Evaluation of Essential Oil Extracted from *Acorus calamus* Rhizomes and Leaves

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Doi-10.51129/ujpah-2021-31-2(2)

Abstract-Essential oils are volatile complex compounds which are characterized by a strong odour and are formed naturally by aromatic plants as secondary metabolites. The practical value of essential oils in daily life is explored through the study of numerous physicochemical characteristics (*Parthiban* et al., 2011). Physicochemical characteristics indirectly influence the quality of the essential oils. The commercial importance of oils mostly depends on these physicochemical characteristics, which provide baseline data to determine its suitability for consumption. Therefore, the present study was taken to evaluate characteristics of the essential oil extracted from the leaves and rhizomes of *Acoruscalamus* which is a wild aromatic plant species growing in the lower Himalayan region and the essential oil extracted from the plant is used frequently in skin care and aromatherapy to uplift mood and relieve stress. Essential oil was extracted from both rhizome and leaves (Ac R and Ac L) of *Acoruscalamus* and are analysed for characteristics like yield, specific gravity, viscosity, refractive index, acid number, saponification value, etc. Results indicated that that essential oil Ac R is more superior to Ac L in quality enhancing its commercial importance and can be taken up for further studies.

Keywords: *Acoruscalamus* , Specific gravity, Viscosity, Refractive index, Acid number

Introduction

Essential Oils are basically a natural oil, obtained from distillation and having a distinctive smell, of the plant or other sources from which it is extracted. They are rich sources of biologically active compounds (*Bishop* et al., 1997)[5]. Essential oils from a broad spectrum of plant species have shown antinociceptive, anti-inflammatory, antimicrobial, antiviral, antitumoral and antioxidant activities (*Rufino* et al., 2010)[6]. Moreover, recently there has been a profound interest in the antimicrobial characteristics of extracts from aromatic medicinal plants, specifically essential oils.

Essential oils are garnering a lot of attention because of their many uses as antioxidants, antifungals, and antiseptics. The exploration of various properties investigates the practical importance of herbal oils in daily life (Parthiban et al., 2011)[7]. Physical characteristics of oil like colour, specific gravity, specific viscosity, refractive index, acid value, saponification value, ester value, carbonyl percentage, test for phenol and solubility etc. indirectly influence the quality of both essential and fixed oils. The overall productivity of oils is primarily determined by their physicochemical properties, which provide standard data to determine their suitability for consumption. The objective of the study is to evaluate characteristics of the essential oil extracted from the leaves and rhizomes of *Acoruscalamus* commonly known as sweet flag is wild aromatic plant species growing in lower Himalayan region.This perennial herb is common on the banks of streams and in damp marshy places.*Calamus* is an essential herb in Ayurvedic medicine, and it is used as a "rejuvenator" for the brain and nerve system, as well as a digestive system remedy. The rhizomes and leaves of *Acorus calamus* contain aromatic oil that has been used medicinally since ancient times and has been harvested commercially. The rhizomes are thought to have antispasmodic, carminative, anthelmintic, nauseate, nervine, sedative, and stimulant properties and are used to treat epilepsy, mental illnesses, chronic diarrhoea, essential oil Ac R and Ac L extracted dysentery, and abdominal pain. Here we are from Rhizome and leaves which indirectly affects the quality of oil.

The whole *Acorus calamus* plant was collected from a local mark of Dehradun authenticated by Systematic Botany Division, Forest Research Institute, Dehradun, Uttarakhand,

Material and Methods

Preparation of substrate

India. The leaves and the rhizomes were cut into small slices followed by oven drying at 60 °C - 80 °C for 48 hrs. The dried parts were then stored at room temperature and then used for the experiment.

Extraction of oil from leaves and rhizomes of *Acorus Calamus*

600 gm of dried leaves and 300 gm of rhizomes of *Acorus calamus* was subjected to hydro-distillation in a Clevenger's apparatus. The volatile fraction, thus obtained after hydro-distillation for 6 - 8 hours, exhibited two distinct layers: an upper aromatic oily layer and a lower colourless aqueous layer.These layers were transferred into separating funnel with addition of diethyl-ether in it. The upper layer was collected and dried over anhydrous sodium sulphate.

