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COMPARATIVE STUDY ON ANTIOXIDANT ACTIVITY OF ESSENTIAL OILS FROM *POGOSTEMON BENGHALENSIS* (BURM.F.) KUNTZE. AND *P.CABLIN* (BLANCO) BENTH

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ABSTRACT

Essential oils (Eos) are complex mixtures of volatile lipophilic components and are obtained from leaf, twigs, fruits, flowers of higher plants. These oils are isolated commonly through hydrodistillation. *Pogostemon* species are well known for their essential oils and therapeutic values. Chemical composition and antioxidant activity of Eos from two species viz. *Pogostemon benghalensis* and *P. cablin* were evaluated. Essential oil from both species was extracted by hydrodistillation using Clevenger type apparatus. The essential oil obtained was subjected to GC-Fid analysis followed by GC-MS. The antioxidant activity was studied using DPPH, ABTS, FRAP, metal chelating, hydrogen peroxide, super oxide radical, reducing power, hydroxyl radical scavenging assays using ascorbic acid and butylated hydoxytoluene as standards. 36 to 41 compounds were identified from the essential oil of *Pogostemon* species. α-Cadinol (35.78%) and patchouli alcohol (34.85%) were the major components in these oils. In addition to these compounds 1,8 cineole, aromadendrene, β-patchoulene, α-caryophyllene, β-caryophyllene, α-patchoulene, germacrene A were identified as the other predominant compounds. The antioxidant activity of Eos was significant when compared with standards. 75.3 to 91.5% inhibition activity was noticed against various free radical scavenging assays. The Eos showed remarkable IC_{50} value which suggests their potency to use as a natural antioxidant in various industries, foods and therapeutics.

KEYWORDS: Essential oils, *Pogostemon*, hydrodistillation, GC-MS, α-Cadinol, patchouli alcohol.

INTRODUCTION

Free radicals are produced in human body by normal and pathological cell metabolism. In most of the living organisms, metabolic pathway includes oxido-reductive events for the production of energy for many biological processes. Oxidative stress due to the uncontrolled production of free radicals triggers several lifestyle diseases such as cancer, arteriosclerosis and neurodegenerative diseases.[1] It is also involved in degenerative processes associated with aging or senescence. The metabolic activities inside the human body release highly reactive free radicals, especially oxygen derived radicals, which are capable of oxidizing biomolecules, resulting in cell death and tissue damages.[2] Cellular systems are protected from free radical damages by oxidative enzymes such as superoxide dismutase (SOD) and catalase (CAT), or by chemicals such as α-tocopherol, ascorbic acid, carotenoids, polyphenols and glutathione.[3] The most commonly used synthetic antioxidants were butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been known for their toxicity and DNA damage induction. Antioxidants play an important role in inhibiting and scavenging the free radicals and the cells from oxidative stress. These antioxidants either directly scavenge or prevent generation of reactive oxygen species formed via various electron transport reactions in living system.[4] Biologists are always involved in a search for identifying novel antioxidant from plant species, but need more information concerning the antioxidant potential of phytochemicals as they are safe and also bioactive. Therefore in recent years, there is vast interest has been directed towards the identification of bioactive natural herbal antioxidants from plants to substitute the synthetic antioxidants.[5] Many natural antioxidants from plant orgin were studied for their capacity to protect organisms and cells from damage, via oxidative stress.[6] Essential oils (Eos) are volatile oils derived from many plants used as functional ingredients in food, drinks, toiletries and cosmetics.[7] Essential oils have been mostly viewed their flavour and fragrance chemistry for flavouring foods, drinks and other goods. Few studies were published on the antioxidant activity of essential oils. In this juncture present study aims to screen the antioxidant properties of essential oils from *Pogostemon benghalensis* and *P.cablin*.

MATERIALS AND METHODS

The plant material used for the study were *Pogostemon benghalensis* and *P.cablin* collected from Munnar Hills of Idukki district.

