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Evaluation of Fungitoxic efficacy and Physicochemical analysis of Essential Oil of *Acorus calamus*

Samuel CO* and Chaudhary Sandeep

Natural Fungicide laboratory, Department of Botany, St. Andrew's P.G. College, Gorakhpur, U.P. (India)
*Corresponding Author - cosamuel5567@gmail.com

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ABSTRACT

The objectives of the study were to determine the fungitoxic efficacy and the physicochemical characteristics of the essential oil extracted from *Acorus calamus*. 500 g of the rhizome pulp was subjected to hydro-distillation using Clevenger's apparatus. The fungitoxic efficacy of the essential oil was tested against twenty-five phytopathogenic fungi by using Poisoned food technique. It showed hundred percent fungitoxicity against six fungi out of twenty-five fungi tested. The physicochemical characteristics of essential oil such as colour, specific gravity, specific viscosity, refractive index, acid value, saponification value, ester value, carbonyl percentage, test for phenol and solubility were also determined. These characteristics are used to determine the quality of extracted essential oil. The oil of *Acorus calamus* was pale yellow and soluble in acetone, ethanol, methanol, benzene and chloroform. The oil exhibited the presence of phenols. The present study indicates that the extraction of essential oil of *Acorus calamus* has a potential of secondary metabolites which can be exploited as a good source of bioactive substances.

Keywords: *Acorus calamus*, Rhizome, Clevenger's apparatus, Essential oil, Fungitoxic efficacy, Physicochemical.

INTRODUCTION

Medicinal plants play an important role from both commercial and healthy lifestyle perspectives. Towards the end of the twentieth century, the World Health Organization (WHO) estimated that an impressive 80% of the world's population probably rely mainly on natural medicines, with plant-originated medicines as the main component of this trend (in developed countries) or tradition (in developing countries) (Aquino *et al.,* 2010). There are two principal kinds of vegetable oils, fixed or fatty oils and essential or volatile oils. Essesitial oil is a fluid of a specific narcotic odor, colorless, highly refractive and easily inflammable. The stain of essential oil on a piece of paper disappears spontaneously. Such oil is generally a mixture of a hydrocarbon with an oxygenated body and the term is used as

a convenient group name for a class of volatile odour bearing substances occurring in various parts of plants and in some animals. It is to these oils that flowers owe their scent and herbs and spices their fragrance.

Essential oils are secreted in different parts of the plant. In scented flowers, such as the rose, they are concentrated in the petals, whereas in spice producing plants the chief deposit may occur in the leaves or bark as in the cinnamon, or in the fruit as in the nutmeg, or in the root as in orris oil.

Volatile oils from a broad spectrum of plant species have shown antinociceptive, anti-inflammatory, antimicrobial, antiviral, antitumoral and antioxidant activities (Rufino *et al*., 2010). Some constituents from oils can act as pro-oxidants (such as free fatty acids and hydroperoxides) or as antioxidants, including tocopherols, phenols, and possibly phospholipids together with other components.

Medicinal plants are the nature's gift to human beings to make disease free healthy life. The medicinal characteristics of plants have been investigated in the recent scientific developments throughout the world, due to their potential activity against several diseases, without side effects and economic viability (Bhanot *et al*., 2011). Several bioactive compounds widely distributed in plants which have been reported to exert multiple biological effect, including antioxidant, anti-inflammatory, anti-carcinogenic etc. (Ganga *et al*., 2011).

Moreover, recently there has been a profound interest in the antimicrobial characteristics of extracts from aromatic medicinal plants, specifically essential oils. Essential oils are volatile complex compounds which are characterized by a strong odour and are formed naturally by aromatic plants as secondary metabolites. They are rich sources of biologically active compounds (Bishop *et al*., 1997).

Thus, plants are a good source of biologically active compounds known as phytochemicals. Phytoconstituents have been found to work as antioxidants by scavenging free radicals and many have curative potential for free radical associated disorders and they also have antimicrobial activity (Molan *et al*., 2012).