Took the weight of small dry empty beaker and pour the solution into it and then put it over water-heater till all the ether evaporated away and only oil was left. Took the weight of the beaker with oil. By this, we can calculated the amount of oil obtained. The obtained oil was stored at low temperature (4 - 6°C) for further use[8].

Studies of characteristics of extracted oil[9]

Characteristics study of an oil provide a base line for aptness of oil. These characteristics of both oils Ac R & Ac L were studied in terms of colour , Specific gravity, Specific Viscosity, Refractive index, Acid number, saponification number[10,11].

Specific gravity

A cleaned and dried empty pycnometer was taken and weighed.

The pycnometer was filled upto the mark with double distilled water and weighed again.

The weight of water was recorded.

After removing the water the pycnometerwas dried in the oven and filled upto the mark with the essential oil under experimentation.

The weight of pycnometer with oil was calculated using the following formula.

$$\textbf{Specific gravity} = \frac{\textbf{wt. of the oil}}{\textbf{wt. of an equal volume of water}}$$

Specific Viscosity

1. An Ostwald viscometer was cleaned and dried. Ten ml of an oil under experimentation was filled in the bulb of the viscometer.
2. The solution was sucked upto upper mark and the viscometer left as much.
3. The time taken by the solution to percolate down from the upper mark of the viscometer to the lower mark was recorded.
4. The process was repeated by filling the viscometer with pure water. The specific viscosity of the oil was calculated as per following formula:

$$\eta_2 = \frac{\rho 2 \times t2}{\rho 1 \times t1} \times \eta_1$$

where,

ρ_1 = density of water (g/ml)

ρ_2 = density of an oil (g/ml)

η_1= Viscosity of water (cp)

η_2= Viscosity of an oil (cp)

t_1 = mean time flow of water within the mark

t_2 = mean time flow of oil within the mark

Refractive index

Abbe type of refractometer was used to determine the refractive indices of the oils.

The double prisms of the apparatus were cleaned with alcohol and one dropoftheoil was placedbetween them.

1. The prisms were closed by tightening the screw heads and the refractometer was allowed to stand for few minute to equate the temperature of the oils and the apparatus.
2. The alidade of the refractometer was moved backward or forward to get a broader line which was a band of colour.
3. A sharp colourless line was obtained by rotating the screw heads of the compensator. Finally, the line was adjusted in such a way that it fell on the point of intersection of the cross-hairs.
4. The refractive index was head directly on the scales of the sector.

$$\text{Acid number} = \frac{\text{Volume of 0.1 N Alkali consumed}}{\text{Weight of 1ml essential oil}} \text{ X } 5.61$$

Acid number

1. One ml of the essential oil was dissolved in 15 ml of 95 % ethanol in a conical flask.
2. Three drops of 1% phenolpthaline were added to the contents of the flask and it was titrated against 0.1 N sodium hydroxide solution.
3. The first appearance of pink colouration that did not fade with in 10 second was considered as the end point.
4. Another set without oil was also run parallel to treatment and the difference in the amount of alkali used while titrating the treatment and the set without oil gave the amount of alkali consumed for determination of the acid number of the oil.
5. The acid number was calculated by
6. the following formula :

$$\text{Acid number} = \frac{\text{Volume of 0.1 N Alkali consumed}}{\text{Weight of 1ml essential oil}} \text{ X } 5.61$$

Saponification number

1. One ml of the essential oil was taken into a 100 ml saponification flask. Ten ml of 0.5 N alcoholic sodium hydroxide solution was added to the flask and an air cooled glass condenser (1 meter in length and 1 cm in diameter) was attached to it.
2. The mixture was refluxed for an hour on a water bath and then allowed to cool down to room temperature.
3. The contents were the titrated against 0.5 N aqueous hydrochloric acid using 3 drops of 1% phenolpathalin solution as the indicator.
4. Another set, without oil was also run parallel to the treatment set and the difference in the amount of acid consumed for the determination of saponification number of the oil, which was calculated by the following formula:

$$\text{Saponification number} = \frac{\text{Volume of 0.5 N Acid consumed}}{\text{Weight of 1ml essential oil}} \text{X } 5.61$$

Results and Discussion

Extraction of essential from rhizome and leaves is carried out using K Lelvengers apparatus and results of yield are summarized Table -1.

