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Estimation of antioxidant activities of fixed and volatile oils extracted from *Syzygium aromaticum* (clove)

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ABSTRACT

The antioxidant activity of fixed and volatile oils were extracted from the dry buds of Syzygium aromaticum (clove) are evaluated by the scavenging activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) and the reducing power assay, total phenolic and flavonoids are also detected. The result showed that the fixed oil had highest TPC and TFC than the volatile oil in comparison with the standard (pyrogallol, quercetin). Also based on the DPPH radical scavenging activity and reducing power assay the fixed oil is rather strong than the volatile oil, the result compared to standard antioxidant L-ascorbic acid and it is found increase with increase in concentration. Finally, the present study suggests that both fixed and volatile extracted from S.aromaticum have potential activity as source of natural antioxidant.

Keywords; syzybium aromaticum (clove), 1,1-diphenyl-2-picrylhdrazyl (DPPH)

INTRODUCTION

Syzygium aromaticum (L.) commonly called clove, which belongs to the family *Myrtaceae*, is an important aromatic spice. Clove is commercially cultivated in India, Madagascar, Sri Lanka, Indonesia and the south of China. Now-a-days it also cultivated in Bangladesh in a small scale. The clove oil especially has been used by traditional folk healers as well as by modern pharmacists and dentists in alleviating the symptoms associated with toothache and dental decay. Also in the treatment of skin ulcers [1].

The clove herbal tea is prepared by boiling and steeping the dried clove buds in water. This tea is seen as a cure for problems such as nausea and as an aid to eliminating excess gas in the stomach and the intestines. Clove bud oil has biological activities, such as antibacterial, antifungal, and antioxidant properties, and are used traditionally as flavoring agent and antimicrobial material in food, It is clear that common spices *S. aromaticum* has broad spectrum pharmacological effects against various microorganism as well as in treatment of different health problems in human beings. Chemicals present in *S. aromaticum* have significant effect against cancer, cardiovascular rich factors, and as antidibetic/antioxidant, [2]. And also shows antibacterial properties against food borne pathogens (S. aureus, P. aeruginosa, E. coli) [3]. Oil of Clove showed maximum antifungal activity against Aspergillus flavus, A. niger, A. terreus, A. oryzae, A. fumigatus, Fusarium moniliforme, F. solani and Penicillium fungal species [4]. With other 16 medicinal plants, clove used in the treatment of Jaundice in the Satra culture people that related to their livelihood as well as socio-economic and spiritual aspect [5].

Ample experimental and epidemiological studies support the involvement of oxidative stress in pathogenesis and progression of many diseases. It is quite known that oxygen, indispensable for maintaining life, sometimes becomes toxic and results in the generation of most aggressive agents such as reactive oxygen species (ROS). The high reactivity of ROS can trigger a host of disorders in biological systems. Endogenous antioxidant enzymes are responsible for preventing and neutralizing the free radical induced damages of tissues. Oxidative stress is an

outcome of imbalance between ROS production and antioxidant defences, which in turn evokes a series of events deregulating the cellular functions [6]. Antioxidant is a substance that has the ability to delay the oxidation of a substrate by inhibiting the initiation or propagation of oxidizing chain reactions caused by free radicals. It plays important roles to prevent fats and oils from becoming rancid and protects human body from detrimental effects of free radicals. Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ) and propyl gallate (PG) have been widely used around the world for decades. However, they are being scrutinized for possible toxic and carcinogenic effects. As a result, an intense new area of research has been developed concerning the search for identification and characterization of naturally occurring antioxidants. Natural antioxidants are more ideal as food additives, not only for their free radical scavenging properties, but also on the belief that natural products are healthier and safer than synthetic ones; thus they are more readily acceptable to the modern consumers [7].

