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PHYSIOCHEMICAL CHARACTERISATION OF ESSENTIAL OILS FROM *POGOSTEMON BENGHALENSIS* (BURM.F.) KUNTZE. AND *P. CABLIN* (BLANCO) BENTH.

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ABSTRACT

Pogostemon benghalensis and *Pogostemon cablin* belongs to the family Lamiaceae and are well known for their essential oil (Eos) content. Essential oils are volatile, hydrophobic liquid in nature with complex mixtures of terpenes and its derivatives biogenerated by the mevalonate pathway. The essential oils from the plants are used traditionally as well as for therapeutic purpose. The *P. cablin* is widely cultivated for the production of Patchouli oil and has got remarkable market value while *P. benghalensis* is an underexploited plant species. The essential oils from the fresh leaves of plants were isolated by hydro-distillation method using Clevenger type apparatus. 0.383% and 0.290% essential oils were obtained from *Pogostemon benghalensis* and *P. cablin* respectively. The Eos were subjected to various physiochemical analysis include colour, odour, viscosity, specific gravity, carbon residue, refractive index, optical rotation and acidic value. The significant result obtained in the physiochemical studies of essential oil, suggest the title plants for pharmaceutical, therapeutical and perfumery industries.

KEYWORDS: Essential oils, *Pogostemon*, Hydrodistillation, Physiochemical, Specific gravity, Refractive Index.

INTRODUCTION

Herbals are wonders of the nature. Currently pharmaceuticals are in search of finding natural phytochemicals with medicinal properties of plants because of their potent activity against several diseases without any harmful side effects. Several biologically active therapeutic

compounds have been identified from the plant species which are used to cure various disorders. Essential oils (Eos) are secondary metabolites obtained from leaves, bark, roots, stem and flowers of many angiosperm plant species. Many Lamiaceae species are used for the extraction of essential oil. These oils have been used for centuries in aroma therapy and also healing effects upon various ailments. The synergetic effect of the active volatile compounds present in these oils is responsible for its characteristic medicinal aroma and therapeutic properties. Essential oils are used in a wide variety of consumer goods such as soaps, detergents, toilet products, perfumes, cosmetics, pharmaceuticals, confectionery food products, soft drinks, distilled alcoholic beverages and insecticides. In the present study *Pogostemon benghalensis* and *Pogostemon cablin* were used for the extraction of essential oil. Both plants are belongs to the family Lamiaceae comprising about approximately 150 species. This genus is a native to tropical Asia and widely grown in India, Malaysia, Philippines and Indonesia.[1] *Pogostemon cablin* is widely cultivated for the production of Patchouli oil. Patcholuli oil is used in perfumes and cosmetic industries. The oil was proven for its medicinal properties like antimicrobial, antiseptic, insecticide, sedative and diuretic.[2] In this juncture Eos from the two *Pogostemon* species were extracted by hydrodistillation method and their physiochemical properties were analysed.

The physiochemical characteristic of the oil is a basic tool to evaluate the quality of oil and based on the data the oil is used for various biochemical analyses. The physiochemical parameters like colour, odour, viscosity, specific gravity, carbon residue, refractive index, optical rotation, acidic value were analysed. For the commercial production of Eos, analysis physiochemical parameters are pre-requisite.

MATERIALS AND METHOD

Plant Material

Pogostemon benghalensis and *P. cablin* were used for the present study. The plants were collected from the Munnar Hills of Idukki district and Agasthyamala of Trivandrum District. The fresh leaves were used for the extraction of essential oils.

Extraction of Essential oil

250 g of fresh leaves were collected from the plants and were washed thoroughly, chopped into small pieces for maximum production of oils. Chopped leaves were put in a Clevenger type apparatus with sufficient amount of distilled water. The steam carrying the oil were condensed and collected in the measuring column. The leaves were subjected to steam

distillation for continuous 5 h. Since the oil is lighter, immiscible and coloured it is collected as an upper layer in the water column. The oil in the column was collected carefully by eliminating the water content and anhydrous sodium sulphate is added to remove the water content. The oil was stored in air tight amber coloured glass bottles at 4^0C for further studies.[3]

Physiochemical Properties of Essential oils

The physiochemical characters of Eos such as colour, odour, percentage yield, viscosity, specific gravity, carbon residue, refractive index, optical rotation, total acid number, iodine number and saponification value were analysed. The physiochemical parameter provides base line knowledge of Eos.[4]

Colour and odour

The colour of Eos was validated by physical observation in day light as well as in UV light of UV chamber and the odour was detected physically.

Calculation of percentage yield

The percentage of yield is a basic parameter used to evaluate the quantity of Eos obtained from plant tissue and is a useful parameter for large scale production of Eos. The percentage of yield was calculated using the following formula.[15]

% yield = (weight of the oil ÷ weight of fresh leaves used) ×100.

