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ISSN 2321 - 6328

Research Article

ANTIOXIDANT AND FREE RADICAL SCAVENGING ACTIVITY OF ESSENTIAL OIL EXTRACTED FROM *ROSMARINUS OFFICINALIS* L.

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Article Received on: 12/08/15 Accepted on: 09/10/15

DOI: 10.7897/2321-6328.03549

ABSTRACT

Free radicals/reactive oxygen species are related to many biological phenomena such as inflammation, aging, and carcinogenesis. In this work, we studied antioxidant activity oils extract of *leaves Rosmarinus officinalis*. Rosemary is well known as a spice and widely used plant in ethno medicine worldwide. Our results obtained from the in vitro antioxidant screened showed that oils from leave *Rosmarinus officinalis* has considerable amounts of polyphenolic and flavonoids compounds which are responsible for the antioxidant properties. And also they give the higher reductive potential due to reducing capacity and DPPH free radical scavenging activity which serves as strong indicator of antioxidant activities.

Key words: *Rosmarinus officinalis*, antioxidant activity, DPPH, Free radical and Radical scavenging.

INTRODUCTION

It is increasingly being realized that many of today's diseases are due to the "oxidative stress" that results from an imbalance between formation and neutralization of pro oxidants. Oxidative stress is initiated by free radicals, which seek stability through electron pairing with biological macromolecules such as proteins, lipids and DNA in healthy human cells and cause protein and DNA damage along with lipid peroxidation. These changes contribute to cancer, atherosclerosis, cardiovascular diseases, ageing and inflammatory diseases[1,2]. All human cells protect themselves against free radical damage by enzymes such as superoxide dismutase (SOD) and catalase, or compounds such as ascorbic acid, tocopherol and glutathione. Sometimes these protective mechanisms are disrupted by various pathological processes, and antioxidant supplements are vital to combat oxidative damage. Recently, much attention has been directed towards the development of ethno medicines with strong antioxidant properties but low cytotoxicity.

Rosemary (*Rosmarinus officinalis L.*) belongs to the family Labiatae (Lamiaceae) and has been an important medicinal plant since earliest times. It is also a commonly used spice and flavoring agent. Its essential oil is used therapeutically and balneologically. Rosemary contains a large number of compounds responsible for its antioxidant, anti-inflammatory, anti-mutagenic, anti-carcinogenic, chemo- preventive, anti-microbial, and anti-viral activities[4-10]. Also, an induction of NGF (nerve growth factor) in human glioblastoma cells using rosemary extract has been described [11].

The main antioxidant compounds found in rosemary extract are rosmarinic acid, carnosic acid, and carnosol[12-14]. Carnosic acid and carnosol are phenolic diterpenes of abietane type, and rosmarinic acid is an ester of caffeic acid and 3,4-dihydroxyphenyllactate (phenolic depside) (see Fig. 1 for structures). Carnosic acid represents the main constituent of the phenolic diterpenes in rosemary.

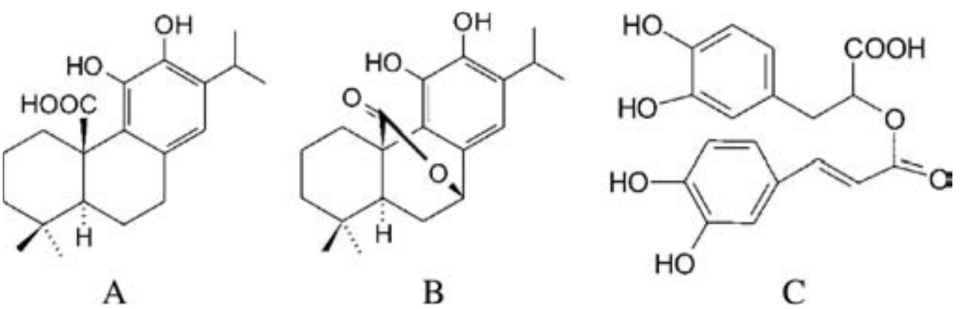

Figure 1. Structures of carnosic acid (A), carnosol (B), and rosmarinic acid (C).

