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Research Article

IN VITRO FREE RADICAL SCAVENGING ACTIVITY OF TEA TREE OIL AND CLOVE OIL

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ABSTRACT

Objective: To investigate the *in vitro free radical scavenging* activity of tea tree oil and the clove oil.

Methods: Both tea tree oil and clove oil were tested by using phytochemical test, estimation of total phenolic content as well as various antioxidants assays such as nitric oxide radical inhibition assay, hydrogen peroxide scavenging assay and *1-diphenyl 2-picrylhyorazyl (DPPH) assay*

Results: Both tea tree oil and clove oil possess antioxidants activity and *in vitro* free radical scavenging activity.

Keywords: *Scavenging* activity, Tea tree oil, Clove oil, Antioxidant.

INTRODUCTION

Essential oils are complex mixture that is obtained from the aromatic plants. Tea tree oil is a yellowish oil which is extracted from the tea plant's leaves known as *Melaleuca alternifolia,* which is an Australian native plant [1]. Tea tree oil contains about 100 substances essentially monoterpenes, sesquiterpenes, and other alcohol derivatives [2]. Terpinen-4-ol, γ-terpinene, α-terpinene, α-terpineol, α-terpinolene, 1,8-cineole, etc., are also presence.

The tea tree oil is extracted through steam distillation process. Nowadays, the tea tree oil is very famous, and it is used in cosmetic and health care. In general, it is used for treating ringworm and athlete's foot, cuts and scrapes, acne, and also dandruff. In addition, tea tree oil also possesses therapeutic benefits such as anti-inflammatory, antioxidants, and anticancer properties [3,4]. Besides, it also has other properties such as antibacterial, antifungal, antiviral, and also analgesic properties [2,5-8].

Clove oil is isolated from the clove tree, known as *Eugenia caryophyllata*. The clove tree, which is an evergreen tree, is a member of Myrtaceae family [9,10] that can be found in Southeast Asian countries especially Indonesia. Clove is also known as aromatic spice, and it is commonly used for seasoning and medicinal purposes in Indian and Chinese culture. During seventh centuries, it is widely used in lots of countries, especially in western hemisphere because it gives a positive impact on health.

The essential clove oil can be extracted through steam distillation from three different parts of the clove tree, which are flower bud, leaf, and stem and produce bud oil, leaf oil, and stem oil based on the parts it is extracted from. Among these oils, clove bud oil is the most beneficial, and it is being used widely for many functions, especially in aromatherapy. Clove oil has been listed as a "generally regarded as safe" substance by the United States Food and Drug Administration when it is administered at levels not exceeding 1500 ppm in all food categories [9,11-14].

In addition, clove oil is composed of 90-95% of the active substance, eugenol [15] that mainly used in dentistry. When the deep decay on the patient's tooth is removed, eugenol will help to relieve pain in the patients. It mixed with zinc oxide to form cement [16] and used for restoration of teeth.

Oxidation mechanisms and role of free radicals have gained lots of attentions starting from the past few decades [17]. During uptake of oxygen for the cellular metabolism, it will lead to the production of reactive oxygen species (ROS) [18]. ROS contain free radicals such as superoxide anion radicals, hydroxyl radicals, and also non-free-radicals such as hydrogen peroxide and singlet oxygen, which are forms of various activated oxygen [19-21]. They are dangerous and results in certain alteration in nucleic acids, protein, and lipid molecules.

ROS are very harmful, and they are capable to cause damage on the crucial biomolecules. If they are not scavenged by the cellular constituents [20,22]. Both clove oil and tea tree oil have the properties to act as antioxidants [23]. The antioxidant has the ability to stop the free radical's harmful action. The antioxidant substance will scavenge the free radicals and detoxify the organism [24]. Therefore, it will protect the body from the free radicals and also ROS effects. The antioxidants also block the progress of many chronic diseases along with lipid peroxidation [25,26]. The study was conducted with the aim to investigate about the *in vitro* free radical scavenging activity of the tea tree oil and the clove oil.