Table-1 Yield percentage of Extracted Oil

Part of the plant used	Initially amount taken (in gm)	Oil obtained (in gm)	% oil yield(w/w)
Leaves (Ac L)	600	3.2850	0.54%
Rhizomes (Ac R)	300	1.9068	0.66%

Table-2 Characteristics of Essential oil of *Acorus calamus*

Parameter studied	Ac L	Ac R
Colour	Dark yellow	Pale yellow
Specific gravity	0.843	0.878
Specific viscosity	0.094	0.104
Refractive index	1.501	1.553
Acid number	14.582	12.756
Saponification number	33.982	35.346

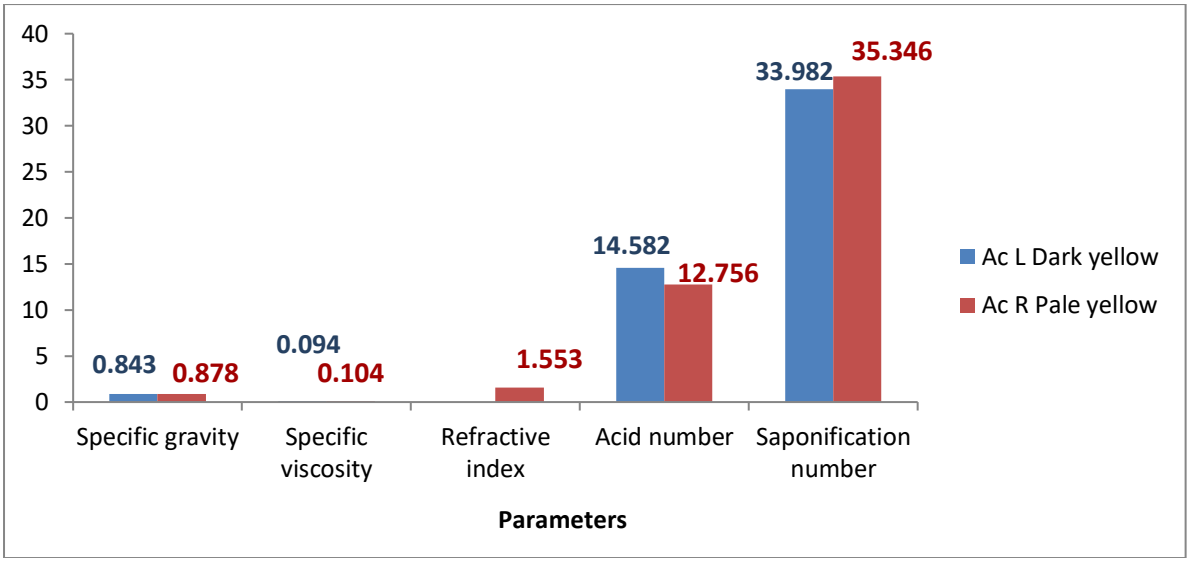

Figure-1 Characteristics of Essential oil of *Acorus calamus* Rhizome and leaves

The essential oil of leaves and rhizomes of *Acorus calamus* was found to be dark-yellow and pale-yellow respectively.Specific gravity is defined as the ratio of the density of a respective substance tothe density of water at 4°C[12]. For most of the findings, specific gravity values of oils are less than 1 except few containing oxygenated aromatic compounds[13]. Our results are little inconsistent with Elert (2000), who described that most of the oils are characterized with specific gravity ranges from 0.9100 to 0.9400. The acid number is the number of milligram of KOH required to neutralize the free fatty acids in one gram of oil. The acid number quantifies the amount of acids in an oil. Acid value is an indirect method for determining the amount of free fatty acid in oil samples and their edibility[14]. Oil with a low free fatty acid content is more widely preferred[15]. The number of milligram of KOH which is required to completely saponify one gram of oil sample is called saponification value[16]. The saponific- ation value of an essential oil of AcR is found be too little bit higher than that of the essential oil of AcL as well as the essential oil of AcR is proved to possess low acid number value than the essential oil of AcL which suggests that the quality of the oil and the suitability for consumption of essential oil from AcR is superior to AcL.

Conclusion

It has been concluded from the above study that the essential oil of Ac R is more suitable for the usage purposes on commercial scale than Ac L. Many research had already been performedwithin the past to assess the traits of essential oil from rhizomes of *Acorus calamus*, however the above study evaluate the characteristics of oil from both leaves and rhizomes and shows that the oil from rhizome is advanced to that of the leaves.

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