Numerous aromatic, spicy and medicinal plants have been examined for their antioxidative potential [8]. Herbs and spices that are usually used to flavour dishes are among the tremendous sources of phenolic compounds, which have been reported to show good antioxidant activity, Chemical constituents with antioxidant activity found in high concentrations in plants determine their considerable role in the prevention of various degenerative diseases [9]. The purpose of this study is to evaluate the antioxidant properties of the fixed and volatile oils extracted from the buds of *Syzygium aromaticum* (clove). A recent study presented the sources, types, mechanism of action and damaged caused by free radicals [10].

MATERIALS AND METHODS

Plant material: The buds of S. aromaticum of good quality were obtained from a local market, Benghazi, Libya, 2012.

Chemicals: 1,1-Diphenylpicrylhydrazyl (DPPH) was obtained from Sigma Chemicals, 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 obtained from biochemistry laboratory of chemistry department.

Sample preparation:
Extraction of fixed oil: The fixed oil from the powdered buds of *S. aromaticum* (100g) was extracted with light petroleum ether (40-60 C°) in a soxhlet apparatus for about 4h and the solvent was removed by rotary vacuum evaporator.

Extraction of volatile oil: The dry powdered buds of *S. aromaticum* (200 g) were subjected to hydrodistillation using Clevenger apparatus for 6h for isolation of volatile oils separately (Clevenger, 1928). The oil samples were stored at 7°C in air-tight containers after drying them over anhydrous sodium sulfate and filtered before analysis.

Antioxidant activities assays and quantitative analysis:
Total phenolic content (TPC): Phenolic compound concentration in the fixed and volatile oils extracted from S.aromaticum (clove) was estimated using the colorimetric method based on Folin-Ciocalteu reagent [11]**.** 0.05 ml of the oils at different concentration "100,200,300,400,500 µg/ml" was mixed 0.05 ml of Folin-Ciocalteu reagent. Then 0.5 ml of 15% sodium carbonate solution was added to the mixture and then the adjusted to 1 ml with 0.4 ml of distilled water. The reaction was allowed to stand for 10 min, after which the absorbance was read at 725 nm by UV-visible spectrophotometer. Quantification was done with respect to stander calibration curve of Pyrogallol the results were expressed as pyrogallol "µg/ml". Estimation of the phenolic compounds was carried out in triplicate. The results were mean values ± standard deviations.

Total flavonoids content: Aluminum chloride colorimetric method was used for determination [12]. 2 ml of Different concentration "100, 200, 300, 400, 500 µg/ml "of oils mixed with 0.1ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with a UV-visible spectrophotometer. The calibration curve was prepared by preparing quercetin solution in methanol at concentrations "100 to 500 µg/ml".

Reducing power assay (RPA): The reducing power was determined according to the [13]. 2ml of the oils with different concentration "100,200,300,400,500µg/ml" was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide then mixture was incubated in water bath at 50 C^{0} for 20 minutes and 2.5 ml of trichloroacetic acid was added to the mixture which was then centrifuged at 3000 rpm for 10 minutes. Finally 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 1 ml $Fecl_3$, substances, which have reduction

potential react with potassium ferricyanide (Fe^{3+}) to form potassium ferricyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700nm by UV-Visible spectrophotometer. 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 $\xrightarrow{antioxidant}$ **potassium ferricyanide + ferrous chloride**

