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GC-MS Chemical Profile, Antioxidant activity, and Sun Protection Factor of Essential oil of Tea Tree *(Melaleuca alternifolia)* and Rosemary *(Rosmarinus officinalis L.)*

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ABSTRACT

The present investigation aimed to determine the oxidative potential, sun protection factor value, and half-maximal inhibitory concentration of rosemary essential oil and tea tree essential oil. These two essential oils have gained popularity as active ingredients in many cosmetic preparations due to their antioxidant activity, whether used individually or in combination. Gas Chromatography-mass spectroscopy is used to identify the presence of different phytochemical constituents in essential oils. The GC-MS (Gas chromatography-mass spectroscopy) chemical analysis of tea tree oil revealed 34 and rosemary oil revealed 35 volatile chemical components with sesquiterpene hydrocarbon, monoterpenes (42.77%), and alcohols (41.01%) as major detected classes. The 2, 2-diphenylpicrylhydrazyl (DPPH) and nitric oxide-free scavenging activity techniques were used to investigate the antioxidant capacity of these oils. It was found that both tea tree and rosemary oil have the potential to slow down skin aging through their anti-oxidative properties using the approach of free radical scavenging activity. The UV spectroscopy method was used to determine the sun protection factor, and the sun protection values of rosemary and tea tree oil were found to be 8.45 and 6.85, respectively. Rosemary oil was an extremely promising tea tree essential oil for anti-aging and sunburn prevention. The study's findings indicated that rosemary and tea tree essential oil can both offer a synergistic sun protection factor effect, antioxidant property, and anti-aging or extra activity of cosmetic preparations.

Keywords: Free radical scavenging activity (FRSA), 2, 2-diphenylpicrylhydrazyl (DPPH), Nitric oxide activity, Sun protection factor, Tea tree essential oil, Rosemary Essential oil.

INTRODUCTION

Essential oils have been around for centuries in many cultures for their medicinal and therapeutic uses and have enhanced lives for thousands of years[1]. Essential oils (EOs) are aromatic oily liquids composed of a complex mixture of volatile compounds and are produced by aromatic plants as secondary metabolites[2]. The use of essential oils as a natural antioxidant is of great interest in cosmetic formulation since most commonly used synthetic antioxidants such as butylhydroxytoluene

(BHT) are suspected to be harmful side effects on human health[5]. The antioxidants are beneficial in two ways: on the one hand, they aid in the declination of active constituents in cosmeceuticals products; on the other hand, antioxidants protect skin damage and slow the aging process by improving skin gleam and minimizing age spots, sun spots, fine lines, and wrinkles[4]. It would seem that vitamins and nutritional factors have an effect on the skin's antioxidative protection and that combining multiple antioxidants at the same time has a synergistic effect[6,7,8]. Sunburn, photodermatoses, hyperpigmentation, photoaging of the skin, and precancerous lesions and cancers are all side effects of UV exposure. The mechanisms discussed in this paper are involved in the formation of these clinical changes in the skin[9]. UV exposure causes skin side effects such as sunburn, photodermatoses, hyperpigmentation, photoaging of the skin, and precancerous lesions and cancers. The mechanisms discussed in this paper are involved in the formation of these clinical differences in the skin[10].

Rosemary *(Rosmarinus officinalis L.)*, from a botanical perspective, this plant is expressive because it contains over 240 active pharmacological and nutritional composites. Antifungal properties of the Rosemary plant have been discovered through pharmacological research. Gas chromatography-mass spectrometry was used to find out the chemical constituents of essential oil (EOs) of rosemary upstanding region collected in Kerman province. Kerman province's rosemary essential yield was calculated to be 3.2 percent. The essential oil included 35 composites that accounted for 98.74% of the total oils[11]. The principal constituents were α-pinene (14.62%), camphor (12.67%), verbenone (10.19%), and 1, 8-cineole (10.63 %)[12]. Carnosic acid, carnosol, rosmarinic acid, and hesperidin dominate these plants' polyphenolic histories. The antioxidant components of rosemary have been identified as cyclic diterpene diphenols, carnosolic acid, and carnosol 2. The antioxidant activity of essential oil is one of the biological properties of highly considerable interest in cosmeceutical formulation.