Extraction and analysis by GC-MS

Hydro-distillation of fresh leaves of *Pogostemon* species was carried out using a Clevenger-type apparatus. GC-MS analysis was done out using Hewlett-Packard 6890 gas chromatograph (Agilent Technologies, USA). 1 µl of essential oils from each species was injected into the machine fitted with an HP-5 (5% phenyl methylpolysiloxane, 30 m 0.32 mm i.d., 0.25 µm film thickness) capillary column coupled with a model 5973 mass detector. GC–MS operation conditions were injector temperature: 220°C; transfer line temperature: 240°C; oven temperature programme: 60– 246°C (3°C/min); carrier gas: helium (1.4 ml/min); detector temperature: 250°C; mass spectra, electron impact (EI+) mode, 70 eV; ion source temperature: 240°C.

AOX methodology

Free-radical scavenging potentialities assay include DPPH, ABTS, FRAP, Reducing power, Hydrogen Peroxide, Hydroxyl radical, Iron chelating activity and Superoxide radicals. The positive controls were Butylated Hydroxytoluene (BHT) and ascorbic acid.

DPPH Method

The essential oil was mixed with 95% methanol to prepare the stock solution. Freshly prepared 100 µM DPPH in methanol solution was taken in test tubes and added different concentrations (20–100 µg) of Eos. After incubation for 30 min in the dark, the discolouration was measured at 517 nm.[8] The IC_{50} value was calculated using the dose inhibition curve.

ABTS radical scavenging activity

ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic) assay was performed by preparing the stock solution by mixing equal volumes of 7 mM ABTS solution and 2.45 mM potassium persulfate solution followed by incubation for 12 h at room temperature in the dark to yield $ABTS^{+}$ radicals.[9] Fresh working solution was prepared using 50% methanol. Serially diluted Eos were added to the working ABTS solution and the absorbance was measured up to 6 min using positive control and blank at 734nm.

FRAP assay

The FRAP (Ferric Reducing Antioxidant Power assay) procedure was carried as described by Benzie and Strain.[10] The principle of this method is based on the reduction of a ferric-tripyridyltriazine complex to its ferrous coloured form in the presence of antioxidants. The

FRAP reagent was prepared freshly using TPTZ (2, 4,6- tripyridyl- S- triazine), ferric chloride(FeCl3) and acetate buffer and warmed at 37ºC. Aliquots of 20 to 100 µg/ml sample were mixed with 3 ml FRAP reagent. After incubation at 37ºC for 10 min, the absorbance of reaction mixture at 595nm was measured spectrophotometrically. The values were expressed in µM equivalent to FeSO4.7H_2O calculating using calibration curve.

Metal chelating activity

The chelation of ferrous ions by Eos was estimated by method of Dinis *et al.*[11] 50 µl of 2 mM ferrous chloride ($FeCl_2$) was added to 1 ml of different concentrations of the Eos (20-100 µg/ml). 0.2 ml of 5 mM ferrozine solution was added to initiate the reaction. The absorbance of the solution was measured at 562 nm against a suitable blank. Using Disodium Ethylene Diamine Tetraacetate (Na_2EDTA) was used as positive control; the percentage inhibition of ferrozine–$Fe2^+$ complex formation was calculated by standard method.

Hydrogen Peroxide Scavenging

Hydrogen peroxide(H_2O_2) at 40 mM concentration in phosphate buffer was used with various concentrations (20-100 µg/ml) of Eos and incubated for 10 min at room temperature according to Nabavi *et al.*[12] The absorbance of hydrogen peroxide at 230 nm was determined. After incubation the absorbance of the reaction mixture was measured against phosphate buffer without hydrogen peroxide as blank. For each concentration, a separate blank sample was used for background subtraction. The percentage scavenging activity of hydrogen peroxide by the Eos was calculated.

Superoxide radical scavenging activity

Superoxide scavenging was determined by the Nitrobluetetrazolium reduction method.[13] The scavenging activity is based on the reduction of Nitrobluetetrazolium (NBT). The reaction mixture consisted of 1 ml of Nitrobluetetrazolium (NBT) solution (l M NBT in 100 mM phosphate buffer, pH 7.4), 1 ml NADH solution and (l M NADH in 100 mM phosphate buffer, pH 7.4) and various concentration of Eos. The reaction was triggered by adding 100 µl of Phenazine methosulfate (PMS) solution (60 µM PMS in 100 mM phosphate buffer, pH 7.4). The tubes were uniformly illuminated with an incandescent visible light for 15 min and the optical density was measured at 530 nm before and after the illumination. The percentage inhibition of superoxide generation was evaluated by comparing the absorbance values of the control and experimental tubes. The abilities to scavenge the superoxide radical were calculated by using standard formula.