METHOD

Material

Tea tree oil and clove oil was purchased from Cyprus enterprises, Arugambakkam, Chennai, India.

Phytochemical test

Phytochemicals are primary and secondary metabolites that are presence in plants [27]. Proteins and common sugars are part of primary components, whereas alkaloids and phenolic compounds are example of secondary components [28]. Therefore, phytochemical test was done to detect the presence of these natural compounds.

Test for carbohydrates [29]

To 2 ml of plant extract, 1 ml of Molisch's reagent, and a few drops of concentrated sulfuric acid were added. The presence of purple or reddish indicates the presence of carbohydrates.

Test for tannins [30]

To 1 ml of plant extract, 2 ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

Test for saponins [31]

To 2 ml of plant extract, 2 ml of distilled water was added and shaken in a graduated cylinder for 15 minutes lengthwise. Formation of 1 cm layer of foam indicates the presence of saponins.

Test for flavonoids [32]

To 2 ml of plant extract, 1 ml of 2N sodium hydroxide was added. The presence of yellow indicates the presence of flavonoids.

Test for alkaloids [33]

To 2 ml of plant extract, 2 ml of concentrated hydrochloric acid was added. Then, a few drops of Mayer's reagent were added. The presence of green or white precipitate indicates the presence of alkaloids.

Test for quinones [34]

To 1 ml of extract, 1 ml of concentrated sulfuric acid was added. Formation of red indicates the presence of quinones.

Test for glycosides [35]

To 2 ml of plant extract, 3 ml of chloroform and 10% ammonia solution was added. Formation of pink indicates the presence of glycosides.

Test for cardiac glycosides [32]

To 0.5 ml of extract, 2 ml of glacial acetic acid and a few drops of 5% ferric chloride were added. This was under-layered with 1 ml of concentrated sulfuric acid. Formation of brown ring at the interface indicates the presence of cardiac glycosides.

Test for terpenoids [32]

To 0.5 ml of extract, 2 ml of chloroform was added and concentrated sulfuric acid was added carefully. Formation of red-brown at the interface indicates the presence of terpenoids.

Test for phenols [34]

To 1 ml of the extract, a few drops of phenol Ciocalteu reagent were added followed by few drops of 15% sodium carbonate solution. Formation of blue or green indicates the presence of phenols.

Test for coumarins [34]

To 1 ml of extract, 1 ml of 10% NaOH was added. Formation of yellow indicates the presence of coumarins.

Steroids and phytosteroids [36]

To 1 ml of plant extract, equal volume of chloroform is added and subjected with few drops of concentrated sulfuric acid appearance of brown ring indicates the presence of steroids and appearance of bluish-brown ring indicates the presence of phytosteroids.

Phlobatannins [32]

To 1ml of plant extract, few drops of 2% HCL were added, and appearance of red precipitate indicates the presence of phlobatannins.

Anthraquinones [32]

To 1 ml of plant extract, few drops of 10% ammonia solution were added, and appearance pink precipitate indicates the presence of anthraquinones.

Estimation of total phenolic content (TPC) [37]

TPC of extracts was assessed according to the Folin-Ciocalteu method (Slinkard and Singleton, 1977) with some modifications. Briefly, different concentrations of oils (200, 400, and 600 µg) were made to 2 ml with distilled water and 1 ml of Folin-Ciocalteu's reagent was seeded in a tube, and then 1 ml of 100 g/l sodium carbonate was added. The reaction mixture was incubated at 25°C for 2 hrs, and the absorbance of the mixture was read at 765 nm. A calibration curve with six data points for catechol was obtained. The results were compared to a catechol calibration curve, and the TPC of extracts was expressed as mg of catechol equivalents per gram of extract.