Tea tree oil (*Melaleuca alternifolia*) is used principally to supplement endeavors to validate its use, and its reputed therapeutic antioxidant properties have been studied *In vitro* and, in some cases, *In vivo*. It is a volatile essential oil derived primarily from the Australian native plant *Melaleuca alternifolia*[13]. TTO is used as the main ingredient in many topical preparations used to treat cutaneous infections due to its anti-aging properties[14,15].

The essential oil is high in triglycerides, free fatty acids, tocopherols, sterols, phospholipids, waxes, squalene, and phenolic compounds. According to top dermatologists, facial oils are the missing piece in traditional beauty routines. Skin moisture levels decline with age, causing dehydration and making fine lines and wrinkles more visible. Oils not only hydrate the skin, but their high antioxidant content protects cells from free radical damage, preventing further ageing[16]. Oils are excellent for delivering desired skin benefits in spot applications[17].

The antioxidant capacity and sun protection factor of tea tree and rosemary oil are primarily attributed to terpinen-4-ol, a major constituent of the EOs (essential oils). As a result, the terpinen-4-ol content was limited to 30% with no upper limit to maximize anti-aging activity. In contrast, a 15% upper limit and no less significant boundary was established for 1,8-cineole, though the reasoning behind this may not have been entirely sound[18]. For many years, cineole was mistakenly thought to be a skin and mucous membrane irritant, which fueled efforts to reduce its concentration in TTO (tea tree oil). This reputation will be built on anecdotal evidence from the past and unsubstantiated statements[8].

MATERIAL AND METHODS

Sample collection and processing

The samples were collected for RMO (Rosemary oil) and TTO (Tea tree oil) testing, and their active chemical ingredients piqued the experimenters' interest due to their antioxidative activity. The essential oils of the tea tree and rosemary plant (leaves) were purchased from natural aroma product private limited New Delhi. As a free experimental sample from Shanghai, China, DPPH (2,2-diphenylpicrylhydrazyl) was obtained. Sigma-Aldrich Chemicals Pvt. Ltd. The naphthyl ethylenediamine dihydrochloride and L-ascorbic acid was given by Limited, USA. All chemicals and solvents were of the highest quality.

Characterization of RMO (Rosemary oil)/TTO (Tea tree oil)

The physical properties of an oil, such as

appearance (color), aroma (odor), specific gravity, saponification number, and acid number, were used to classify it[5].

Saponification number

To determine the saponification value, add one ml of essential oil to 50 mL of ethanol-mixed KOH(potassium hydroxide) solution (0.5N) and reflux for thirty minutes to ensure complete dissolution of all chemicals. After adding one mL of phenolphthalein indicator, the sample solution was titrated with 0.5 N Hydrochloric acid solutions until the solution's color changed. A similar procedure was followed with a blank sample solution. The saponification number was then calculated using the following formula:

Formula for Saponification number=56.1xNormality/ $Weight_{(sample)}$ (Vs-Vb)

Where,

Molecular weight of potassium hydroxide=56.1, Vs= Sample titer value, and Vb=blank titer value.

Acid value

One g of EOs (essential oils) was accurately measured and added to a solution mixture of 25 milliliters of diethyl ether and 25 milliliters of C_2H_5OH (ethyl alcohol) to calculate the acid value. After adding 1 mL of the indicator phenolphthalein, the solution was titrated with 0.1 N potassium hydroxide solution and filled in the burette until the color was obtained[19]. The acid number was determined as the following formula:

Formula for Acid value = 56.1(molecular weight of KOH) x Normality x Volume/Mass

GC-MS analysis of TTO/RMO

The chemical constituents present in the EOs (essential oils) samples were identified using GCMS apparatus (Agilent-5973, Aligent Technologies Inc., USA); an ABINNO Wax capillary Colum (30 m length, Inner diameter 0.25mm & 0.25mm film consistency) was used under the following conditions column roaster temperature was 70.0°C & sample injection heat was 250.0°C The gas chromatography operating conditions were as follows: gas carrier flow rate 2.0 mL/min, column temperature programming from 70°C to 270°C at 4°C/min, injector temperatures of 216 and 275°C, respectively, and mass spectroscopy operating process was scanned over the 40.850 m/z range. To differentiate individual oil constituents, the chromatogram data of both EOs element peaks were compared to the standard database from (Wiley 8 database library).

