ASSESSMENT OF GERANIUM OIL FOR THEIR ANTIFUNGAL AND ANTIOXIDANT POTENTIAL EXTRACTED BY DIFFERENT TECHNIQUES

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ABSTRACT:
The present work aimed to evaluated the antifungal and antioxidant potential of geranium oil extracted by different methods simultaneously. The geranium oil was extracted by Clevenger method, solvent extraction by soxhlet apparatus and microwave assisted hydrodistillation method. The percent yield oil obtained by microwave assisted method was found to be more than clevenger method. Solvent extraction method produced less amount of oil. The samples were further studied for their antifungal action, which was found better in oil obtained by clevenger method. The same samples when analyzed for their antioxidant potential, showed comparatively higher value. The study also involved development and validation of HPLC method for determination of geraniol constituent in oil samples. It was concluded that oil extracted through clevenger method exhibited better antifungal and antioxidant action. The developed and validated method for evaluation of geraniol was found to be precise and accurate and robust, also utilized in routine analysis of raw material.

KEYWORDS:
Geranium oil, essential oils, antioxidant, antifungal, HPLC, development, validation, geraniol.

1. INTRODUCTION

Fungi and yeast may cause a variety of skin diseases, which are collectively known as dermatomycoses. *Trichophyton* species produce the dermatophytoses, which are a collection of frequent fungal diseases of the skin and nails caused by various distinct fungus and categorised by the location on the body. Ringworm, often known as *tinea*, is a dermatophyte infection. *Tinea capitis*, *tinea barbae*, *tinea corporis*, *tinea cruris*, *tinea manus*, *tinea pedis*, and *tinea unguium* are among the fungal infections caused by dermatophyte[1]. Dermatophytes are moulds (fungi) that rely on the protein keratin for their survival. The outer layer of human skin is made up of keratin, which is a structural substance. Hair and nails are made up primarily of this substance. Dermatophytes must feed on skin, hair, or nails to thrive. The symptoms of a dermatophyte infection differ depending on where the fungal infection is located. The infected spots are usually

moderately irritating with a scaling, slightly elevated border and little or no inflammation. These patches might appear and disappear at any time. In certain cases, the inflammation is more severe, resulting in big and small fluid-filled areas (typically on the foot) or a swollen, inflammatory patch on the scalp that occasionally bleeds pus (kerion).

Essential oils are volatile aromatic liquids collected from different parts of plants such as root, flower, leaves, bark, fruits, peels and seeds or whole plant [2]. The use of these essential oils have been decreased from last few years. Now-a-days, herbal medicinal system gaining the value due to reduced side effects. The essential oils are found to be useful in cooking to enhance the taste and health benefits of food, in the manufacture of perfumes and cosmetics.Essential oils are defined as mixtures of fragrant materials and odorless materials formed in cytoplasm and are normally present in the form of tiny droplets within the cells [3]. These are the chemically pure compounds, volatile under normal conditions. These are extracted by steam distillation or by mechanical processes. Essential oils are composed of lipophilic and highly volatile secondary plant metabolites, reaching a mass below a molecular weight of 300. Essential oils are used in the cosmetics, for the preparation of soap, detergent, also in perfume industry. It is known to effectively eliminate dead cells and to promote the regeneration of newer, healthier skin, thus benefitting acne-prone skin. Its astringent property helps tighten the skin and to diminish the appearance of the symptoms of aging.Geranium Oil has also been used to promote hair growth by nourishing the scalp and balancing the production of sebum and natural oils. These are also used in pharmaceutics for their potential as medicinal agent [4, 5].

Geranium Essential Oil is known to reduce feelings of stress, anxiety, sadness, fatigue, and tension, thereby enhancing the general sense of well-being and relaxation, while offering relief to those suffering from insomnia. Its sweet, uplifting floral scent makes it an ideal ingredient in the manufacturing of soaps and cosmetics, such as creams and perfumes. Furthermore, the scent of Geranium Oil is known to enhance concentration, improve cognitive function, and balance the emotions as well as the hormones. Its calming and tonic properties are known to regulate several body systems, including the respiratory and circulatory functions. This facilitates functions such as nutrient absorption and digestion, and as a result it improves general health.Linalool, geranyl formate, citronellol, and geraniol are the major constituents of geranium oil. The different constituents present in geranium oil are responsible for different actions different such as antimicrobial [6, 7, 9],antifungal [8- 10], Insecticidal [8], antioxidants [10], anti-inflammatory[11, 12].