Antioxidant assays

Nitric oxide radical inhibition assay [38]

Sodium nitroprusside in an aqueous solution at physiological pH spontaneously generates nitric oxide; it interacts with oxygen to produce nitrite ions, which can be estimated by the use of Griess-Ilosvay reaction (Garrat, 1964). In the present investigation, Griess-Ilosvay reagent was modified using naphthyl ethylenediamine dihydrochloride (0.1% w/v) instead of 1-naphthylamine (5%). The reaction mixture (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml), and different concentrations of oils (200-600 µg) or standard solution (0.5 ml) were incubated at 25°C for 150 minutes.

After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted and mixed with 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 minutes for completing diazotization. Then, 1 ml of naphthyl ethylenediamine dihydrochloride (1%) was added, mixed, and allowed to stand for 30 minutes. A pink-colored chromophore was formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank. Ascorbic acid was used as positive control. The scavenging activity was calculated using the formula.

% of Inhibition = (A of control - A of test)/A of control * 100

1-diphenyl 2-picrylhyorazyl (DPPH) assay [39]

The antioxidant activity of the extracts was measured on the basis of the scavenging activity of the stable 1, DPPH free radical according to the method described by Brand-Williams *et al*. with slight modifications. 1 ml of 0.1 mM DPPH solution in methanol was mixed with 1 ml of essential oil solution of varying concentrations (200, 400, and 600 µg). Corresponding blank sample were prepared, and L-Ascorbic acid (1-100 µg/ml) was used as reference standard. Mixer of 1 ml methanol and 1 ml DPPH solution was used as control. The decrease in absorbance was measured at 517 nm after 30 minutes in dark using a Ultraviolet-visible spectrophotometer. The inhibition % was calculated using the following formula.

% of Inhibition = (A of control – A of Test)/A of control * 100

Hydrogen peroxide scavenging assay [40]

The ability of the extract to scavenge hydrogen peroxide was determined according to the method of Ruch *et al*. (1989). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically absorption at 230 nm (8500 II, Bio- Crom GmbH, Zurich, Switzerland). Extracts (200-600 µg) in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40 mM). Absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of scavenging of hydrogen peroxide of extract, and standard was calculated using the following equation:

% of inhibition = (A of control – A of test)/A of control * 100

RESULTS AND DISCUSSION

Phytochemical test

Clove and tea tree oils in Table 1 showed a strong presence of carbohydrate, tannins, quinones, cardiac glycosides, phenols, and saponins and showed a weak presence, flavonoids, alkaloid, glycosides, phlobatannins, and anthraquinones were absent. Steroids were also presence in both oils.

In general, both tea tree oil and clove oil have lots of similarities on their properties and function. Basically, if reaction takes place, the changes in the solution will be positive, by means, there is the presence of a compound in the oil solution. As is seen in Table 1, the outcome of this experiment shows that both of tea tree oil and clove oil have same compound presence in them. Tea tree oil and clove oil have similar components present.

TPC

TPC of tea tree oil and clove oil was estimated by Folin-Ciocalteu method through some modification. In this experiment, the amount of phenol content was measured in both clove oil and tea tree oil was demonstrated in Table 2 and Graph 1. 200 µg of tea tree oil and clove oil showed a TPC of 4.3 and 3.2, respectively. As the concentration of oils increased, their TPC also concomitantly increased.

After the TPC for of oil were determined, the tea tree oil and clove oil were then examined for their antioxidants activity.

Antioxidant activity assay

Antioxidant activity of tea tree oil clove oil was analyzed using nitric oxide scavenging assay (Table 3, Graph 2), DPPH assay (Table 4, Graph 3), and hydrogen peroxide assay (Table 5, Graph 4).

In nitric oxide scavenging assay, at a concentration of 200 µg, both tea tree oil and clove oil showed 22% and 16% inhibition, respectively. As the concentration of oil increased percentage of inhibition also increased simultaneously. For a concentration of 600 µg, tea tree oil showed an inhibition of 63% and clove oil showed 60% inhibition. Here, ascorbic acid was taken as a standard. Both of oil showed approximately equal inhibitory activity.