Density

The density of oil is calculated as the ratio of mass to volume. The density of Eos is calculated using the following equation.[6]

Density, D= Mass/ volume

Viscosity

Viscosity is the property of a fluid which opposes the relative motion between two surfaces or is the friction between the molecule of the fluid and the surface. Viscosity of the oil is calculated with the help of a viscometer. The spindle of the viscometer is immersed in the oil. Further the meter is turned on and stable reading on the meter was recorded.[5]

Determination of Specific Gravity

Specific gravity is the ratio of density between the Eos and the reference substance. For the determination of specific gravity of Eos, a clean known volume of the bottle was taken and

weighed (W_0). Then the bottle was filled fully with water and closed the lid with a stopper and weighed (W_1). Similarly, instead of water, Eos is taken in the bottle and again weighed (W_2). The refractive index is calculated using the following formula.

Specific gravity of Eos = $(W_2 - W_0) \div (W_1 - W_0)$

Carbon residue

A known amount of sample was taken in a preweighed silica crucible. Then the crucible was heated strongly till the oil gets completely vaporised in a heating mantle. Then, the crucible was allowed to cool down in a desiccator. Further the weight of crucible was taken and the carbon residue was calculated by the following equation.[5]

Carbon residue (%) = (W1 / W2) × 100

Whereas

W1= Carbon residue in the crucible

W2= Weight of sample

Measurement of refractive index

Refractive index is a measurement of how much light is refracted when it enters from one media to another. Refractive index was calculated based on the Snell's law. Refractive index of Eos was measured using Shimadzu refractive Index detector. A single drop of Eos was placed inside the instrument to read the refractive index. Refractive index of a medium (N) was calculated by the formula

N = c/v

Whereas, c = velocity of light in vacuum (299792458 m/s), v= phase velocity of light in the medium.

Measurement of optical activity

Optical rotation or optical activity is the ability of a substance to rotate the plane of polarized light to left or right and is measured using standard instruments. The rotation of the plane of polarization may be either clockwise, to the right (dextrorotary, +), or to the left (levorotary, -). The direction of rotation was analysed by the instrument, i.e., if the rotation was levo (-) then the light beam moves in anti-clock wise direction and dextro (+) if it was clock wise. Polarimeter was used to calculate the optical activity of Eos.

Total Acid Number (TAN)

The total acid number indicates the acidity of a substance. It is determined by the amount of potassium hydroxide in milligram that is needed to neutralize the acid present in one gram of oil. 1g of Eos was taken and the final volume was made to 50 ml using ethyl alcohol. The mixture was titrated against 0.1N potassium hydroxide (KOH). Phenophthalein was used as an indicator. Total acid number was calculated using the formula.[5]

Acid Number = (V × N × 56.1) ÷ W

Where, V = volume of potassium hydroxide

N = normality of potassium hydroxide

W = weight in gm of sample

Iodine number (Iodine Index)

Iodine number was determined as the total amount of iodine in g used by 100 g of sample. It is the measurement of total unsaturated fatty acid present in the oil. 1g of Eos, 10 ml carbon tetra chloride, 20 ml iodine chloride solution was taken in a conical flask. 20 ml of 10% potassium iodide solution was added to above prepared solution and final volume was made upto 150 ml using distilled water. The resulting solution was titrated against 0.1 M sodium thiosulphate ($Na_2S_3O_3$). Starch was used as an indicator. A blank solution was titrated Simultaneously and reaction was initiated with carbon tetra chloride. Iodine value was calculated by the given formula.[7]

Iodine value = [(A-B) × N ×12.9] ÷ Weight of sample

A = 0.1N sodium thiosulphate required by blank

B = 0.1N sodium thiosulphate required by sample

N = Normality of sodium thiosulphate

Saponification value

Saponification value indicates the mg of potassium hydroxide required to saponify 1 g of fat. The value is generated based on the type and chain length of fatty acid present in the oil. The quantitative analysis was based on the titration reaction. 1 g of Eos was treated with 25 ml of 0.5 mol/L potassium hydroxide in ethanol and was heated for 30 min. Phenolphthalein was used as an indicator. The sample solution was titrated against 0.5M hydrochloric acid. A blank titration was also carried out at the same time. The saponification value was calculated as follows.[5]

Saponification value = [(b - a) ÷ m] × 8.5

Where,

a = 0.5M HCl required (ml) by the sample

b = 0.5M HCl required (ml) by the blank

M= Mass of the sample.

RESULTS AND DISCUSSION

The physiochemical studies were the preliminary analysis to determine the quality of Eos. These preliminary analyses help for future studies on therapeutic and biochemical properties of the Eos. The physiochemical properties include colour, odour, yield, density, viscosity, specific gravity, carbon residue, refractive index, optical activity, total acid number, iodine Value and saponification value were expressed in Table1.