Due to the anti-oxidative effect, rosemary extracts have been widely used in the food industry as a natural antioxidant as a stabilizer of fat and fat-containing foods. The potential of the anti-oxidative compounds of rosemary has also been researched with increasing interest for its application in pharmaceuticals. The purpose of the present work is to determine the antioxidant activity of the essential oils of *Rosmarinus officinalis* grow in Al-Jabal Al Akhdar, and house in Benghazi and commercial.

MATERIALS AND METHODS

Plant material: The leaves of *Rosmarinus officinalis*, were collected from Al-Jabal Al Akhdar area in Benghazi, Libya 2013 (sample 1), and The leaves of *Rosmarinus officinalis* were collected froam house in Benghazi ,Libya 2013 (sample 2), but oil of *Rosmarinus officinalis* commercial from a super market, Benghazi, Libya 2013(sample3).

Chemicals: 1,1-Diphenylpicrylhydrazyl (DPPH˙) , and Ethanol alcohol were supplied from Sigma and Merck company. Ascorbic acid, Folin-Ciocalteu reagent, ferric chloride, potassium ferricyanide, monobasic dihydrogen phosphate, dibasic monohydrogen phosphate, trichloro acetic acid, sodium carbonate, petroleum ether, anhydrous sodium sulfate and pyrogallol were obtained from the biochemistry laboratory of chemistry department-Benghazi University.

Extraction of essential oil from leaves of *Rosmarinus officinalis* (sample 1 and sample 2): The dry powdered leaves of *Rosmarinus officinalis* (sample 1 and sample 2) (500g) were subjected to hydrodistillation using Clevenger apparatus. The isolation of volatile oils was complete within 6 hours[15]. The oil samples were stored at 7°C in dark air-tight containers after drying over anhydrous sodium sulfate and filtered before injecting to GC-MS analysis.

Oil analysis: The oil samples extracted from leaves of *Rosmarinus officinalis* were subjected to ;

Gas chromatography/ Mass spectra. Thermo Scientific, Trace GC Ultra & ISQ Single Quadruple MS, DB-5 bonded-phase fused-silica capillary column was used in for GC/MS analysis of essential oils.

Antioxidant activities assays and quantitative analysis: All of these experimental have been conducted in biochemistry laboratory at Benghazi University.

Total phenolic content (TPC): Total concentration of phenolic compound in the essential oils obtained from *Rosmarinus officinalis* was estimated using the colorimetric method based on Folin-Ciocalteu reagent[16]. Quantification was done with respect to standerd calibration curve of Pyrogallol the results were expressed as pyrogallol "µg/ml".

Total flavonoids content (TFC): Aluminum chloride colorimetric method was used for determination[17]. The calibration curve was obtained by preparing different quercetin solutions in methanol at concentrations "100 to 500 µg/ml".

Reducing power assay (RPA): The reducing power was determined according to the[18]. Quantification was done with respect to stander calibration curve of ascorbic acid the results were expressed as ascorbic acid "µg/ml".

Potassium ferricyanide + ferric chloride $\xrightarrow{\text{antioxidant}}$ potassium ferricyanide + ferrous chloride.

DPPH free radical scavenging activity (RSA): The antioxidant activity of the essential oils was measured in terms of hydrogen donating or radical-scavenging ability using the stable DPPH˙ method as modified by [19]. Radical scavenging activity was expressed as percent of inhibition and was calculated using the following formula:-

%DPPH "RSA" = [Abs. of Control – Abs. of Sample / Abs. of Control] x 100

RESULTS

The GC-MS of the essential oil from rosemary leaves collected from Al-Jabal Al Akhdar, and house in Benghazi: Table (1) represents the chemical composition of the essential oil extracted from rosemary leaves collected from Al-Jabal Al Akhdar, and house in Benghazi. As can be seen from this table 12 compounds representing about (66.2%, 55.8%) respectively. The major components are as follows: α-pinene (18%, 14%) respectively, camphene (8%, 2.5%) respectively, 1,8-cineol (16% , 24%) respectively, β-pinene (2%, 4%) respectively and camphor (13% , 5%) respectively.