Estimation of oxidative activities for TTO (Tea tree oil)/RMO (Rosemary oil)

TTO/RMO antioxidant activity was examined using the following methods:

- Nitric oxide (NO) assay
- 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay

Nitric oxide free radical scavenging activity:

The ability of antioxidant-containing samples to scavenge nitric oxide free radicals is measured by this assay. At physiological pH, sodium nitroprusside (SNP) spontaneously initiates the formation of nitric oxide, which then reacts with oxygen to form nitrite ions, as measured by the Griess Illosvoy reaction. As a positive control, standard ascorbic acid was dissolved in methanol, just like essential oils. Oil samples and standard concentrations ranging from 10 to 1000 g/mL were mixed with 2 mL of a 10 mM SNP solution prepared in 0.5 mM PBS at pH 7.4. The final solution was incubated at 25°C for 120 minutes. Following incubation, 0.5 mL of oil sample and 0.5 mL of Griess reagent were mixed. In a UV-Visible double beam spectrophotometer, the abs of the colored solution were measured at 546nm in comparison to the control (10 mmol/L SNP in phosphate buffer solution without essential oil or standard sample). The rate of inhibition of nitric oxide free radical scavenging activity by essential oil was calculated using the equation below[20].

$$\text{rate of Inhibits} = \frac{\text{ABS (control)}-\text{ABS (sample)}}{\text{ABS (control)}} \times 100$$

ABS-Absorbance

The percentage inhibition was calculated for each test solution dilution, and a chart with concentration (g/mL) and free radical inhibit percentage was generated to obtain a linear equation. The half-maximal inhibitory concentration value was calculated using the linear equation. The IC_{50} value is defined as the sample concentration required for scavenging 50% of the nitric oxide free radical. The experiment was carried out three times.

2,2-diphenylpicrylhydrazyl (DPPH) free radical scavenging activity

After a few minor adjustments, the DPPH (2, 2-diphenylpicrylhydrazyl) free radical scavenging assay was used to assess DPPH activities in a 96-well microtiter plate. A sample stock solution volume of 100L produced final concentrations of 500, 250, 125, 63, 16, 8, 4, and 2 g/mL after being diluted twice. The positive control was ascorbic acid. Following that, each well received 100 L of DPPH (2, 2-diphenylpicrylhydrazyl) at a concentration of 0.04 percent (w/v). The mixture was blended and incubated for 30 min at room temperature in the dark. A microplate reader was used to calculate the abs value at 515nm. The methanol sample serves as a blank for the test sample. The control well contained a DPPH solution made of methanol. Each well's total volume is 200 L. On each sample, three copies of each test were run. The percentage of inhibition was calculated[21].

$$\text{Inhibition percentage} = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} \times 100$$

The rate of inhibition was calculated for each test solution dilution, and a chart was built to achieve a linear equation, with concentration and rate of inhibition, and the half maximal inhibitory concentration was measured using the equation. The IC_{50} (half maximal inhibitory concentration) value is the sample concentration needed to scavenge 50% of the NO (nitric oxide) assay.

Estimation of SPF (sun protection factor) value

There are two types of *In vitro* SPF techniques. A very simple mathematical equation was developed to replace the *In vitro* method used in methods that measure UV absorption or transmission through hydrogel product films in quartz plates or biomembranes, as well as methods that rely on spectrophotometric analysis of diluted solutions to determine the absorption properties of essential oil agents[22].

The COPILA standard was used to calculate in vitro SPF, which involves calculating the percentage of a product's transmittance over the UV spectra, subjective by the EE weighting factor at a range of absorbance[23].