DPPH assay was done with ascorbic acid as standard. For a concentration of 200 µg, both tea tree oil and clove oil showed an inhibition of 38% and 30%, respectively. As the concentration of oils increased concomitantly radical scavenging ability also increased which was recorded as percentage of inhibition.

Hydrogen peroxide assay of both tea tree oil and clove oil showed a 31% and 22% inhibitory activity, respectively. 600 µg of tea tree oil and clove oil showed 29% and 57% of inhibition, respectively. From the results, it is evident that both oils scavenged free radicals and their ability to scavenge the free radical in antioxidants activity was approximately the same.

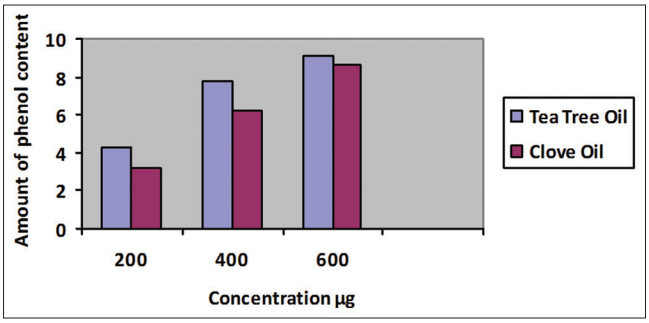
Table 1: Phytochemical test on tea tree oil and clove oil

S. N	Phytochemical tests	Tea tree oil	Clove oil
1	Carbohydrates test	+	+
2	Tannins test	+	+
3	Saponins test	Weakly +	Weakly +
4	Flavonoids test	–	–
5	Alkaloid test	–	–
6	Quinones test	+	+
7	Glycosides test	–	–
8	Cardiac glycosides test	+	+
9	Terpenoids test	+	+
10	Phenols test	+	+
11	Coumarins test	–	–
12	Steroids and phytosteroids	Steroids	Steroids
13	Phlobatannins test	–	–
14	Anthraquinones test	–	–

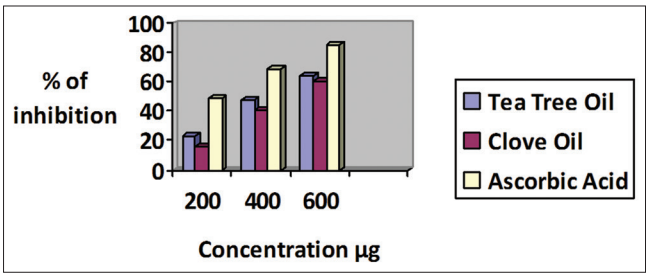
+: Present, –: Absent

CONCLUSION

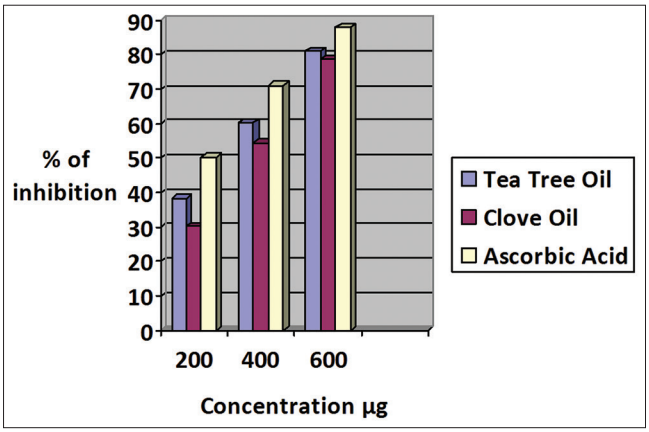
Based on the experiment conducted, it was proven that both tea tree oil and clove oil possess antioxidants activity. According to data



Graph 1: Amount of phenolic content in both tea tree oil and clove oil



Graph 2: The percentage of nitric oxide radical inhibition of tea tree oil and clove oil