The present work was performed to evaluate the geranium oil for their antifungal and antioxidant action. The study also involved the development of simple HPLC method for standardization of geraniol content present in the oil.

2. MATERIAL AND METHOD

2.1 Material:

The geranium oil was procured as gift sample from Amsar Pvt. Ltd., Goa.

Microbial strains

Essential oil extracts were bio-assayed for activity against C. albicans (ATCC 90028; ATCC). C. albicans WAS cultured in Sabouraud dextrose broth (Sigma-Aldrich, St. Louis, MO) and incubated at 37°C.

2.2 Methodology:

2.2.1 Botanical description of raw material

Botanical Name: *Pelargonium graveolens / Pelargonium asperum*

Plant Family: Geraniaceae

Common Method of Extraction: Steam Distilled
Plant Part Typically Used: Leaves
Color: Clear to Pale Yellow/Green
Consistency: Thin
Strength of Initial Aroma: Very Strong
Aromatic Description: Geranium Essential Oil smells floral, fresh, sweet and slightly fruity

2.2.2 Extraction Method

The raw sample of geranium oil was extracted by three different methods-

1) Hydrodistillation by Clevenger apparatus
2) Solvent extraction with Soxhlet apparatus
3) Microwave assisted hydrodistillation

Hydrodistillation by Clevenger apparatus:

Approximately 10 gm of raw material placed inside the 1000 ml round bottom flask. Then 750 ml of distilled water was poured over the roots inside the flask. The Clevenger arm was placed onto of the round bottom flask and tighten together. The whole assembled apparatus was placed in the heating device and supported with clamps. The condenser is attached to the top socket of the apparatus. The pump and chilling device for the cooling fluid is turned on, the temperature of the cooling fluid is set to 20°C. The heater was set on and allowed to reach steady state for 1 hour and then extracted for 4 hr. After extraction was complete the concrete (solvent which contains the extracts) was collected by opening the tap on the Clevenger arm. At first the water was allowed to drain out first and then the oil was collected in a vial. The sample was then weighed to obtain a yield of geranium oil.

Solvent extraction with Soxhlet apparatus

The soxhlet column was packed with a layer of cotton wool, thereafter approximately 10 gm of respective raw material was added, followed by another layer of cotton wool. Approximately 150 ml of hexane solvent in the round bottom flask was placed with few anti-bumping granules. The soxhlet column was placed on top of the round bottom flask and tighten together. An additional 100 ml hexane was poured onto the bed to saturate the bed. The assembled apparatus was placed in the heating device and supported with clamps. The condenser was attached to the top of the lid of the apparatus. The pump and chilling device for the cooling fluid is turned on; the temperature of the cooling fluid is set to 20°C. The heating mantle is also turned on to a setting of 3. Then extraction was carried out for three cycles of 4 hrs each. After each cycle the concrete was collected and fresh solvent was added in assemble. The solvent was then evaporated in a rota-evaporator and then placed in a fume cupboard to remove any residual hexane left in the extract. The sample is then weighed to obtain the yield of geranium oil.

Microwave assisted hydrodistillation

The study involved utilization of domestic microwave oven with wave frequency of 2450 MHz. The dimensions of the PTFE-coated cavity of the microwave oven were 48.5 cm x 37.0 cm x 29.25 cm. The microwave oven was modified by drilling a hole at the top. A round bottom flask with a capacity of 1000 ml was placed inside the oven and was connected to the Clevenger apparatus through the hole. Then, the hole was closed with PTFE to prevent any loss of the heat inside. Some feed to solvent ratio (0.30; 0.40; 0.50 gm/ml) were placed in the reaction flask and heated by microwave irradiation with various power (300; 450; 600 W) and various extraction time (60; 120; 180 min). The different densities and their immiscibility required that the water and geranium oil be separated from each other by

separating funnel and the excess water be refluxed to the extraction vessel in order to provide uniform conditions of feed to solvent ratios for extraction. The oil was collected in amber vials, dried under anhydrous sodium sulphate and stored at 4° C.