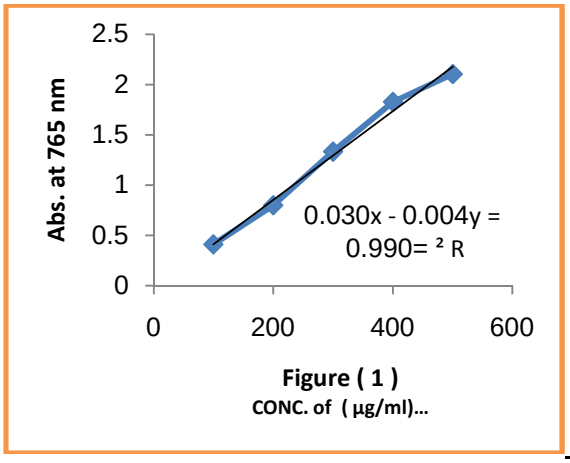
DPPH free Radical Scavenging activity (RSA): The antioxidant activity of the fixed and volatile oils was measured in terms of hydrogen donating or radical-scavenging ability using the stable DPPH method as modified by [14]. The reaction mixture containing 2 ml of the oil at different concentration"100,200,300,400,500µg/ml" and 2ml of DPPH (0.2mM) was vigorously shaken and incubated in darkness at room temperature for 30 minutes. When the DPPH reacted with an antioxidant compound in an oil that can donate hydrogen, it was reduced and resulting decrease in absorbance at 517nm using UV-visible spectrophotometer, and the mean values were obtained from triplicate experiments. The percentage of the remaining DPPH was plotted against the sample concentration. A lower value indicates greater antioxidant activity. Radical scavenging activity was expressed as percent of inhibition and was calculated using the following formula:-

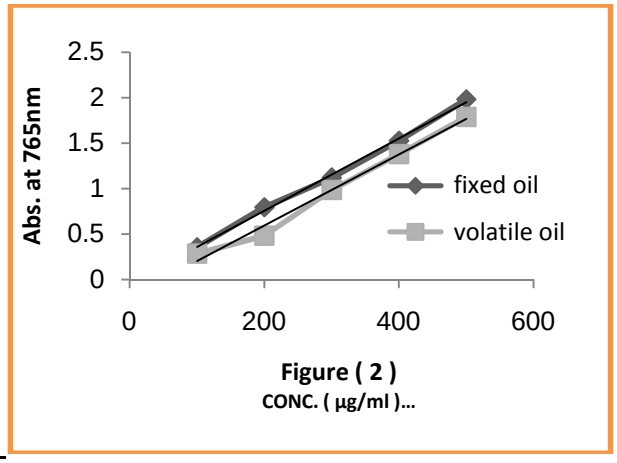
%DPPH " RSA" = [Abs. of Control – Abs. of Sample / Abs. of Control] x 100

RESULTS AND DISCUSSION

A similar strategy was used recently to study the Antioxidant activity and to estimate the total phenolic and flavonoid contents of the root extract of *Amaranthus spinosus* [15].

Total phenolic content: Folin-Ciocalteu reagent, a mixture of phosphotungstic ($H_3PW_{12}O_{40}$) and phosphomolybdic ($H_3PMo_{12}O_{40}$) acids, is reduced to blue oxides of tungstene (W_8O_{23}) and molybdene (Mo_8O_{23}) during phenol oxidation. This reaction occurs under alkaline condition provided by sodium carbonate. The intensity of blue colour reflects the quantity of phenolic compounds, which can be measured using spectrophotometer [7]. Figure (2) shows the total phenolic compound founds in fixed and volatile oil where the fixed oil contains high TPC, the results suggest that the TPC varied significantly between fixed and volatile oil may due to in the difference in the extraction process. The colour measurement which was non-specific on phenol and perhaps there were other component that can react with folin-Ciocalteu reagent such as saponin. The results expressed according to Pyrogallol as a phenolic compound in figure (1).





Total flavonoids content: Due to their low redox potentials flavonoids are thermodynamically able to reduce most oxidizing free radicals relevant to biological systems such as superoxide, peroxyl, alkoxyl, and hydroxyl radicals [16]. The principle of aluminum chloride colorimetric method is that aluminum chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition, aluminum chloride forms acid labile complexes with the ortho-dihydroxyl groups in the A- or B-ring of flavonoids [12]. The results obtained in this study in the figure (4) indicate that the fixed and volatile oils contain slightly amount of flavonoids compounds as compared with the quercetin in figure(3) which used as standard.