Reducing Power

Different aliquotes of the Eos (20-100 µg) were mixed 1% Potassium ferricyanide ($K_3Fe(CN)_6$). The mixture was incubated at 50°C for 20 min. Followed by adding 10% TCA to the mixture and centrifuged at 3,000 rpm for 10 min. The upper layer of the solution was separated and was mixed with equal volume of distilled water. 0.1% Ferric chloride ($FeCl_3$) was added to the aliquots and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Butylated hydroxytoluene (BHT) and ascorbic acid was used as a reference standard.[14]

Hydroxyl radical-scavenging activity

Hydroxyl radical scavenging activity of Eos was measured according to the method of Halliwell *et al*.[15] One ml of the final reaction solution consisted of aliquots 20-100 µg of various concentrations of the Eos, 1 mM Ferric chloride($FeCl_3$), 1 mM Ethylene Diamine Tetraacetic Acid (EDTA), 20 mM hydrogen peroxide (H_2O_2), 30 mM deoxyribose in potassium phosphate buffer (pH 7.4). The reaction mixture was incubated for 1 h at 37°C, and further heated in a boiling water bath for15 min after addition of 1 ml trichloro acetic acid and 2-thiobarbituric acid. The colour developed was measured at 532 nm against a suitable blank and scavenging activity was measured using the standard formula.

RESULTS AND DISCUSSION

The antioxidant activity of Eos was due to the active aromatic compounds present in the oil. The GC-MS analysis identified 41 and 36 volatile compounds from the leaves of *P. benghalensis* and *P. cablin* respectively. α-Cadinol (35.78%) and patchouli alcohol (34.85%) were the major components in these oils. The predominat components of *P. benghalensis* were 1,8 cineole (7.14%), aromadendrene (4.16%), þ-cymene (1.6%), bornylacetae (2.15%), longicyclene (2.74%), β–elemene (1.56%), longifolene (1.18%), α-caryophyllene (1.08%), β-caryophyllene (1.14%), trans-β-farnesene (1.56%), α-patchoulene (2.39%), gurjunene (2.86%), valencene (2.21%), epi-cubedol (1.03%), bicyclogermacrene (2.78%), trans-β-guaiene(1.26%), α-bisabolene (1.16%), ɗ-cadinene (2.45%), elemol (1.01%), spathulenol(1.16%), caryophyllene oxide (1.15%), guaiol (1.79%), isolongifol (1.20%), cubenol-1-epi (1.56%), α-murolol (1.45%), bulsenol(1.51%) and cadalene- 8,9-epoxide (2.95%).

In addition to patchouli alcohol, the essential oil of *P. cablin* contained α-pinene (2.38%), 1,8 cineole (4.21%), β-patchoulene (8.45%), α-caryophyllene (1.02%), β-caryophyllene (3.21%),

α-guaiene (5.73%), aromadendrene (2.78%), α-patchoulene (6.18%), alloaromadendre (2.17%), seychellene (1.84%), cis-β-guaiene (1.37%), α-selinene (4.36%), trans-β-guaiene (1.03%), germacrene A (5.48%), α-bulnesene (1.42%), globulol (2.37%) and guaiol (2.47%) as the major components.

The DPPH is widely used to determine the antioxidant activity of natural plant products. The DPPH radical scavenging activity of Eos showed significant data when compared with the synthetic antioxidants BHT and ascorbic acid. The radical scavenging activity of Eos against DPPH radical resulted the IC_{50} values of 23.8 µg/ml and 27.6 µg/ml respectively for *P. benghalensis* and *P. cablin.* It was observed a higher value of 91.5% and 88.8% for the *Pogostemon* species at a concentration of 100 µg/ml and was at par with the synthetic antioxidant. The synthetic antioxidant ascorbic acid and BHT reached the values of 96.5 and 94.8 respectively at 1mg concentration (Table 1 & 3).

Table 1: Antioxidant activity of essential oil from *P.benghalensis* by different radical scavenging assays.