2.2.3 Antifungal Studies:

Determination of MIC:

MIC values were obtained using the tube dilution bioassay following Donaldson et al. (2005) and Eloff (1998). To reduce essential oil volatility and increase solubility, 2% agar (w/v) was added to each broth. Essential oil (20 ml) was serially diluted across five borosilicate test tubes (13 x 100 mm) resulting in final oil concentrations of 5.00, 2.50, 1.25, 0.63, and 0.31 µl/ml. Each test tube was inoculated with 20 ml of microbial broth and controls consisted of test tubes containing 20 ml of the microbial broth without oil. All tubes were incubated as noted above and control and experimental groups were replicated three times. After 24 h, 800 ml of p-iodonitrotetrazolium chloride dye solution (INT) (Sigma-Aldrich, India) was added to each tube. INT is a colorimetric indicator that changes from clear to purple after exposure to CO_2 indicating bacterial respiration, metabolic activity, and growth. Color changes were observed after 30 min and samples in tubes without color change were plated to confirm growth inhibition. Samples of controls also were plated to confirm growth.

MIC was defined as the lowest concentration of essential oil that inhibited greater than 95% growth of the microorganism and the MIC of 0.31 µl/ml with no variation among replicates was considered as highly inhibitory.

Determination of maximum fungicidal concentration (MFC)

The maximum fungicidal concentrations (MFCs) were determined by subculturing of 2 µl from each of the wells showing no growth into microliter plates containing 100 µl of broth per well and further incubation for 72 h at 28 °C. The lowest concentration with no visible growth was defined as MFC indicating 99.5% killing of the original inoculum. Commercial standards, Fluconazole (Sigma), was used as positive control (1–3000 µg/ml) and negative control (DMSO—99.9%) for fungus. All experiments were performed in duplicate and repeated three times for reproducibility.

2.2.4 In-Vitro Antioxidant Assay:

Scavenging Action of 2, 2-Diphenyl-1-Picrylhydrazyl

Stock solution of DPPH was prepared by dissolving 150 mg of DPPH in 500ml methanol. Working solutions were prepared by taking different concentrations of extract with DPPH and ethanol. The change in deep violet color of solution was then analyzed at 520 nm spectrophotometrically and compared with reference standard compound being used was ascorbic acid.

ABTS Radical Scavenging Assay:

ABTS solution (2mM) and potassium persulphate solution (17mM) were prepared separately, mixed and allowed to stand in dark for about 12-16 hrs. This results in ABTS cation radical formation. Different concentrations of extract solution were reacted with above ABTS solution and Absorbance was recorded at 750 nm compared with ascorbic acid as standard reference.

Bleaching Action on Pyrogallol Red by Peroxynitrite Nitrate:

Peroxynitrite was prepared by reacting hydrogen peroxide, nitric acid, and sodium nitrite (2M each) followed by addition of 4 M sodium hydroxide in frozen conditions (at -700 C). Pyrogallolred solution (100µm) was prepared using 100 mM phosphate buffer (pH 7.4). Peroxynitrite solution was then reacted with mixture of extract solution and pyrogallol

solution with immediate vortexing for 15 min. Absorbance was measured at 540 nm and Ascorbic acid was used as standard antioxidant for comparison.

Ascorbate Iron Induced Lipid Peroxidation:

Bovine Serum Albumin (BSA) suspension in phosphate buffer (pH 7.4) was prepared by ultra-sonication. Ferric chloride solution (1mM), extract solution, phosphate buffer (pH 7.4) and ascorbate solution (1mM) were mixed together and incubated at 37°C for 1 hr. After incubation, trichloroacetic acid(10%) was mixed and centrifuged at 1800rpm for 10 min. Equal amount supernatant liquid and thiobarbuturic acid (0.67%), were treated by boiling at 100°C for 20 min. Rapidly cooled and measured the absorbance at 532nm.Atube containing all the reaction mixture except the plant extract was used as control. Blank was phosphate buffer. The percent inhibition was calculated with the formula

$$\text{Percent inhibition (\%)} = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} \times 100$$

2.2.5 Development and validation of HPLC method for Geraniol

2.2.5.1 HPLC Method Development

The determination was done using Agilent Tech. (1100) system. The separation of chromatogram was carried out on column Fortis C18 (100 x 4.6 mm id with 2.5µm particle size) using various solvent systems such as acetonitrile, methanol, water were tried for development of HPLC method for analysis of geraniol. The effect of flow rate was determined by setting flow rates at 0.5ml/min, 0.7 ml/min, 1.0 ml/min, 1.1 ml/min, 1.2 ml/min and 1.5 ml/min. The solution was scanned between the wavelength range 400-200 nm using the UV spectrophotometer.Quantification of drug was estimated by calculating peak are using CHEMSTATION 10.1 software.