Table 1: **Gas chromatographic analysis for essential oil from leaves rosemary collected from Al-Jabal Al Akhdar, and house in Benghazi**

No	R. Time	Chemical constituents	Essential oil from leavesof *Rosmarinus officinalis*, were collected from Al-Jabal Al Akhdar ,%	Essential oil from leaves of *Rosmarinus officinalis*, were collected from house in Benghazi,%
1	6.162	α-pinene	18	14
2	7.009	β-pinene	2.0	4.0
3	8.109	camphene	8	2.5
4	9.856	myrcene	1.5	1.0
5	10.594	limonene	2.5	1.5
6	15.447	1,8-cineol	16.0	24
7	15.631	p-cymene	1.0	0.8
8	18.188	camphor	13.0	5.0
9	18.677	bornyl	0.5	0.1
10	20.239	terpineol	1.0	1.0
11	21.265	borneol	2.0	1.5
12	21.817	verbenone	0.7	0.4

The antioxidant activities of essential oils extracted of leaves *Rosmarinus officinalis* grows in Al-Jabal Al Akhdar, house in Benghazi and commercial oil of *Rosmarinus officinalis* are evaluated by:

Total phenolic content (TPC): Figure (3) show the total phenolic content that found in essential oils extracted from leaves *Rosmarinus officinalis* grows in Al-Jabal Al Akhdar, house in Benghazi and commercial oil were the essential oils extracted

from leaves grows in Al-Jabal Al Akhdar and house in Benghazi contain high total phenolic content, the results expressed according to pyrogallol as phenolic compound in Figure (2).

Total flavonoids content (TFC): The results obtained in this study as shown in Figure (5) indicate that the essential oils extracted from leaves grows in Al-Jabal Al Akhdar and house in Benghazi contain medium amount of flavonoids compounds as compared with the qurecetin whish used as standard (Figure 4). While the commercial oil contain slightly amount of flavonoids compounds

Reducing power assay (RPA): As shown in Figure (7) the reducing power assay of essential oils extracted from leaves grows in Al-Jabal Al Akhdar and house in Benghazi exhibit higher reducing activity than the ascorbic acid but the commercial oil not showed reducing power assay.

The DPPH˙ radical scavenging activity: The result of the DPPH˙ radical scavenging activity of essential oils are shown in Figure (8), this result compared with the well-known antioxidant ascorbic acid were the percent of the inhibition is 94% at 500 µg/ml of the essential oil from leaves grows in Al-Jabal Al Akhdar , 92.3% at 500 µg/ml of the essential oil from grows in house and 45.6**%** at 500 µg/ml of the essential commercial oil