	DPPH assay	**ABTS assay**	**FRAP assay**	**Metal chelating activity**
Conc. (µg/ml)	%Inhibition ±SD	%Inhibition ±SD	µM equivalent to $FeSO_4.7H_2O$	%Inhibition ±SD
20	47.2 ± 0.08	32.4±0.06	128.2± 0.03	35.6±0.04
40	73.9±0.04	46.8±0.04	132.4±0.04	56.2±0.05
60	84.9 ± 0.02	69.4±0.01	149.5 ± 0.36	64.3±0.06
80	90.2 ± 0.03	76.3±0.02	150.1 ± 0.42	70.8±0.02
100	91.5± 0.06	80.4±0.08	158.2 ± 0.6	75.3±0.04
IC_{50} (µg/ml)	23.8± 0.01	45.1±0.02	-	34.5±0.08
Ascorbic acid (1mg)	96.5± 0.05	78.4±0.02	158.8±0.08	76.4±0.03
BHT(1mg)	94.8± 0.03	80.5±0.4	140.2±0.07	79.7±0.04

Table 2: Antioxidant powers of essential oil from *P.benghalensis* by various scavenging assays.

	Hydroxgen peroxide scavenging	**Superoxide radical scavenging**	**Reducing power activity**	**Hydroxyl radical scavenging**
Conc. (µg/ml)	%Inhibition ± SD	%Inhibition ±SD	%Inhibition ±SD	%Inhibition ±SD
20	35.8 ± 0.06	39.4 ± 0.03	31.7± 0.06	43.8 ± 0.03
40	58.7 ± 0.05	65.9 ± 0.04	59.4± 0.03	70.5 ± 0.04
60	79.6± 0.03	78.5 ± 0.02	65.7± 0.02	79.7 ± 0.12
80	80.3± 0.02	86.4± 0.07	72.9 ± 0.09	84.6 ± 0.07
100	83.2± 0.05	89.3±0.01	78.6± 0.07	85.9±0.03
IC_{50} (µg/ml)	32.7±0.02	27.4±0.01	33.7±0.01	24.8±0.05
Ascorbic acid (1mg)	84.1 ±0.03	90.5±0.04	78.7±0.04	90.4± 0.03
BHT(1mg)	83.5 ±0.07	89.4±0.05	72.5±0.05	91.7± 0.06

ABTS assay showed a dose dependant inhibition of free radicals. 80.45% and 81.75% of inhibition were displayed by Eos of *P. benghalensis* and *P. cablin* respectively. The IC_{50} values of scavenging ability were 45.1 µg/ml and 39.1 µg/ml for Eos of *P. benghalensis* and *P. cablin* respectively. Whereas, the synthetic antioxidant ascorbic acid and BHT yielded 78.4% and 80.5% at 1mg concentration. The reducing potentialities of Eos were determined by ferric reducing antioxidant power (FRAP) method and the results were shown in the table 1&3. The results were expressed in µM equivalent to $FeSO_4.7H_2O$. The oil obtained from *P. benghalensis* yielded values ranging from 128.2 µM to 158.2 µM at concentration 20-100 µg/ml. While, *P. cablin* showed the values ranged from 104.1 µM to 149.5 µM for the same range of concentrations. In the present study the ion chelating activity of the oils at concentrations of 20 µg/ml to 100 µg/ml were evaluated. The IC_{50} values for the metal chelating activity were 34.5 µg/ml and 32.5µg/ml for *P. benghalensis* and *P. cablin* respectively. The chelating activity increased with concentration of Eos.

Table 3: Antioxidant activity of essential oil from *P.cablin*.

	DPPH assay	**ABTS assay**	**FRAP assay**	**Metal chelating activity**
Conc. (µg/ml)	%Inhibition ± SD	%Inhibition ±SD	µM equivalent to $FeSO_4.7H_2O$	%Inhibition ±SD
20	44.5 ± 0.04	35.4±0.064	104.1± 0.03	38.4±0.08
40	69.9±0.05	52.8±0.04	115.9±0.01	59.7±0.03
60	84.7 ± 0.07	71.2±0.01	137.6 ± 0.14	68.6±0.01
80	88.4 ± 0.01	80.5±0.08	142.8 ± 0.21	73.3±0.05
100	88.8± 0.06	81.7±0.02	149.5 ± 0.04	82.4±0.04
IC_{50} (µg/ml)	27.6± 0.05	39.1±0.04	-	32.5±0.03
Ascorbic acid (1mg)	96.5± 0.05	78.4±0.02	158.8±0.08	76.4±0.03
BHT(1mg)	94.8± 0.03	80.5±0.4	140.2±0.07	79.7±0.04

Table 4: Antioxidant activity of essential oil from *P.cablin*.

