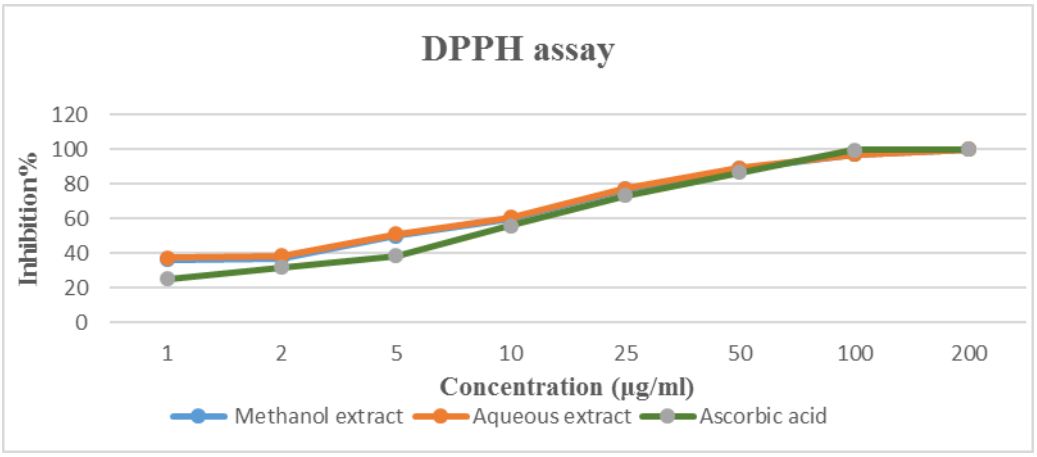

Figure No. 2: DPPH radical scavenging activity of methanolic and aqueous extracts of *M. communis* at different concentrations

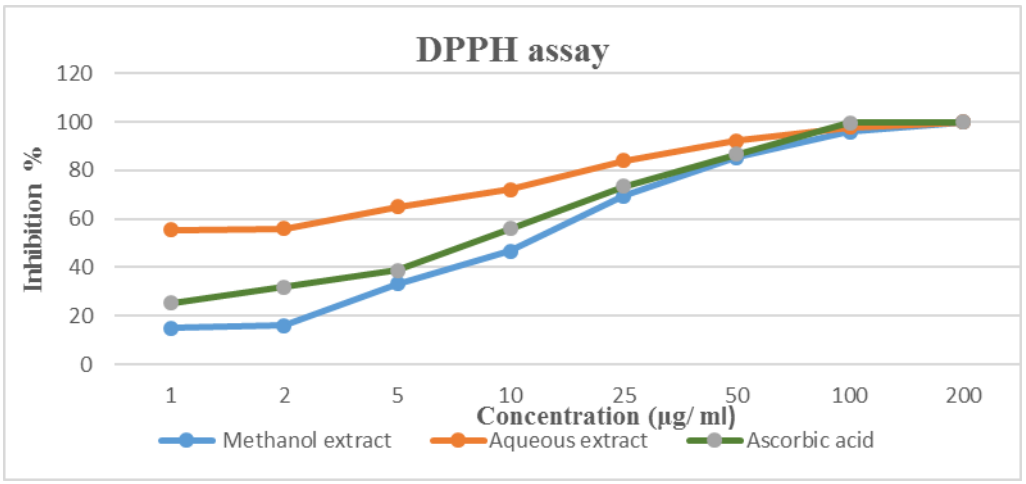

Figure No. 3: DPPH radical scavenging activity of methanolic and aqueous extracts of *F. grahamiana* at different concentrations

CONCLUSION

The present study of *Myrtus communis* leaves and *Flemingia grahamiana* Warrus indicate that the methanolic and aqueous extracts possess a good scavenging activity. Further studies are recommended to isolate and identify antioxidant components in *Myrtus communis* and *Flemingia grahamiana*, which could be used as an easily accessible source of natural antioxidants; possible food supplement and in the pharmaceutical industry.

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