In the present study the Eos form *Pogostemon* species were provided a characteristic medicinal aroma. The colour and odour of the oil was very important in the manufacture of perfumes, cosmetics, soaps items.[8] Also the colour of the oil was an important factor which attract the demand of the oil by the consumers.[9] The oil from *Pogostemon benghalensis* was brownish yellow in colour while oil from *Pogostemon cablin* was pale yellow. Various terpenoid derivatives present in these oils deliver the specific colour. Eos were isolated from different part of plants such as flower, fruit, leaves, twigs, seed.[10] 0.383% and 0.290% yield were noticed from essential oils from *Pogostemon benghalensis* and *P.cablin* respectively. 0.96 ml oil was obtained from 250 g of fresh leaves from *P. benghalensis* and *P.cablin* yielded 0.73 ml. The yield of Eos depends on genotype, ecological variation, temperature, altitude and climatic conditions. Ramzi and Starvoz[11] reported that the winning parameters and dryness significantly affect the essential oil yield. The yield of Eos from *P. benghalensis* was slightly higher when compared with *P.cablin.*

Density of the Eos from *P. benghalensis* was noticed as 0.9450 g/ml while that of *P. cablin* was 0.9295 g/ml. Essential oils commonly have a lower density when compared to water. It was found that the density of oil is inversely proportional to temperature.[12] Viscosity is a property of liquid generated by the friction of molecules in the liquid or to the surface. The relative motion of fluid depends on its viscous nature. In the present study the viscosity was expressed in centipoise (cP) with a value of 125.29 and 114.90 for the *Pogostemon* species studied. The *Jatropha* and *Citrus* seed oils showed a comparable result with the present study.[13] The refractive index and density commonly impart dark colours to the oil.[6] Also the increased density value of oil indicates the presence of resinous aromatic compounds.

These types of resinous compounds as well as terpenoid derivatives of Eos make them unique from the rest of the oils.

Specific gravity is another criterion which determines the quality and purity of the oil.[8] In the present analysis the oils displayed values0.895 and 0.903 at 20^0C for *P. benghalensis* while *P. cablin* respectively. Most of the essential oils had specific gravity less than one and the oxygenated aromatic compounds were one of the factors which determine the specific gravity.[14] The specific gravity of the essential oil of *R. centifolia* was 0.823 at 30^{o}C.[15] The present results were comparable with the early study of Allen[16] with specific gravity values of 0.848 to 0.861 at 30^{o}C and was lower when compared with the study of Lozzi,[17] who obtained the values .0.953 to 0.986 at 20^{o}C.

The carbon residue value of *P. benghalensis* was 1.921% and that of *P. cablin* was 1.825%. It indicated the carbonaceous substance remains after the complete evaporation of volatile substances present in the oil. The refractive index (RI) gave inference on compounds present in the oils as well as the purity.[18] The refractive index also varied with the length of chain and carbon number. Hence it is used to determine the saturation level of essential oils.[19] The measured refractive index was 1.5291 and 1.5486 for *P. benghalensis* and *P. cablin* respectively. Both the Eos showed laevorotatory with values of -0.568 and -7.660 for *P. benghalensis* and *P. cablin* respectively.

The total acid number (TAN) is a measure of acidity of the Eos. It is used for the prediction of quality, age, edibility of the oils.[20] The free fatty acid in the Eos can be indirectly determined by the total acid number. It is a measure of total acid present in the oil.[21] The free fatty acid composition is inversely proportional to the quality of oil.[22] The KOH molecule was used to neutralise the free acid molecule present in the oil. The total acid value of *P. benghalensis* was 1.36 and that of *P. cablin* was 1.58. The higher TAN value indicates the instability of the oils.

The Eos of *P. benghalensis* yielded an Iodine value of 116.25 while that of *P. cablin* was 108.34. Iodine number was an important tool used to measure the drying property of oil.[23] The unsaturation in the oils is directly correlated with the iodine number and is very useful in the cosmetic production and in various industrial purposes.[24] The iodine value is also used to calculate the rancidity of oil and the level of oxidation by the chemicals or enzymatic

substance.[25] The iodine number of oil is useful to analysis whether the oil is suitable for the production of soap.[23]

The saponification value is positive measure of molecular weight of acidic material in the oil. The lower value indicates the lower molecular weight.[26] The saponification value is an ideal tool for the use of the oils in cosmetic industry for the production of liquid soap, shampoo, shaving creams.[27] The saponification value of *P. benghalensis* found to be 185.12 and that of *P. cablin* was 178.55 which suggest that these oils are suitable for the production of bathing soaps.[8]

Table 1: Physiochemical properties of essential oil.

Sl No	Parameter	*P. benghalensis*	*P. cablin*
1	Colour	Brownish yellow	Pale yellow
2	Odour	Characteristic medicinal odour	Characteristic medicinal odour
3	Percentage Yield (%)	0.383	0.290
4	Density (g/ml)	0.9450	0.9295
5	Viscosity (cP)	125.29	114.90
6	Specific gravity @20^{0}C	0.895	0.903
7	Carbon residue(%)	1.921	1.825
8	Refractive index	1.5291	1.5486
9	Optical activity	-0.568	-9.660
10	Total acid number (mg of KOH/g oil)	1.36	1.58
11	Iodine number	116.25	108.34
12	Saponification value (mg of KOH/g oil)	185.12	178.55

CONCLUSION

The physiochemical analysis of essential oil from *P. benghalensis* and *P. cablin* indicates its remarkable potential value in the pharmaceutical and fragrance industry and helps for further biochemical and *in vivo* analysis.

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