	Hydroxgen peroxide scavenging	**Superoxide radical scavenging**	**Reducing power activity**	**Hydroxyl radical scavenging**
Conc. (µg/ml)	%Inhibition ± SD	%Inhibition ±SD	%Inhibition ±SD	%Inhibition ±SD
20	39.2 ± 0.04	37.3 ± 0.03	28.6± 0.05	46.8 ± 0.06
40	60.5 ± 0.03	61.7 ± 0.01	50.3± 0.03	75.4 ± 0.14
60	76.7± 0.07	70.9 ± 0.08	61.5± 0.07	80.9 ± 0.9
80	83.4± 0.01	84.3± 0.02	68.5 ± 0.06	86.7 ± 0.01
100	84.5± 0.04	86.7±0.06	72.3± 0.05	88.4±0.08
IC_{50} (µg/ml)	31.8±0.08	28.1±0.06	39.8±0.05	22.6±0.07
Ascorbic acid (1mg)	84.1 ±0.03	90.5±0.04	78.7±0.04	90.4± 0.03
BHT(1mg)	83.5 ±0.07	89.4±0.05	72.5±0.05	91.7± 0.06

Further, free radical scavenging activity of essential oil was evaluated with hydrogen peroxide scavenging method. Here also a dose dependent increase in the scavenging activity was noticed and the values were comparable with ascorbic acid and BHT. The oil from *P. cablin* showed 84.5% which was higher than that of ascorbic acid and BHT. Similarly significant result was obtained from *P. benghalensis* with 83.2% inhibition at a concentration of 100 µg/ml. With regard to IC_{50} values the superoxide radical scavenging activity of the essential oils from both the plant species reveals its potency as natural free radical scavengers. The minimum and maximum values of inhibition ranged from 39.4 to 89.3% for the oil from *P. benghalensis* and 37.3 to 86.7% for *P. cablin.* Reducing power of Eos from *P. benghalensis* and *P. cablin* were described in Table 2 & 4. The reducing power of the Eos increased with concentration. The reducing power of both species showed excellent result in comparison with synthetic antioxidants. The values ranged from 31.7 -78.6% and 28.6-72.3% for *P. benghalensis* and P. *cablin* respectively. The reducing power of ascorbic acid and BHT were 78.7 and 72.5% respectively. From the analysis it was noticed that in hydroxyl radical scavenging activity, percentage of inhibition increased with concentration. The activity was tested with concentration ranging from 20-100 µg/ml. Highest values of 85.9 and 88.4% were observed at concentration 100 µg/ml for *P. benghalensis* and *P. cablin* respectively. It is interesting to note that a positive correlation with the concentration and the volatile compounds present in the Eos results in the active radical scavenging capacity.

A large number of synthetic antioxidants were widely used in post modification of food quality including Butylated hydroxytoluene (BHT), Butylated hydroxyanisole (BHA), propyl galate (PG) and tertiary butylhydroquinone (TBHQ) promote negative health effects.[16] There were reports that specify commonly used food additives such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) should be restricted because of their toxicity and DNA damaging property.[17] Hence there is a growing demand to identify novel natural antioxidant from plants. The volatile components present in the Eos served as a reducing agent for scavenging the biologically active oxidative radicals.[18] Free radicals are those molecule which had an unpaired electron. The free radical are formed due to bacterial and parasitic infections, inflammation, lung damage, atherosclerosis, reperfusion injury, cardiovascular disorders, aging and neoplastic disease.[19]

The DPPH assay is wildly used to evaluate the antioxidant activity.[20] DPPH is a stable free radical molecule with nitrogen in its center part. The reduction of this molecule by hydrogen

or electron donation by an antioxidant molecule resulted in the colour change from violet to yellow, change in colour due to scavenging molecules helps to evaluate the antioxidant activity.[21] It was found that the free radical scavenging activities of Eos were increased with concentration and was due to the terpenoid compounds present in the oil. Using the ABTS scavenging method both the Eos showed similar trend with potent radical scavenging activity. But the obtained values were lower than that of DPPH assay. The volatile aromatic chemical compounds present in the Eos may inhibit the potassium persulfate activity and resulted in the controlled production of ABTS•+ radical.