$SPF_{spectrophotometric}$=(CorrectionFactor),$_{10}$x $\sum^{290}_{320}$ EE(λ) x Intensity (λ) x absorbance (λ)

Where,

EE=Erythemogenic Effect of radiation at a wavelength (λ), I (λ)=Intensity of solar light at wavelength, abs=Absorbanc by a test sample of the standard solution. It was calculated that a standard microemulsion preparation containing 8% homosalate presented an SPF value of 4, calculated by a UV spectrophotometer.

The values for the term (I) λ x (EE) λ are constant, which were calculated, and are shown in Table 1.

Table 1: Value of Intensity (λ) & EE (λ) used SPF calculation

Wavelength	(I)λ x (EE)λ
290	0.0165
295	0.0819
300	0.2912
305	0.3217
310	0.1867
315	0.0778
320	0.0197
Total	1

(I) λ is the sunlight spectra intensity, (EE) λ is the erythemal action spectrum the intensity spectrum & erythemal spectrum are constant

The sample (1 g) was weighed, transferred to a volumetric flask of 100 mL, filled to volume with ethanol, mixed for 15 min, and then filtered through Whatman filters. A 5.0 mL sample was transferred to a 25 mL volumetric flask after being diluted to volume with methanol. The absorption values were measured between 290 and 320nm (every 5nm). Each measurement was taken three times, and the final number represents the average of those three measurements. The Mansur equation was then used to calculate the sun protection factor values for the formulations. The *In vitro* method is replaced by a combination of two relatively simple mathematical equations[24]

RESULTS

TTO & RMO physicochemical characterization

Tea tree oil yield was discovered to be between one and two percent. Tea tree oil, also known as *Melaleuca alternifolia* oil, is a water-soluble essential oil with a distinct camphoraceous aroma and a yellowish to nearly clear color. At 25°C, the acid and saponification numbers of the EOs were 1.01-1.22 (mg KOH/g), 177.56-200.45 (mg KOH/g), and 0.8950-0.9050 g/mL, respectively.

Rosemary oil yield was found to be between 0.5 and 2%. *Rosmarinus officinalis Linn*, a member of the Lamiaceae family, flowering tops and leaves were used to create rosemary essential oil. Colorless to pale yellow transparent liquid of herbal camphoraceous minty aromatic woody balsamic medicinal phenolic essential oil. At 25°C, the acid values of the oil were 0.5-1.12 (mg KOH/g), 185-195 (mg KOH/g), and 0.908g/mL, respectively.

(Gas chromatography-mass spectroscopy) Analysis

A complete GCMS analysis of tea tree and rosemary essential oils was performed on the obtained essential oil.

GCMS analysis of RMO

The gas chromatogram of rosemary oil is shown in Fig. 1(A), and its chemical composition is shown in Table 2. RMO's gas chromatography-mass spectroscopy analysis revealed 35 chemical constituents. Carnosol (28%), terpinen-4-ol (20.6%), β-pinene (10.55%), caryophyllene (2.54%), epi-camphor (9.97%), camphor (7.26%), α-pinene (1.9%), and 1,8-cineole were the most prevalent components of RMO oil. Because of their age-defying and antioxidant properties, these chemicals can reduce or prevent oxidative stress, and they can be used in skincare routines to slow skin aging. As a result, monoterpenes became prevalent, which is why they are important in cosmeceuticals and perfumes[25].

GCMS analysis of TTO

Figure 1(B) depicts the gas chromatogram of rosemary oil, and Table 3 lists its chemical components. TTO's gas chromatogram revealed a total of 34 chemical constituents. The majority of the oil (44.5%) was composed of Cineole-1, 8 (4.51%) Cyclohexanol, 8.78% Terpineol, 2.65% Terpineol-4-ol, 12.11% (10.5%) α-pinene, β-pinene (5.81%) Terpinolene. Because of their anti-aging and antioxidant properties, these substances can be used in skincare regimens to delay skin aging and possibly reduce or prevent oxidative stress. Other elements were present in lesser amounts. The primary goal of the GCMS study was to detect the presence of monoterpenes, oxygenated monoterpenes, alcohols, sesquiterpene hydrocarbons, and diterpenes in essential oils because they are primarily responsible for antioxidant activity, sun protection factors, and skin aging[26].