Essential oils are gaining remarkable interest for their potential multipurpose use as antioxidant, antifungal, and antiseptic agent; Study of various physicochemical characteristics explores the practical importance of herbal oils in daily life (Parthiban *et al.,* 2011). Physicochemical 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 commercial importance of oils mostly depends on these physicochemical characteristics, which provide baseline data to determine its suitability for consumption. The objective of the study to determine the fungitoxic efficacy and the physicochemical characteristics of the essential oil extracted from the rhizomes of *Acorus calamus.*

MATERIALS AND METHODS

Isolation of Essential Oil

Five hundred grams of rhizome of *Acorus calamus* was surface sterilized by dipping in 2 % sodium hypochlorite solution for 5 minutes and thoroughly washed with sterilized double distilled water followed by pulverization using a sterilized pestle and mortar. The pulp 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. The lower odorless aqueous layer was collected and dried over anhydrous sodium sulphate. The amount of oil thus recovered was noted in terms of per cent recovery on fresh /dry weight basis according to the material used. The oils were stored at low temperature (4 - 6°C) for further use (Essien *et al.,* 2008).

Determination of Fungitoxic efficacy of *Acorus calamus* essential oil

The fungitoxic efficacy of the essential oil extracted from the rhizome of *Acorus calamus* was determined by 'Poisoned Food Technique' (Arora and Dwivedi, 1979). For treatment sets, 1 ml of the essential oil was mixed with 9 ml of molten PDA medium in a pre-sterilized Petri plate and the contents were agitated in a circular mode in order to mix the essential oil homogeneously. In control sets, 1 ml of sterilized double distilled water was added in place of the

essential oil. A fungal disc (5 mm in diameter) cut from the periphery of 7 days old culture of different test fungi with the help of flame sterilized cork borer, served as inoculums. The plates were incubated for 7 days at 27 ± 2°C.

Colony diameters in mutual perpendicular directions were measured on the seventh day in assay plates. Fungitoxicity was recorded in terms of the percent inhibition of mycelial growth and calculated using the following formula (Vincent 1947):

$$Percent\ Inhibition = \frac{dc - dt}{dc} X100$$

Where, dc = average diameter of fungal colony in control sets

dt = average diameter of fungal colony in treatment sets

The experiments were repeated twice and each set contained four replications.

Determination of Physicochemical characteristics of oil:

Physicochemical characteristics provide a base line for aptness of oils (Kimbonguila *et al.* 2010). The physicochemical characteristics of the oil determined were Specific gravity, Specific Viscosity, Refractive index, Acid number, Saponification number, Ester number, Carbonyl percentage, Test for phenols, Solubility in organic solvents.

(a) 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 pycnometer was 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.

$$Specific\ gravity = \frac{Weight\ of\ the\ oil}{Weight\ of\ an\ equal\ volume\ of\ water}$$

(b) Specific Viscosity :

An Ostwald viscometer was cleaned and dried. Ten ml of 1% solution (in acetone) of the oil under experimentation was filled in the bulb of the viscometer. The solution was sucked upto upper mark and the viscometer left as much. The time taken by the solution to percolate down from the upper mark of the viscometer to the lower mark was recorded.

The process was repeated by filling the viscometer with pure acetone. The relative viscosity of the oil was calculated as per following formula:

$$\eta rel = \frac{Flow\ time\ of\ 1\%solution\ of\ essential\ oil\ in\ solvent}{Flow\ time\ of\ pure\ solvent}$$

The value of relative viscosity thus obtained, was converted to specific viscosity by the formula as under:

$\eta sp = \eta rel^{-1}$

Where, ηsp = **Specific viscosity**

ηrel^{-1} = **relative viscosity**

(c) 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 drop of the oil was placed between them. The prisms were closed by tightening the screw heads and the refractometer was allowed to stand for few minutes to equate the temperature of the oils and the apparatus. The alidade of the refractometer was moved backward of forward to get a broader line which was a band of colour. 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. The refractive index was head directly on the scales of the sector.

(d) Acid number:

One ml of the essential oil was dissolved in 15 ml of 95 % ethanol in a conical flask. Three drops of 1% phenolpthaline were added to the contents of the flask and it was titrated against 0.1 N sodium hydroxide solution. The first appearance of pink colouration that did not fade with in 10 second was considered as the end point. 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. The acid number was calculated by the following formula :

$$Acid\ number = \frac{Volume\ of\ 0.1\ N\ Alkali\ consumed}{Weight\ of\ 1ml\ essential\ oil} X5.61$$

(*The weight of 1 ml essential oil of *Acorus calamus* = 0.940 gm).