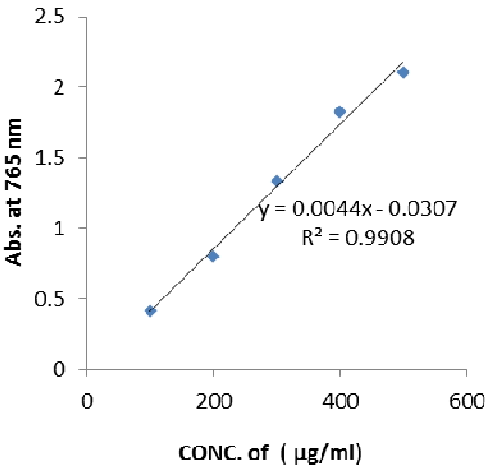

Figure 2: Total phenolic content of pyrogallol

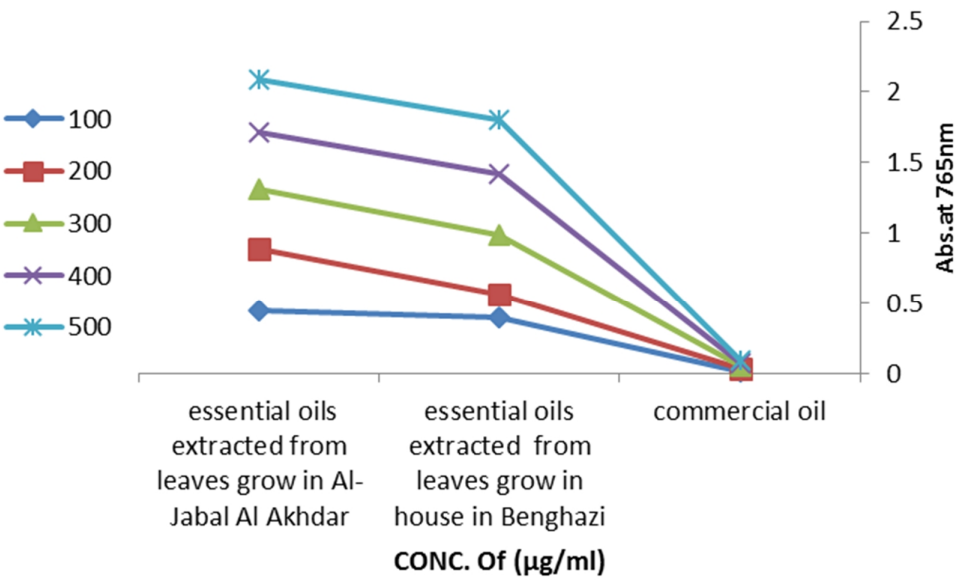

Figure 3: Total phenolic content (TPC) of essential oils extracted of leaves grow in Al-Jabal Al Akhdar, grow in house in Benghazi and commercial oil from *Rosmarinus officinalis*

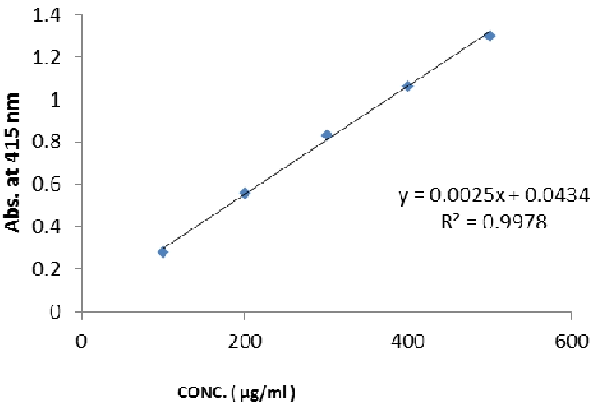

Figure 4: Total flavonoids content of quercetin

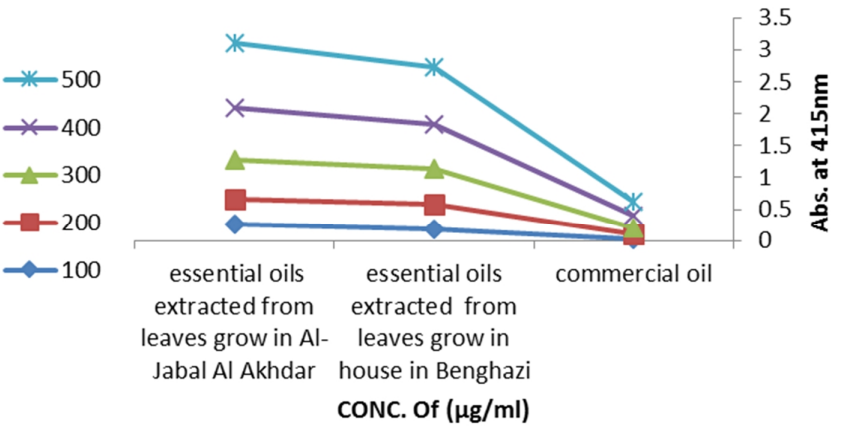

Figure 5: Total flavonoids content of essential oils extracted of leaves grow in Al-Jabal Al Akhdar, grows in house in Benghazi and commercial oil from *Rosmarinus officinalis*