Table 2: Rosemary oil chemical constituent's details by GC-MS analysis

Sr. No	RT (Retention Time)	Content percentage (%)	Chemical constituents
1	8.81	0.9	α-thujene
2	9.092	1.9	α-pinene
3	9.684	1.26	Camphor
4	9.882	10.55	β-pinene
5	11.221	1.44	Myrcene
6	12.700	1.888	p-cymene
7	13.082	0.344	Limonene
8	14.805	0.444	Linalool
9	16.983	0.324	Citronellol acetate
10	17.578	1.999	0-cymene
11	18.065	20.6	Terpinen-4-ol
12	20.795	1.688	α-terpineol
13	22.892	1.99	Citronellol
14	23.053	0.139	Z-linalol oxide
15	23.645	1.54	Caryophyllene
16	24.686	0.12	γ-terpinene
17	26.777	0.999	Citronellyl Acetate
18	27.139	2.97	Epi- camphor
19	28.001	18.76	1,8 cineole
20	28.575	2.7	rosmanol,
21	29.604	1.77	epirosmanol,
22	30.616	1.44	hesperidin,
23	31.802	0.567	Camphene hydrate
24	33.329	1.345	E-citronellyl tiglat
25	34.045	0.9999	Aromatic oxygenated Monoterpenes
26	34.587	0.67	rosmarinic acid
27	35.297	0.522	Sesquiterpene Hydrocarbons
28	36.165	1.777	(E)- caryophyllene
29	36.255	2.098	isorosmanol.
30	37.113	1.339	Carophyllnol
31	39.291	1.666	Oxygenated Sesquiterpenes
32	40.579	1.700	Citronellyl butyrate
33	47.994	28.0	Carnosol
34	49.073	1.44	Oxygenated Monoterpenes
35	49.165	1.111	Carsonic acid

Table 3: Chemical constituent composition of Tea tree oil by GCMS analysis

Sr. No	RT (Retention Time)	Content percentage (%)	Chemical constituents
1	6.872	1.44	Camphene
2	7.138	1.66	Sabinene
3	7.346	0.344	Limolene
4	8.588	0.22	alpha-phellendrene
5	8.868	10.5	β –pinene
6	9.130	1.33	Verbenone
7	9.576	0.22	Trans-β-ocimene
8	10.476	12.11	α-pinene
9	11.091	5.81	Terpinolene
10	11.895	0.44	Linalool
11	12.881	1.55	α-pinene-epoxide
12	14.278	-	Neo-allo-ocimene
13	16.965	0.4	Trans-myoxide
14	16.395	-	Cis-tagetone
15	17.775	2.33	Camphor
16	17.163	3.55	α- Terpineol
17	18.359	-	Viridiflorol
18	19.897	2.65	Terpinene-4-ol
19	19.936	0.99	B-caryophyllene
20	20.663	1.4	ϒ-terpinene
21	20.536	0.444	Trans-pinocarveol
22	21.595	1.000	α-terpinene
23	22.165	-	Trans-ocimenone
24	23.043	1.44	Aromadendrene
25	23.545	0.22	Citronellyl Formate
26	25.805	0.55	Isopiperitenone
27	29.604	0.22	(E)-caryophyllene
28	30.346	4.51	Cyclohexanol
29	30.616	0.99	Citronellyl propionate
30	31.802	-	Globulol
31	35.136	44.5	1,8 Cineole
32	35.920	1.33	β-Terpineol
33	36.297	1.33	P-Cymene
34	40.073	8.78	Terpineol