(e) Saponification number:

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. The mixture was refluxed for an hour on a water bath and then allowed to cool down to room temperature. The contents were the titrated against 0.5 N aquous hydrochloric acid using 3 drops of 1% phenolpathalin solution as the indicator. 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:

$$\text{Saponification number} = \frac{\text{Volume of 0.5 N Acid consumed}}{\text{Weight of 1ml essential oil}} \text{ X 28..05}$$

(* The weight of 1 ml essential oil of *Acorus calamus* = 0.940 gm)

(f) Ester number :

The ester number was calculated using the saponification number and the acid number by the following formula:

Ester Number = Saponification number - Acid number

(g) Carbonyl percentage :

One ml of the oil was taken in 100 ml saponification flask and 35 ml of 0.5 N hydroxylamine hydrochloride solution was added to it. The flask was left overnight at room temperature. The liberated hydrochloric acid was estimated by titrating the contents of the flask against 0.5 N alcoholic sodium hydroxide solution using two drops of bromophenol blue as the indicator. Another set, without oil, was run parallel to the treatment set 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 determining the carbonyl percentage, which was calculated using the formula :

$$\text{Carbonyl percentage}^{*} = \frac{\text{Amount of 0.5 N alkali}}{\text{Weight of the essential oil}} \text{ X Mol. Wt. of Asaronaldehyde}^{**}$$

[* The carbonyl percentage was calculated in terms of Asronaldehyde
** Mol. wt. of Asronaldehyde = 196.2]

(h) Test for phenols :

One ml of the oil was taken in the test tube containing 1 ml of 95 % ethanol. Three drops of alcoholic ferric chloride solution were added to it. The appearance of brown colour indicated the presence of phenols in the oil.

(i) Solubility in organic solvents :

One ml of the oil was taken in a test tube and one ml of the organic solvent was added drop and shaken after each addition. Appearance of the clear solution indicated of the oil in the solvent.

RESULTS AND DISCUSSION

The results recorded in Table - 1 showed that the essential oil of *Acorus calamus* completely inhibited the growth of the six test fungi viz., *Aspergillus flavus, A.niger, A.nidulans, Helminthosporium oryzae, Pyricularia oryzae* and *Rhizoctonia solani.*

Table 1: Fungitoxic Efficacy of Essential oil of *Acorus calamus*

Sl. No.	Test Fungi	Percent Inhibition of Mycelial Growth
1	*Alternari alternata*	72.2
2	*A. solani*	76
3	*Aspergillus flavus*	100
4	*A.niger*	100
5	*A.nidulans*	100
6	*Cladosporium cladosporioides*	52.1
7	*C. herbarum*	59.5
8	*Colletotrichum falcatum*	95.2
9	*Curvularia genicula*	46
10	*C. lunata*	48
11	*Fusarium acuminatum*	70
12	*F.moniliforme*	68.5
13	*F. udum*	73.2
14	*F. solani*	73
15	*Gibberella fujikuroi*	84

Table 1: Continued...

Sl. No.	Test Fungi	Percent Inhibition of Mycelial Growth
16	*Helminthosporium oryzae*	100
17	*Penicillium notatum*	62.8
18	*P. chrysogenum*	52.5
19	*Phytophthora infestans*	48.5
20	*Pyricularia oryzeae*	100
21	*Pythium aphanidermatum*	82.5
22	*Rhizoctonia solani*	100
23	*Rhizopus nigricans*	63.2
24	*Sclerotium oryzae*	54.2
25	*Sclerotium rolfsi*	59.7

Table 2: Physiochemical Characteristics of Essential oil of *Acorus calamus*

Parameter studied	Value
Colour	Pale yellow
Specific gravity	0.886
Specific viscosity	0.105
Refractive index	$n^{25}{}_{D}$ 1.4030
Acid number	11.936
Saponification number	34.319
Ester number	22.383
Carbonyl percentage	48.0*
Test for phenols	Positive
Solubility	Soluble in acetone, ethanol, methanol benzene & chloroform.

Studies of various physicochemical characteristics identify the practical importance and provide basis for suitability and utility of various oils of plants origin in daily life. Physicochemical characteristics of the essential oil like Specific gravity, Specific Viscosity, Refractive index, Acid number, Saponification number, Ester number, Carbonyl percentage, Test for phenols, Solubility in organic solvents (Table -2) indirectly tells about the quality of essential oil.

The essential oil of *Acorus calamus* was pale yellow and dark yellow repectively. The oil was soluble in acetone, ethanol, ethanol, methanol, benzene and chloroform. The oil also exhibited the presence of phenols.

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CONCLUSION

These results indicated that the essential oil of *Acorus calamus* is suitable for the extraction as antifungal purposes on commercial scale. However, further investigation for the identification of bioactive compounds under *in vitro* and *in vivo* conditions is required.

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