Preparation of mobile phase:

Mobile phase was prepared by mixing HPLC grade acetonitrile and water in the ratio of 98:2 v/v. The content was sonicated for 15 min and filtered through 0.45 µm membrane filter. Mixed solvents were degassed and used as mobile phase.

Preparation of reference standard and sample solution:

Geraniol is the main active content present in geranium oil. Therefore geraniol is used as reference standard. 1000 mg of oil equivalent to 10 mg of geranium oil used to prepared standard and sample solution.

2.2.5.2 Method validation parameters

- **Linearity**

The calibration curve was plotted over the concentration range from 2 to 10 µg/ml of geraniol. The aliquots of each solution were injected under the optimized chromatographic conditions. The regression equation and correlation coefficient were evaluated by plotting peak area versus concentration of geraniol.

- **Accuracy**

The accuracy of method was evaluated by the standard addition method. The known amounts of standard solutions such as 80, 100, 120% levels were added to previously analyzed sample solutions. The percent of individual recovery and % RSD at each level for the drug was measured. The solutions were analyzed triplicate in each level.

- **Precision**

Method precision (repeatability)

The repeatability of instrument was tested by repeatedly injecting sample solution of concentration 6 µg/ml. The % RSD should not be more than 2%.

Intermediate precision

Intermediate precision was evaluated through intraday and interday precision. The intraday precision was examined using three different concentrations. The intraday and interday precision was studied by analyzing the corresponding concentration 3 times on the same day and on different days. The results were described in terms of % relative standard deviation (% RSD).

- **Robustness**

The effect of small changes in the composition of the mobile phase were used to examine the robustness of method. In present study the method was examined by deliberate alterations in the chromatographic conditions such as detection wavelength (± 1 nm), flow rate (± 0.1 ml/min) and mobile phase. At 3 altered concentration the robustness study was carried out. The % RSD was determined.

- **Limit of Detection (LOD) and Limit of Quantification (LOQ)**

The limit of detection (LOD) and limit of quantification (LOQ) of the method were determined by visual method on the basis of trial and error. Calculation of LOD and LOQ were carried out from signal-to-noise ratio for etoposide. S/N ratio was determined at various concentration until S/N ratio of LOD was 3 and of LOQ 10.

2.2.5.3 Percent Content of Geraniol in Geranium oil

The determination of percent content of geraniol was carried out using previously developed and validated HPLC method.

3. RESULT AND DISCUSSION:

3.1 Extraction yield:

Among all extraction method microwave method was found to be more effective in terms of yield. Geranium oil was obtained from leaves, results concluded that microwave assisted method was more effective followed by Clevenger apparatus and then soxhlet extraction process (Table 1).

Table 1: Percent yield of geranium oil by different methods

Sr. No.	Extraction methods	% yield of geranium oil
1.	Hydrodistillation by Clevenger apparatus	0.82±1.99
2.	Solvent extraction with Soxhlet apparatus	0.75±1.85
3.	Microwave assisted hydrodistillation	2.05±1.97

3.2 Anti-Fungal Study

The minimum inhibition concentration and maximum fungicidal concentration of geranium oil was found to be 0.21 µg/ml and 150 µg/ml respectively (Table 2). The percent yield of oil obtained through microwave extraction method was found to be higher however, efficiency of oil was found to lower than oil obtained by Clevenger method.

Table 2: Antifungal action of geranium oil

Antifungal Activity	Geranium oil extracted through different methods		
	Hydrodistillation by Clevenger apparatus	Solvent extraction with Soxhlet apparatus	Microwave assisted hydrodistillation
MIC in µg/ml for each method against C.Albicans	0.21±1.99	1.09±1.32	1.18±1.36

MFC in µg/ml for each method against C.Albicans	150	180	180

3.3 Antioxidant Study

Antifungal studies concluded that, oil obtained by Clevenger method was found to be more potent than the oil extracted by solvent extraction method and microwave assisted method. Based on the above results oil extracted through Clevenger method was selected further for evaluation of anti-oxidant potential. Overall antioxidant activity was found to be very good given in table 3 and figure 1.