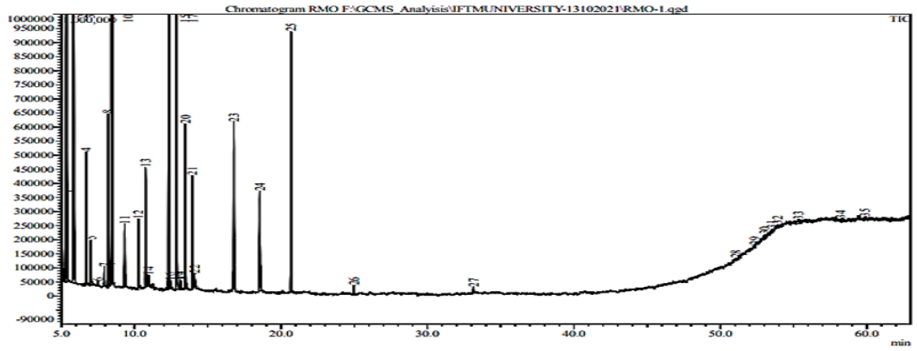

Fig. 1(A). Gas chromatogram of RMO by GCMS analysis

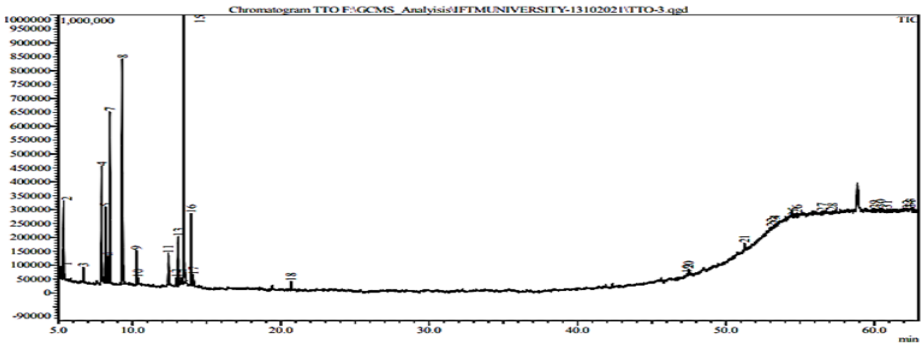

Fig. 1 (B). Gas chromatogram of TTO by GCMS analysis

DISCUSSION

Determination of *In vitro* antioxidant capacity

Significant quantities of essential oils are naturally responsible for antioxidant capacity. We used two techniques in this investigation: To ascertain the antioxidative potency of rosemary oil and tea tree oil

- Nitric oxide assay
- 2, 2-diphenyl-1-picryl-hydrazyl-hydrate assay

Nitric oxide free radical scavenging activity

Table 4 and Fig. 2 (A) show that the nitric oxide radical scavenging of oils ranges between 23.13 and 81.57% and 36.55 and 90.04%, respectively. Tea tree essential oil (23.13%) and rosemary essential oil (36.55%) had the lowest activity at 0.10 g/mL, while at 1.0 mg/mL, tea tree essential oil (81.57%) and rosemary essential oil (90.04%) had the highest activity. At this dose, the EOs have greater than 50% scavenge efficacy, with an IC_{50} value of 0.50 mg/mL (62.76%)[2]. The essential oils TTO and RMO have oxidation properties, as evidenced by the decreased absorbance of the reaction mixture.

2, 2-diphenyl-1-picryl-hydrazyl-hydrate free radical scavenging activity

The free radical scavenging activity (FRSA) of essential oils ranges from 28.42 to 83.34% and 30.96 to 88.44%, according to TTO and RMO measurements. TTO has the lowest free radical scavenging activity of 28.42% at 0.10 mg/mL and the highest at 83.34% at 1.00 mg/mL when compared to regular ascorbic acid. RMO has the lowest (30.96% at 0.10 mg/mL) and highest (88.44% at 1.00 mg/mL) free radical scavenging activity when compared to regular ascorbic acid. The IC_{50} value was 0.25 mg/mL, and the free radical scavenging activity was 63.23%. The results shown in Table 5 and Fig. 2(B) show that the antioxidants' free radical scavenging abilities cause the constant and violet-blue color core to change to a yellowish color. When the results are compared, we can conclude that TTO and RMO essential oils both have beneficial antioxidant properties, and their concentration increases their ability to scavenge free radicals[25].