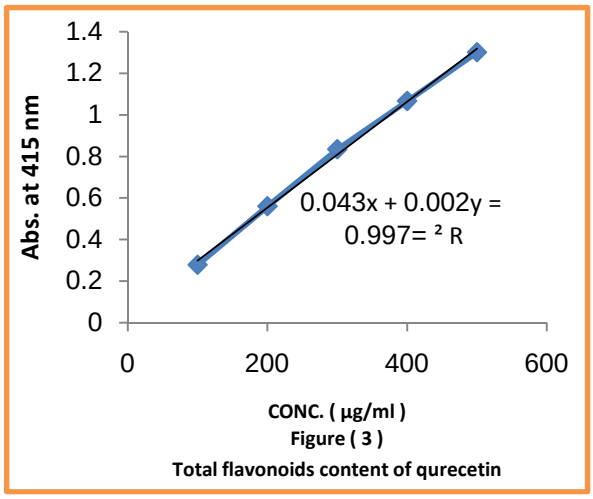


Figure (3)
Total flavonoids content of qurecetin

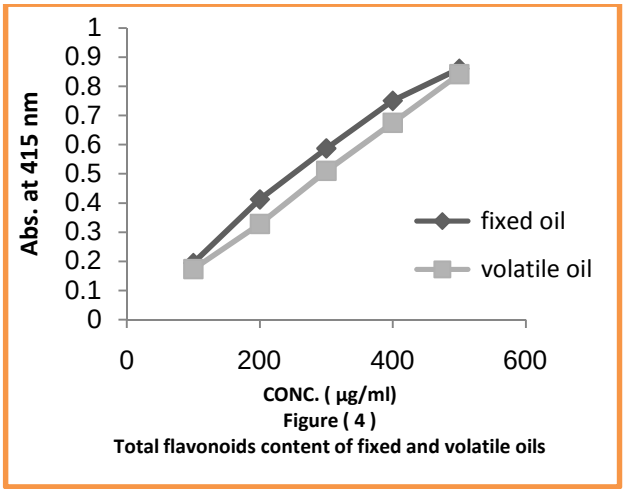


Figure (4)
Total flavonoids content of fixed and volatile oils

Reducing power assay: The reducing power assay is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates. In this assay the yellow colour of the test solution change to various shades of green and blue depending on the reducing power of each compound. Presence of the reducers causes the conversion of the ferricyanide to the ferrous form [17].

Standard curve of ascorbic acid is shown in figure (5).and the figure (6) shows the reducing power assay of fixed and volatile oils of S.aromaticum. The reducing power assay increase with increase in concentration and the fixed oil exhibit higher reducing activity than the volatile oil.