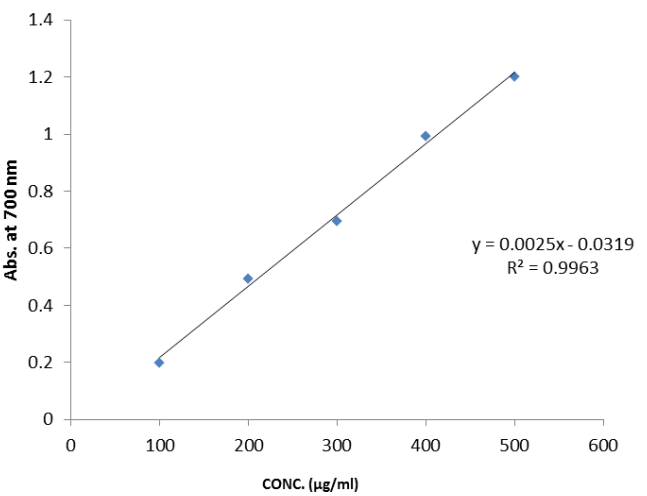

Figure 6: Reducing power assay of Vitamin C

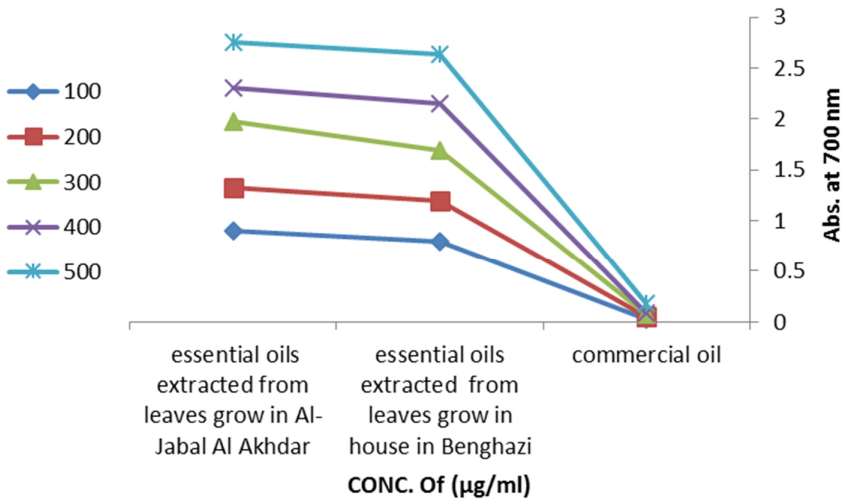

Figure 7: Reducing power assay of essential oils extracted of leaves grow in Al-Jabal Al Akhdar, grows in house in Benghazi and commercial oil from *Rosmarinus officinalis*

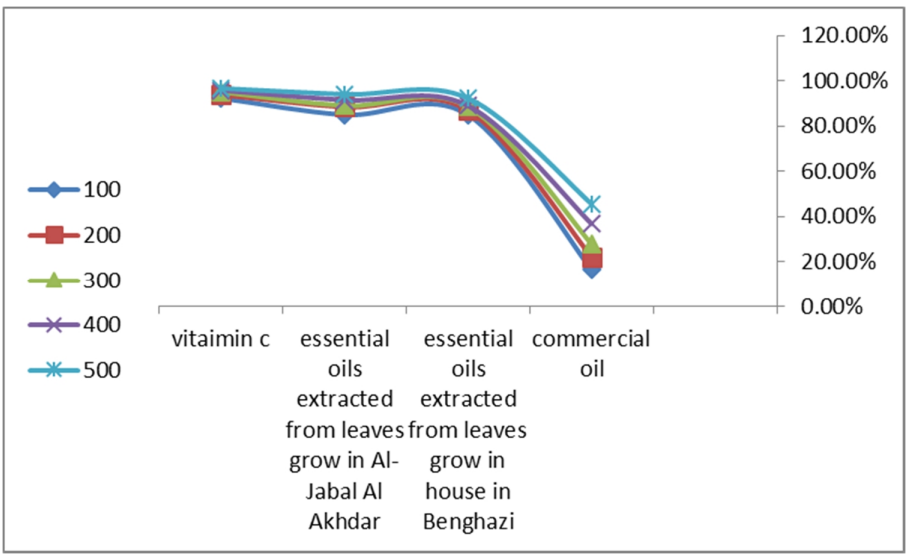

Figure 8: DPPH radical scavenging activity of vitamin C, essential oils extracted of leaves grow in Al-Jabal Al Akhdar, grows in homes in Benghazi and commercial oil from *Rosmarinus officinalis* according to % inhibition

DISCUSSION

There is an increasing interest in phytochemicals as new sources of natural antioxidant and antimicrobial agents. The use of synthetic antioxidants in the food industry is severely restricted as to both application and level[20]. Currently, there is a strong debate about the safety aspects of chemical preservatives, since they are considered responsible for many carcinogenic and teratogenic attributes, as well as residual toxicity [21].

Plant-derived polyphenols receive considerable attention because of their potential antioxidant and antimicrobial properties[21]. Phenolic compounds exhibit a considerable free-radical scavenging (antioxidant) activity, which is determined by their reactivity as hydrogen- or electron- donating agents, the stability of the resulting antioxidant-derived radical, their reactivity with other antioxidants and, finally, their metal chelating properties[22].