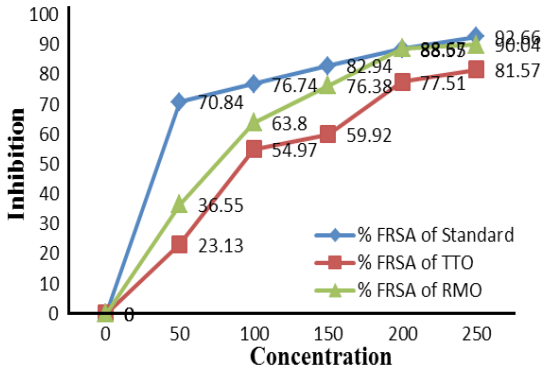

Fig. 2(A). Nitric oxide free radical scavenging assay

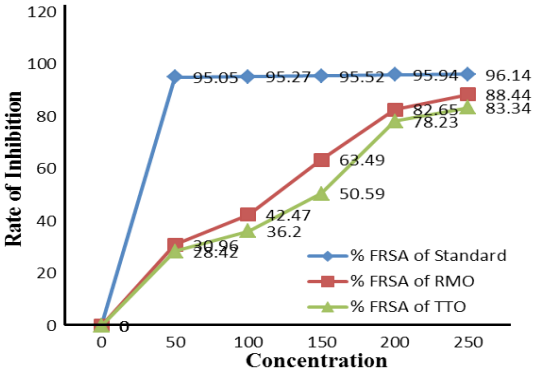

Fig. 2(B). DPPH free radical scavenging assay of TTO & RMO

Table 4: Nitric oxide free radical scavenging activity for TTO & RMO

Concentration (mg/mL)	Absorbance of Blank	Absorbance of standard	Absorbance of (Tea tree oil)	Absorbance of (Rosemary oil)	%FRSA of Standard	%FRSA of TTO	%FRSA of RMO
0	0	0	0	0	0	0	0
0.1	1.677	0.489	1.289	1.064	70.84	23.13	36.55
0.25	1.677	0.390	0.755	0.607	76.74	54.97	63.80
0.5	1.677	0.286	0.672	0.396	82.94	59.92	76.38
0.75	1.677	0.192	0.377	0.190	88.55	77.51	88.67
1.00	1.677	0.123	0.309	0.167	92.66	81.57	90.04

Table 5: DPPH assays of Tea tree oil & Rosemary oil:

Concentration (mg/mL)	Absorbance of Blank	Absorbance of standard	Absorbance of Sample oil (Rosemary oil)	Absorbanceof Sample oil (Tea tree oil)	% FRSA of Standard	% FRSA of RMO	% FRSA of TTO
0	0	0	0	0	0	0	0
0.1	4.024	0.199	2.778	2.880	95.05	30.96	28.42
0.25	4.024	0.190	2.315	2.567	95.27	42.47	36.20
0.5	4.024	0.180	1.470	1.988	95.52	63.49	50.59
0.75	4.024	0.163	0.698	0.876	95.94	82.65	78.23
1.00	4.024	0.155	0.465	0.670	96.14	88.44	83.34

FRSA-Free radical scavenging Activity

Using the DPPH assay technique, the IC_{50} values for Tea tree essential oil and Rosemary essential oil are 0.25 g/mL and 0.25 g/mL, respectively. Tea tree essential oil has half maximal concentrations of 0.25 g/mL and 0.50 g/mL in the Nitric Oxide Scavenging technique. Both essential oils were 50% effective against free radicals at these concentrations[17]. When the results were compared, it was discovered that both rosemary and tea tree essential oils had potent antioxidant properties that increased with concentration.

Table 6: IC_{50} (half maximal inhibitory concentration) Value of Tea tree oil

IC_{50} (mg/mL)	Standard	Tea tree essential oil
Nitric Oxide Scavenging Method	<0.1	0.25
DPPH Scavenging Method	<0.1	0.25

Table 7: IC_{50} (half maximal inhibitory concentration) Value of Rosemary oil

IC_{50} (mg/mL)	Standard	Rosemary essential oil
Nitric Oxide Scavenging Method	<0.1	0.50
DPPH Scavenging Method	<0.1	0.25

Figure 3: IC_{50} (half maximal inhibitory concentration) value of TTO/RMO by DPPH/Nitric oxide activity.