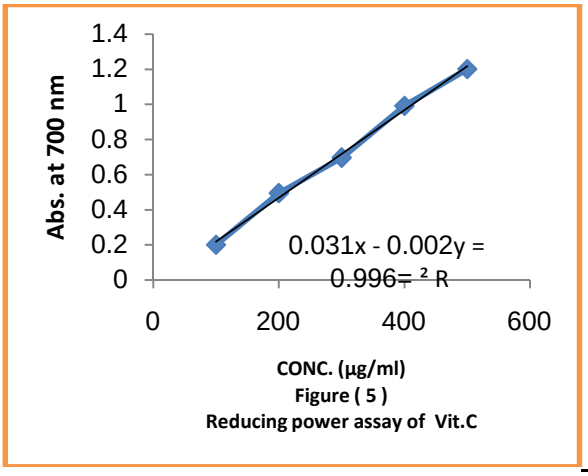


Figure (5)
Reducing power assay of Vit.C

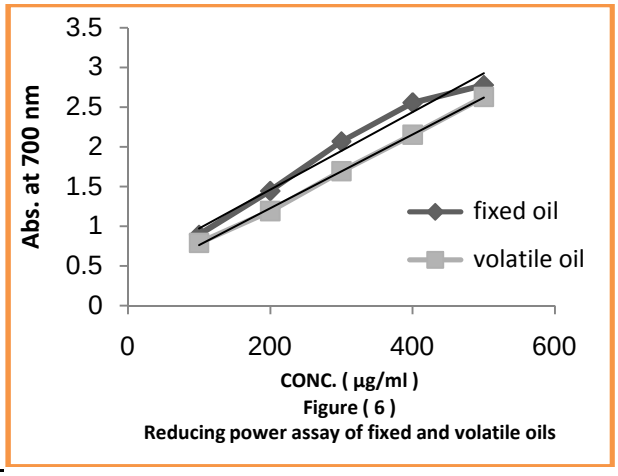


Figure (6)
Reducing power assay of fixed and volatile oils

DPPH free radical scavenging activity (RSA): DPPH radical scavenging activity assay assessed the ability of the extract to donate hydrogen or to scavenge free radicals. DPPH radical is a stable free radical and when it reacts with an antioxidant compound which can donate hydrogen, it is reduced to diphenylpicrylhydrazine. The changes in colour (i.e. from deep-violet to light-yellow) can be measured spectrophotometrically [7].

The clove oil buds when mixed with the DPPH decolorized it due to hydrogen donating ability, it was observed that the scavenging activity of fixed oil of clove buds at all concentrations from 100 to 500 µg/ml is rather strong than the volatile oil. Figure (7) show the radical scavenging activity of fixed and volatile oil and the ascorbic acid which used as standard antioxidant.

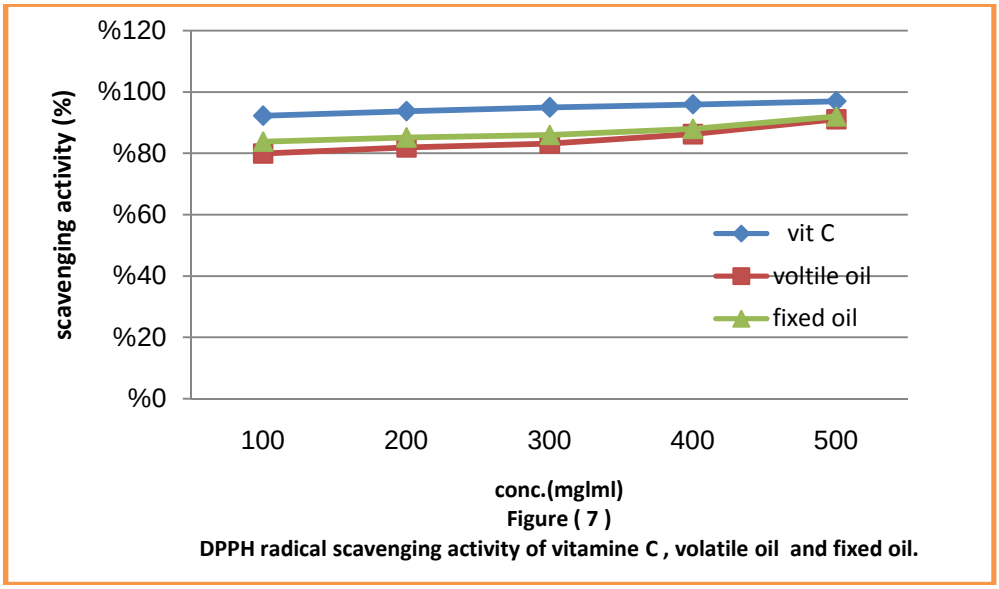


Figure (7)
DPPH radical scavenging activity of vitamine C , volatile oil and fixed oil.

1. Total phenolic content:

Concentration of Pyrogallol " µg/ml "	*Mean ± Standard Deviation*	concentration of fixed oil " µg/ml "	*Mean ± Standard Deviation*	concentration of volatile oil " µg/ml "	*Mean ± Standard Deviation*
100	***0.410 ± 0.0320***	***100***	***0.356 ± 0.0322***	***100***	***0.287 ± 0.00342***
200	***0.799 ± 0.0220***	***200***	***0.795 ± 0.0220***	***200***	***0.483 ± 0.0087***
300	***1.333 ± 0.0045***	***300***	***1.120 ± 0.0120***	***300***	***0.987 ± 0.0253***
400	***1.828 ± 0.0117***	***400***	***1.525 ± 0.0056***	***400***	***1.380 ± 0.0236***
500	***2.105 ± 0.0225***	***500***	***1.980 ± 0.0048***	***500***	***1.789 ± 0.0338***