Reported that rosemary plants are rich sources of phenolic compounds with high antimicrobial activity against both Gram-positive and Gram-negative bacteria. High percent of the antimicrobial activity they attributed to carnosic acid and carnosol. It is clear that rosemary extracts have bioactive properties, but their antimicrobial activities have not been deeply characterized [8].

Rosemary is a spice and medicinal herb widely used around the world. Of the natural antioxidants, rosemary has been widely accepted as one of the spices with the highest antioxidant activity [23]. Rosemary essential oil is also used as an antibacterial, antifungal[10] and anticancer agent [24].

Yesil-Celiktas et al [25] investigated the antibacterial activity of selected essential oils against some food spoilage organisms. They concluded that the essential oils of cinnamon, clove and rosemary were the most active. Similar results were obtained by[26] for the antibacterial activity of rosemary essential oil against *Bacillus cereus* strains grown in carrot broth.

Our results obtained from the in vitro antioxidant screened showed that essential oil (grow in Al-Jabal Al Akhdar and house) has considerable amounts of polyphenolic and flavonoids compounds which are responsible for the antioxidant properties. And also they give the higher reductive potential due to reducing capacity and DPPH free radical scavenging activity which serves as strong indicator of antioxidant activities. [27]found that Rosemary had the high radical-scavenging activity.

Many compounds have been isolated from rosemary, including flavones, diterpenes, steroids, and triterpenes. Of these, the anti-oxidant activity of rosemary extracts has been primarily related to two phenolic diterpenes: carnosic acid and carnosol[25]. The main compounds responsible for the antimicrobial activity are α-pinene, bornyl acetate, camphor and 1,8-cineole[28,29] . The result obtained from the GC-MS technology found that the most important components are α-pinene, β-pinene, 1,8-cineol, camphene, limonene and camphor. The high concentration of 1,8-cineol in both oils (grow in Al-Jabal Al Akhdar and in house) makes it potentially useful in the medicines because they exhibit antibacterial, antifungal, anti-inflammatory activity and antioxidant properties according to[22] .

The phenolic and the flavonoids compounds are groups of secondary metabolites with broad range of biological properties such as: antioxidant, antibacterial ,anti-atherosclerosis, cardiovascular protection and improvement of the endothelial function, it has been reported that antioxidant activity of the phenolic compounds is mainly due to their redox properties which allow them to act as reducing agents, hydrogen donors play an important role by adsorbing and neutralizing reactive free radicals, and chelating ferric ions which catalyses lipid peroxidation, and regarded as promising therapeutic agent for free radical-linked pathologies [30].

Antioxidant components are micro constituents present in the diet that can delay or inhibit lipid oxidation, by inhibiting the initiation or propagation of oxidizing chain reactions, and are also involved in scavenging free radicals[31]. Thus, phenolic compounds may help protect cells against the oxidative damage caused by free radicals, [27] reported that the extract plant rich in phenolic compounds leads to antibacterial activity.

The effectiveness of rosemary extracts as antioxidants have caused their commercial use. It has a powerful inhibitory action on lipid peroxidation production and, a stimulatory action on the synthesis of cellular antioxidants[22]. The activity of rosemary has been ascribed to the diterpene content, mainly carnosic acid and carnosol[32], as well as to the essential oil constituents [33]. Carnosic acid provides protection from the liver carcinogen aflatoxin [27].

Rosemary extracts are widely used in the food. Their major bioactive components have shown antioxidant, antimicrobial, anti-inflammatory, antitumorigenic and chemopreventive activities [33].

CONCLUSION

We suggest the two type oils extracts of rosemary (collected from Al-Jabal Al Akhdar and house) more rich in phenolic constituent such as α-pinene, β-pinene, 1,8-cineole, camphene, limonene and camphor than commercial oil. . It could be concluded that Rosemary displays a wide variation in essential oil chemical composition in correlation with the climatic conditions under which it is grown.

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Cite this article as:

Maraia F. Elmhdwi and Idress Hamad Attitalla. Antioxidant and free radical scavenging activity of essential oil extracted from *Rosmarinus officinalis* L. J Biol Sci Opin 2015;3(5):223-229
http://dx.doi.org/10.7897/2321-6328.03549

Source of support: Nil; Conflict of interest: None Declared