Table 3: Data on Antioxidant action

Sr. No	Activity	IC50 value in µg/ml
1.	DPPH	1.65±0.02
2.	ABTS	1.721±0.21
3.	Pyrogallol red	1.69±1.42
4.	Lipid peroxidation	1.92±1.75

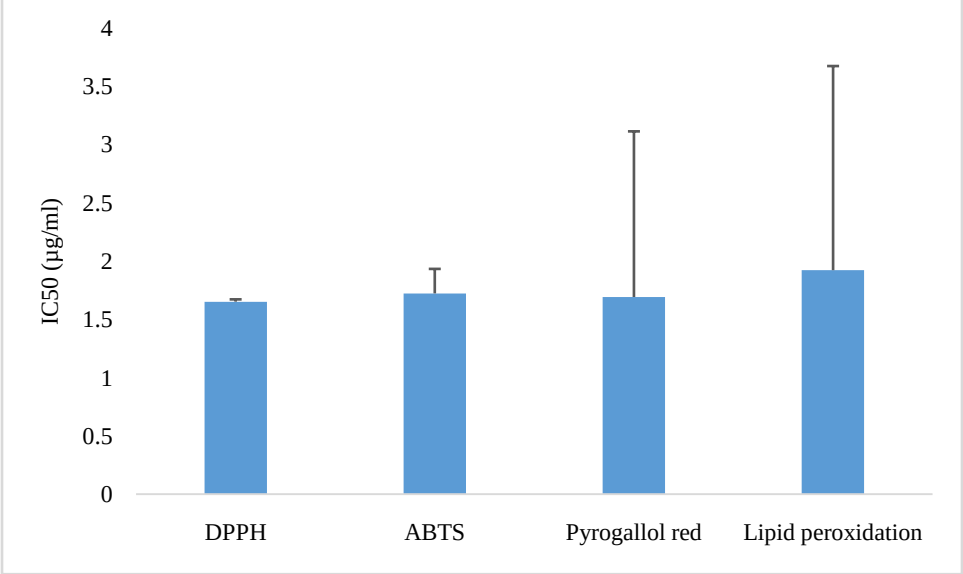

Figure 1: Determination of IC_{50}

3.4 Development and validation of HPLC method

3.4.1 Optimization of RP- HPLC method

The optimization of the RP-HPLC chromatographic parameters were carried out by using different compositions of mobile phase and flow rates. The separation was carried out on Agilent Tech. (1100) system using acetonitrile and water in the ratio of 98:2 v/v with the flow rate 0.7 ml/min as it gave well resolved peak. Based on peak area quantification was carried out at 252nm. The Rt for geraniol was found to be 6.26 min given in figure 2, 3.