2. Total flavonoids content:

Concentration of qurecetin " µg/ml "	*Mean ± Standard Deviation*	concentration of fixed oil " µg/ml "	*Mean ± Standard Deviation*	concentration of volatile oil " µg/ml "	*Mean ± Standard Deviation*
100	***0.279 ± 0.0920***	***100***	***0.194 ± 0.0022***	***100***	***0.173 ± 0.0033***
200	***0.560 ± 0.0350***	***200***	***0.412 ± 0.0020***	***200***	***0.328 ± 0.0020***
300	***0.834 ± 0.0034***	***300***	***0.587 ± 0.0450***	***300***	***0.510 ± 0.0220***
400	***1.066 ± 0.0098***	***400***	***0.750 ± 0.0336***	***400***	***0.675 ± 0.0276***
500	***1.300 ± 0.0065***	***500***	***0.860 ± 0.0348***	***500***	***0.842± 0.0355***

3. Reducing power assay:

Concentration of vitamin C " µg/ml "	*Mean ± Standard Deviation*	concentration of fixed oil " µg/ml "	*Mean ± Standard Deviation*	concentration of volatile oil " µg/ml "	*Mean ± Standard Deviation*
100	***0.201 ± 0.0280***	***100***	***0.895 ± 0.0122***	***100***	***0.791 ± 0.0922***
200	***0.495 ± 0.0350***	***200***	***1.446 ± 0.0120***	***200***	***1.192± 0.0920***
300	***0.697 ± 0.0087***	***300***	***2.070 ± 0.0550***	***300***	***1.694 ± 0.0430***
400	***0.992 ± 0.0727***	***400***	***2.560 ± 0.0436***	***400***	***2.155 ± 0.0036***
500	***1.201 ± 0.0305***	***500***	***2.780 ± 0.0218***	***500***	***2.630± 0.0308***

4. DPPH- radical scavenging activity:

A. According to absorbance:

Concentration of vitamin C " µg/ml "	*Mean ± Standard Deviation*	concentration of fixed oil " µg/ml "	*Mean ± Standard Deviation*	concentration of volatile oil " µg/ml "	*Mean ± Standard Deviation*
100	***0.174 ± 0.0830***	***100***	***0.402 ± 0.0982***	***100***	***0.445 ± 0.0013***
200	***0.140 ± 0.0450***	***200***	***0.335 ± 0.0650***	***200***	***0.395 ± 0.0095***
300	***0.112 ± 0.0097***	***300***	***0.319 ± 0.0420***	***300***	***0.380 ± 0.0299***
400	***0.094 ± 0.0076***	***400***	***0.291 ± 0.0936***	***400***	***0.309 ± 0.0236***
500	***0.075 ± 0.0075***	***500***	***0.174 ± 0.0918***	***500***	***0.201± 0.0098***

B. According to % of inhibition:

Concentration of vitamin C " µg/ml "	*Percent of inhibition* (%)	concentration of fixed oil " µg/ml "	*Percent of inhibition* (%)	concentration of volatile oil " µg/ml "	*Percent of inhibition* (%)
100	***92.3%***	***100***	***82.2%***	***100***	***80.3%***
200	***93.8%***	***200***	***85.2%***	***200***	***82.5%***
300	***95.1%***	***300***	***85.8%***	***300***	***83.2%***
400	***95.8%***	***400***	***87.1%***	***400***	***86.3%***
500	***96.7%***	***500***	***92.3%***	***500***	***91.1%***

CONCLUSION

It is observed that the fixed oil of clove buds at all concentration from "100 to 500 µg/ml" has higher activities than the volatile oil. In general, it is found that the fixed and volatile oil contain phenolic and flavonoids compounds which is 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.

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