The FRAP assay is fast with reproducible results. In the FRAP assay measures the ability of an antioxidant to reduce the ferric tripyridyltriazine (Fe+3-TPTZ) complex to reduced ferrous tripyridyltriazine (Fe+2-TPTZ) complex and the blue colour developed was detected at 595 nm.[10] The Eos from both the plant species resulted a dose dependent increase in the scavenging activity in FRAP assay. The activity of Eos from *P. benghalensis* was slightly higher than the Eos from *P. cablin* in terms of the values represented by µM equivalent to $FeSO_4.7H_2O$.

Hydrogen peroxide is a strong oxidant molecule with a high oxidation potential. It is relatively stable molecule and reaction is slow unless catalyzed by transition metal salts. The high levels of H_2O_2 as being cytotoxic to a wide range of animal, plant and bacterial cells in culture. It induced apoptosis or necrosis in the living system.[22] Hence it must be rapidly eliminated from the body system via enzymes such as catalases, peroxidases.[23] In the present study remarkable H_2O_2 scavenging activity by Eos were recorded with IC_{50} values of 32.7 µg/ml and 31.8 µg/ml. The oil from *P.cablin* displayed slightly higher activity than that of *P. benghalensis.*

Iron is required for respiration, oxygen transport and activity of many enzymes. But the active metal catalyzes oxidative changes in lipids, proteins and other cellular components.[24] The food particles are deteriorated by the chain reaction initiated by metal ions as the lipid peroxidation.[25] The catalysis which were involved in this reaction of metal ions correlates with incidents of cancer and arthritis.[26] Ferrous ions are the most effective pro-oxidants found in food systems.[27] In the present study, the chelating ability of the Eos increased with concentration. In the reductive assay the Fe3+ is reduced to Fe2+ by the electron donors (antioxidant) and the formation of iron complex is monitored spectrophotometrically to

assess the activity.[28] In cellular oxidation the secondary free radicals produced from super oxide radicals damage the cells. The H_2O_2 produced from superoxide radical initiates strand scission in DNA molecule.[22] The Eos yielded significant IC_{5o} values against the superoxide scavenging activity.

The hazardous free radicals generated inside the living system are eradicated by reducing these molecules by the antioxidant substances. The natural antioxidant molecules present in the Eos reduce $Fe3^+$ ferricyanide complex to the ferrous form and the $Fe2^+$ was then monitored at 700 nm.[29] Duh *et al*.[30] suggests that the antioxidant effect is naturally associated with the development of reducing power. Thus the antioxidant activity of Eos was correlated with reducing power. The hydroxyl (OH.) belongs reactive oxygen species (ROS) is one of the most reactive and physiologically destructive free radicals in the biological system. Free radicals are the most prevalent initiators of oxidative reactions that may generate harmful effects.[31] Natural antioxidants have the ability to scavenge free radicals, and eliminate the deleterious effect of the free radicals. Hence, the natural antioxidants received attention as herbal medicines by its radical scavenging potentiality and are used for pharmaceutical research for the production of novel medicines.[32] The Eos from *Pogostemon* species exhibited a concentration dependant scavenging of hydroxyl radicals with an IC_{50} value of 24.8 µg/mL and 32.5 µg/mL and was significant when compared with ascorbic acid and BHT.

COCLUSION

The essential oils from the investigated Lamiaceae plants were rich in oxygenated terpenes and showed considerable radical scavenging and antioxidant activities revealing their potentiality for therapeutic uses. Various in vitro antioxidant activity assays were carried to determine scavenging activity of free radicals by essential oils from *Pogostemon*. Significantly, essential oil of *Pogostemon benghalensis* showed comparatively higher antioxidant activity than *Pogostemon cablin*. Based on the results it can be concluded that two *Pogostemon* oil might be a good candidate for further investigation in developing new antioxidant agents when compared with synthetic antioxidants. These oils can be used as a natural additive in food, cosmetic and pharmaceutical industries as herbal medicines.

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