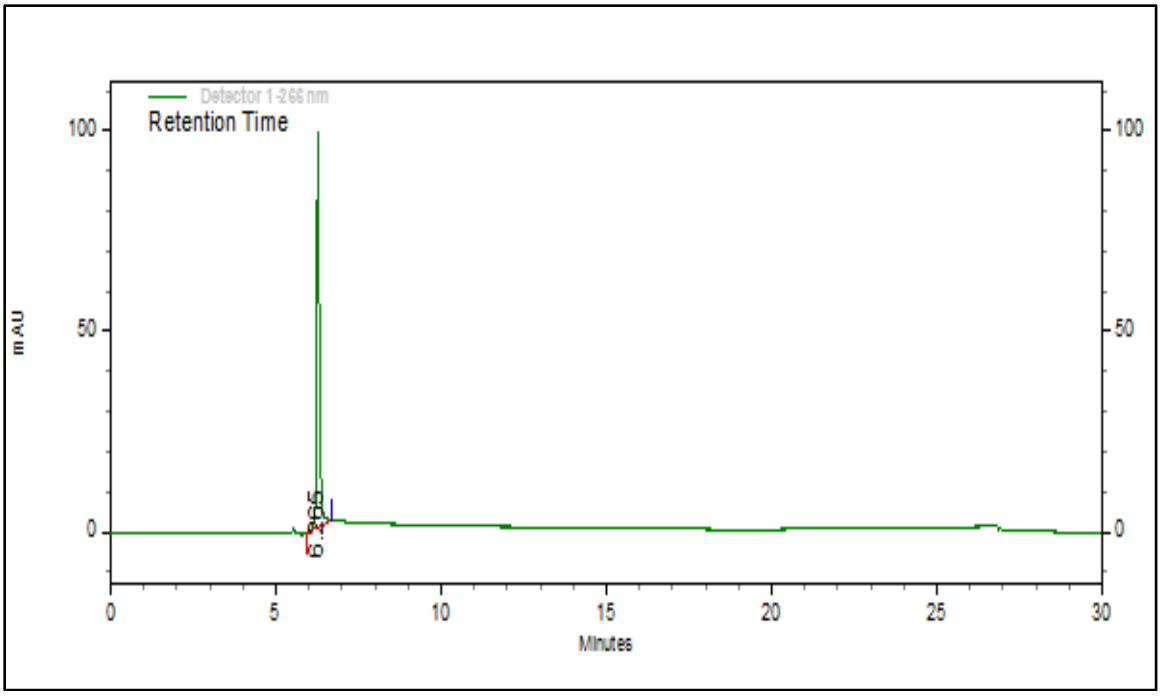

Figure 2: Chromatogram of standard geraniol

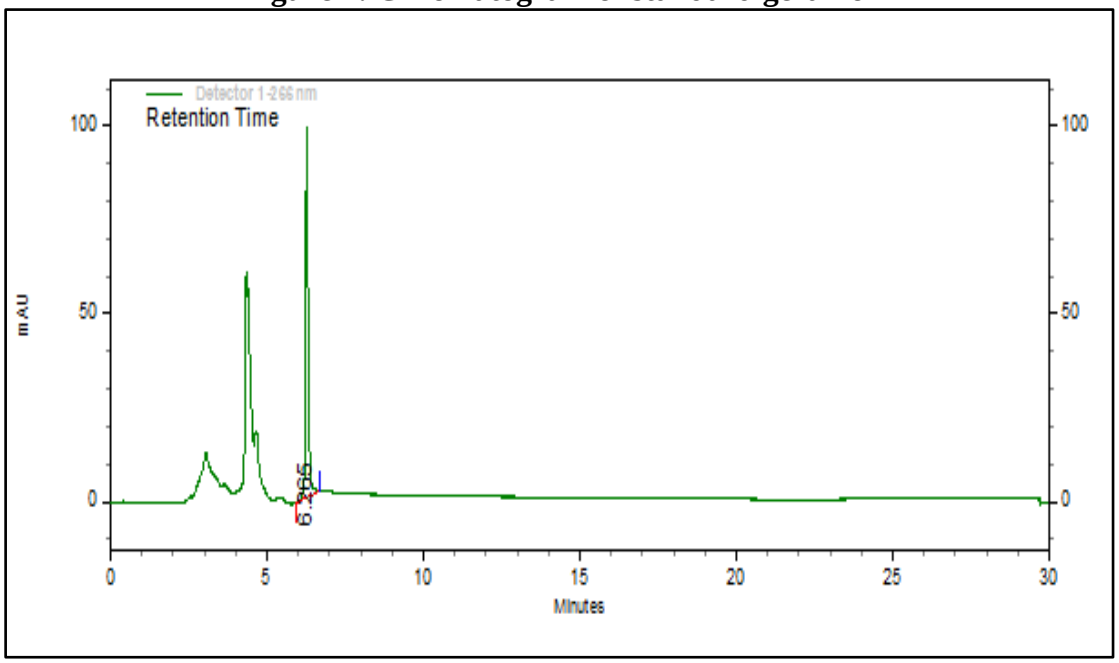

Figure 3: Chromatogram of geraniol in geranium oil

3.4.2 Method validation parameters

International Conference on Harmonisation (ICH) guidelines has been developed by expertise in accordance with the ICH process. This document presents a discussion of the characteristics for considerationduring the validation of the analytical procedures included as part of registrationapplications submitted within the EC, Japan and USA. The document covers the collection of terms, and their definitions, and is not intended to providedirection on how to accomplish validation.

The objective of validation of an analytical procedure is to demonstrate that it issuitable for its intended purpose. The validation of analytical procedures is directed to the fourcommon types of analytical procedures including identification tests, quantitative tests for impurities, limit tests for the control of impurities, quantitative tests of the active moiety in samples of drug substance.

- **Linearity**

The linearity of the method was determined by diluting the standard stock solution to produce the concentration ranges from 2 to 10 µg/ml. The results show excellent correlation existed between peak area and concentration of analyte. By plotting the AUC versus the concentration of analyte, the calibration curve was prepared and analyzed through linear regression (Figure 4).