Graph 3: The percentage of inhibition of tea tree oil and clove oil through 1-diphenyl 2-picrylhyorazyl scavenging assay

Table 2: Total phenolic content of tea tree oil and clove oil

Total phenolic content					
Concentration (µg)	**Tea tree**	**Clove oil**	**Catechol**	**Phenol content of tea tree**	**Phenol content of clove oil**
200	0.0398	0.0291	1.844	4.31670282	3.156182213
400	0.0691	0.0554	3.565	7.75315568	6.21598878
600	0.0845	0.0794	5.552	9.13184438	8.580691643

Table 3: Nitric oxide scavenging assay of tea tree oil and clove oil

Nitric oxide scavenging assay							
Concentration (µg)	**Control**	**Tea tree**	**Clove oil**	**Ascorbic acid**	**Percentage tea tree**	**Percentage of clove oil**	**Percentage of ascorbic acid**
200	0.784	0.604	0.657	0.405	22.95918367	16.19897959	48.34183673
400	0.784	0.415	0.463	0.246	47.06632653	40.94387755	68.62244898
600	0.784	0.284	0.309	0.118	63.7755102	60.58673469	84.94897959

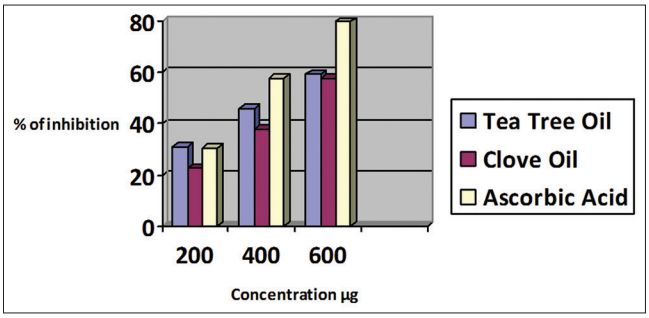
Table 4: DPPH scavenging assay of tea tree oil and clove oil

DPPH scavenging assay							
Concentration (µg)	**Control**	**Percentage of inhibition**					
		Tea tree	**Percentage of inhibition**	**Clove oil**	**Percentage of inhibition**	**Ascorbic acid**	**Percentage of inhibition**
200	1.068	0.659	38.29588	0.745	30.24344569	0.53	50.374532
400	1.068	0.425	60.205993	0.486	54.49438202	0.31	70.973783
600	1.068	0.203	80.992509	0.229	78.55805243	0.13	87.827715

DPPH: 1-diphenyl 2-picrylhyorazyl

Table 5: Hydrogen peroxide assay of tea tree oil and clove oil

Hydrogen peroxide assay							
Concentration (µg)	**Control**	**Tea tree**	**Clove oil**	**Ascorbic acid**	**Percentage tea tree**	**Percentage of clove oil**	**Percentage of ascorbic acid**
200	0.582	0.401	0.451	0.405	31.09965636	22.50859107	30.41237113
400	0.582	0.314	0.362	0.246	46.04810997	37.80068729	57.73195876
600	0.582	0.238	0.246	0.118	59.10652921	57.73195876	79.72508591



Graph 4: Percentage of inhibition of hydrogen peroxide assay of tea tree oil and clove oil

obtained, both clove oil and tea tree oil showed approximately same antioxidant activity. They also displayed effective antioxidants activity when compared to the standard antioxidant compound in different antioxidant assays such as nitrogen peroxide, DPPH, and also hydrogen peroxide. Thus, due to their antioxidant activity, they are capable to scavenge the free radicals, which are dangerous for the body. Other than antioxidant activity, tea tree oil, and clove oil also possess other therapeutic benefits such as anti-inflammatory, antibacterial, and anticancer activity. Hence, they can be used in drug formulations, and more research can be carried out in future to explore the medicinal properties of these oils.

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