Determination of *In vitro* sun protection factor for TTO/RMO

Sun protection factor determination is a useful test for vetting substances when developing cosmeceutical products. The SPF rating demonstrates the efficacy of the microemulsion. The higher the sun protection factor, the better the formulation's UV protection against light. To be effective in preventing photoaging, sunburn, skin wrinkles, and other skin damage, essential oils must absorb a significant amount of UV-radiation (290-400nm). An examination of the literature revealed a scarcity of studies on how to calculate the SPF of essential oils. This is due to two factors. The first is that essential oils are highly flammable and have very different properties than thick, fatty vegetable oils; as a result, they do not provide adequate sun protection when used alone. Money is another consideration[23]. Plant-derived essential oils contain a wide range of chemical constituents. The percentage of these chemical components varies by harvest and batch depending on the growing conditions of the plants. Due to the conflicting information on essential oils, any SPF study would cost millions of dollars. This is one of the primary reasons why the scientific community has decided against funding essential oil SPF research. The TTO and RMO SPF values were 6.85 and 8.45, respectively, according to the current investigation (Table 4). It was discovered that the evaluated essential oils have low SPF ratings for the antioxidant agent primarily used for producing defense against oxidative stress and combating the bothersome free radicals that frequently accompany prolonged sun exposure. Even through this, they always provide numerous skin benefits[26].

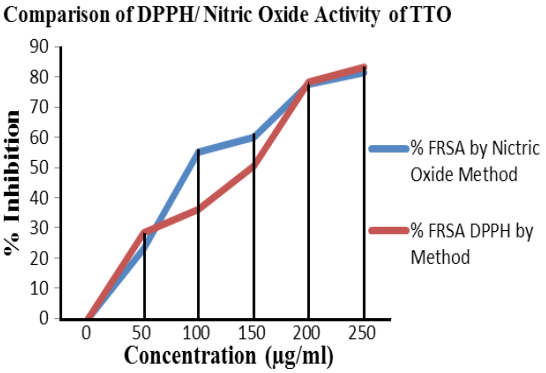

Fig. 3(A). Comparison of DPPH/Nitric Oxide Activity of TTO

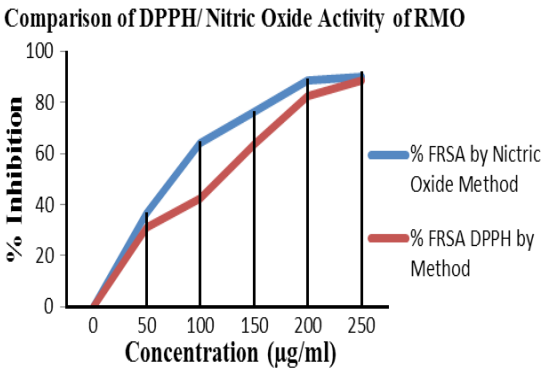

Fig. 3(B). Comparison of DPPH/ Nitric Oxide Activity of RMO

Table 8: SPF (Sun Protection Factor) Value of TTO/RMO

Wavelength (nm)	Absorbance of TTO	Absorbance of RMO
290	0.905± 0.08	0.955± 0.14
295	00867± 0.11	0.939± 0.08
300	0.785± 0.07	0.899± 0.16
305	0.630± 0.16	0.830± 0.19
310	0.523± 0.09	0.799± 0.18
315	0.445± 0.07	0.732± 0.12
320	0.433± 0.11	0.675± 0.14
Calculated Sun Protection Factor	6.85	8.45
n = 3		

CONCLUSION

According to the present research work, tea tree and rosemary oil can prevent or reduce oxidative stress and can be used for anti-aging purposes due to their anti-oxidative properties. The essential oil of TTO and its majority chemical constituent 1, 8 cineole, terpineol-4-ol, β-pinene α-pinene, and above all rosemary essential oil and its chemical constituent's carnosol, camphor, terpinen-4-ol, are active against *In vitro* free radical scavenging activity & skin aging. The sun protection factor results show that tea tree oil/rosemary oil can be used to make microemulsions that protect the skin from sunburn. From the result, it can be concluded that Tea tree oil had a lower sun protection factor than rosemary essential oils. TTO and RMO essential oils have good antioxidant properties and their free radical scavenging activities (in DPPH and Nitric oxide scavenging methods) increase with concentration and have a synergistic photo-protective effect on exposure to UV light.

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