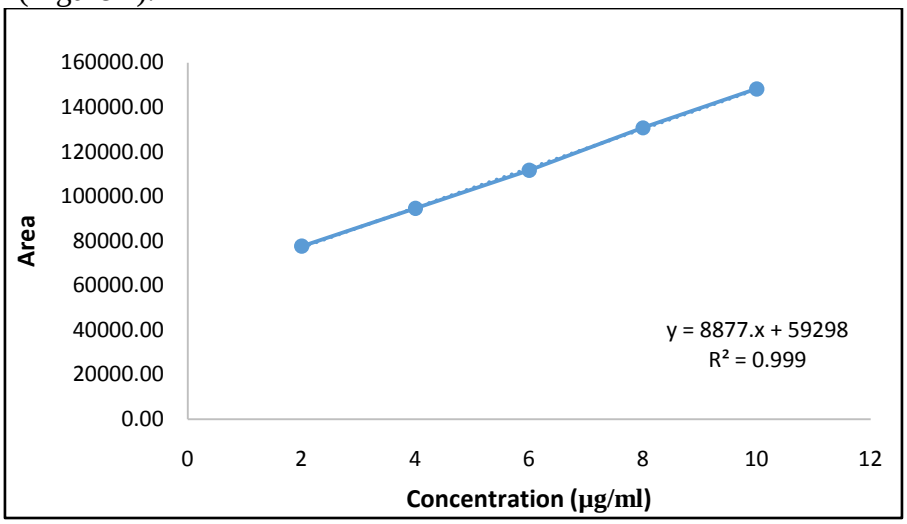

Figure 4: Linearity curve of geraniol

- **Accuracy**

Good recovery study of the drug was carried out at three different concentrations levels indicating the method was accurate. A known amount of standard drug (80, 100, 120%) was added into pre-analyzed sample and subjected them to the proposed HPLC method. The % recovery was found to be within the limits. (Table 4)

Table 4: Recovery data of Geraniol

Level (%)	Drug Conc (mcg)	Amt added (mcg)	Average Amt recovered (mg)	% Recovery	RSD*
80%	6	4.8	4.84	100.81	1.17
100%	6	6	20.58	101.54	1.28
120%	6	7.2	7.16	99.50	0.79

***- N= 3**

- **Precision**

Method precision (repeatability)

The results of repeatability (method precision) are given in Table 5. Method precision was evaluated by repeatedly introducing 6 µg/ml concentration of geraniol. The developed method was found to be precise as % RSD was found to be 1.85.

Table 5: Precision study (repeatability) of geraniol

Conc µg/ml	Area	AVG	SD	% RSD *
6	120252	122452.00	2262.00	1.85
6	124652			
6	123363			

***- N= 3**

Intermediate precision

The results for both intraday and interday were given in Table 6, 7. The analysis of three different concentration (4, 6, 8 µg/ml) of standard solution showed good reproducibility. The % RSD was found to be 0.15, 1.39 and 1.03 for interday precision and 0.42, 1.00, and 0.34 for intraday precision.

Table 6: Precision study (Intraday) of geraniol

Intraday Precision		Area							
Sr. No.	Conc	I	II	III	Mean	Amt Found	% AmtFnd	SD	%RSD*
1	4	92998	93695	93645	93446.00	3.85	96.16	388.78	0.42
2	6	112312	110245	112025	111527.33	5.88	98.05	1119.77	1.00
3	8	131020	130246	130258	130508.00	8.02	100.27	443.45	0.34

***- N= 3**

Table 7: Precision study (Interday) of geraniol

Interday Precision		Area							
Sr. No.	Conc	I	II	III	Mean	Amt Found	% AmtFnd	SD	%RSD*
1	4	92252	92357	92531	92304.50	3.72	92.95	140.91	0.15
2	6	113264	112524	110252	112894.00	6.04	100.62	1569.59	1.39
3	8	130254	132693	132514	131473.50	8.13	101.62	1359.43	1.03

***- N= 3**

- **Robustness**

Robustness was done by small changes in the chromatographic conditions like mobile phase, flow rate and wavelength. It was observed that there were no marked changes in the chromatograms. The developed method was found to be robust as the % RSD values were< 2.0 %. (Table 8, 9, 10)

Table 8: Robustness study with change in flow rate

Flow Rate						
Flow Rate = 0.8 ml/min				Flow Rate = 0.6 ml/min		
Sr. No.	µg/ml	Area		Sr. No.	µg/ml	Area
1	6	121002		1	6	120235
2	6	123107		2	6	120584
3	6	121742		3	6	121568
	Mean	121950.3			**Mean**	120795.7
	SD	1488.46			**SD**	246.78
	%RSD*	1.22			**%RSD***	0.20

***- N= 3**

Table 9: Robustness study with change in wavelength

Wavelength						
Wavelength= 250				**Wavelength= 253**		
Sr. No.	**µgm/ml**	**Area**		**Sr. No.**	**µgm/ml**	**Area**
1	6	123210		1	6	120252
2	6	121441		2	6	121312
3	6	122521		3	6	120441
	Mean	122390.7			**Mean**	120668.3
	SD	1250.87			**SD**	749.53
	%RSD*	1.02			**%RSD***	0.62

***- N= 3**

Table 10: Robustness study with change in mobile phase

Mobile Phase						
Acetonitrile: water (97: 3)				**Acetonitrile: water (99: 1)**		
Sr. No.	**µgm/ml**	**Area**		**Sr. No.**	**µgm/ml**	**Area**
1	6	120121		1	6	121321
2	6	121362		2	6	120251
3	6	120252		3	6	123120
	Mean	120578.33			**Mean**	121564.0
	SD	877.52			**SD**	756.60
	%RSD*	0.73			**%RSD***	0.62

***- N= 3**

- **Limit of Detection (LOD) and Limit of Quantification (LOQ)**

This data showed that the sensitivity of method to determine the geranium oil. The LOD and LOQ was found to be 0.10 µg/ml and 0.30 µg/ml respectively.

3.4.3 Evaluation of Percent Content of Geraniol from Geranium oil

The developed and validated method used for determination of percent content of geraniol in geranium oil. The developed method provided a well resolved chromatogram, with no alterations in peaks of geraniol (Table 11).

Table 11: Percent content of Geraniol

Sr. No.	**Oil obtained by extraction method**	**% Content of Geraniol**
1	Hydrodistillation by Clevenger apparatus	15.02
2	Solvent extraction with Soxhlet apparatus	5.44
3	Microwave assisted hydrodistillation	8.07

4. CONCLUSION

Essential oils are a natural oils typically obtained by distillation and having the characteristic odor of the plant or other source from which it is extracted. Essential oils are used in the aromatherapy and act as antioxidant, antimicrobial, antifungal, pain relievers, anxiety, depression. In the field of cosmetics and industries, the essential oils are used rapidly and mostly used in the perfume industries which are growing increasingly. Essential oils are used in the food preservations and many food items.Geranium oil is known as the 'poor-man's rose' and is extracted from the leaves of variouscultivars of Pelargonium species. Geranium oil is rich in antibacterial, antimicrobial, and antiseptic properties which help to heal wound and soothe inflamed skin.The astringent nature and age-defying property of Geranium Oil help to reduce acne break outs, balance oily or congested skin, and tighten skin. The contractions of the skin muscles and tissues are increased by Geranium. The use of antioxidant improves different immune functions exhibiting an important protective role in infections caused by bacteria, viruses or parasites.

The microwave assisted technique (2.05%) yielded a higher percentage of oil than the clevenger method (0.82%). The solvent extraction method (0.75%) yielded less oil. The samples were then tested for antifungal activity, which was shown to be superior in the oil obtained using the clevenger technique.The percent yield of oil obtained through microwave extraction method was found to be higher however, efficiency of oil was found to lower than oil obtained by Clevenger method. The same sample was utilized to assess antioxidant potential, it revealed that it had higher antioxidant potential. It was discovered that oil extracted using the clevenger process has superior antifungal and antioxidant properties. Furthermore the HPLC method was developed and validated for determining the geraniol component in oil sample. The developed and validated method was found to be simple, accurate, robust and cost effective can be used in regular raw material analysis.

The overall study concluded that Clevenger method was found to be better than solvent extraction by soxhlet apparatus and microwave assisted hydrodistillation method, the oil extracted by clevenger method shown better antioxidant and antifungal action. Furthermore, the evaluation of geranium oil by analytical techniques determine one of the main component found to be higher 15.02% in clevenger method.

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