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## Formulation of simple syrup from *Acorus calamus* and *Ocimum kilimandscharicum* based on their antioxidant and antimicrobial activity

R. S. A. Sorna Kumar\*, R. Rajeswari, S. Rajeswari, C. V. Jemima Romola, R. Suga Priya, K. Rajeshwari and S. Sukasani

Department of Biotechnology, P.S.R Engineering College, Sivakasi-626140, Tamil Nadu

### ABSTRACT

*Acorus calamus* Linn. is a herbaceous plant with rhizome that is indefinite branched, smooth, pinkish or pale green. The leaf and rhizome part of *Acorus Calamus* possess antibacterial activity. *Ocimum kilimandscharicum* is a short herb, native to India. In traditional medicine, it is used to treat various ailments including colds, coughs, abdominal pains, measles and diarrhoea. The present study was to study the basic properties of *Acorus calamus* and *Ocimum kilimandscharicum* leaf extracts and to formulate a simple syrup and studying its activity on pathogens. The aqueous extracts of the leaves were studied for their total protein, free phenolic and hydroxyl free radical scavenging activity. The extracts and prepared syrup were tested for their antimicrobial activity against *P. aragenosa*, *B. cereus*, *E. coli* and *N. meningitis*. Both the prepared formulations were evaluated for various parameters such as pH, appearance, colour, odour and taste. We found that both the extracts possessed excellent antioxidant and antibacterial activity. The formulated syrup too was found to be effective on clinical pathogens.

**Keywords:** *Acorus calamus*, *Ocimum kilimandscharicum*, simple syrup, clinical pathogens, antioxidant

### INTRODUCTION

*Acorus calamus* Linn., is a herbaceous plant with rhizome that is indefinite branched, smooth, pinkish or pale green. The leaves are few, spongy and distichously alternate, 0.7 and 1.7 cm wide with. The flowers are 3 to 8 cm long, cylindrical, greenish brown and contains multitude of rounded spikes covering it. The fruits are found to be small and berry like with few seeds. *Acorus calamus* is a native of eastern countries and indigenous to the marshes of the mountains of India [1,2]. The leaf and rhizome part of *Acorus Calamus* possess antibacterial activity. The aqueous and alcoholic extract of *Acorus Calamus* showed activity against the bacterial strains of *Salmonella typhi*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus* [3,4]. The plant was also studied for its antidiabetic properties. [5,6]

*Ocimum kilimandscharicum* is a short herb, native to India with green leaves of 2-4cm width and 5-9 cm length. It bears flowers with long flower stems, up to 18 inches long [7]. *Ocimum kilimandscharicum* seeds are black and very small, oval shaped and about 1mm in the middle and 2mm long. In traditional medicine, it is used to treat various ailments including colds, coughs, abdominal pains, measles and diarrhoea. The leaves are also used against viral infections, foul ulcers, anorexia and for healing wounds. *Ocimum kilimandscharicum* is active against a number of bacteria such as *Bacillus saccharolyticus*, *Bacillus stearothermophilus*, *Bacillus thurengiensis*, *Bacillus subtilis*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Micrococcus luteus*, *Sarcinalutea*, *Staphylococcus aureus* etc. [8,9]. It also showed activity against fungal species such as *Aspergillus fumigates*, *Aspergillus niger*, *Candida albicans*, *Cryptococcus neoformans*, *Microsporium cassis*, *Sporotrichum schenkii* [10].

The present study was to study the basic properties of *Acorus calamus* and *Ocimum kilimandscharicum* leaf extracts and to formulate *Acorus calamus* and *Ocimum kilimandscharicum* extract based simple syrup and studying its activity on pathogens.

## MATERIALS AND METHODS

### Collection of sample

Organically grown *Acorus calamus* and *Ocimum kilimandscharicum* plants were collected from local market and leaves were separated and washed in running water and shade dried. These were then powdered and used for further studies.

### Extraction from the leaves

50g of leaf powder was extracted with 50ml of solvent. The extracts were dried in a desiccator and re-suspended in the solvent. 5mg/ml was used as stock solution.

### Antioxidant assay

The total protein and free phenol in the sample was determined by Lowry's method [11] and Folin-Ciocalteu method [12] respectively. Hydroxyl scavenging activity of the extracts were determined at 30 min by Axelrod method [13].

### Preparation of the herbal syrup

**Preparations of simple syrup:** The simple syrup (65.67% w/v) was prepared as per Indian pharmacopoeia. 66.7 gm of sucrose was weighed and added to 1 litre of water. This was heated with occasional stirring until sucrose dissolves. [14].

**Method of preparation of decoction:** 50g of leaf powder was mixed with 400 ml of water and boiled until total volume became one fourth of previous. After boiling the mixture was cooled and filtered. Filtrate was taken to prepare the final herbal syrup [15].

**Method of preparation of formulation:** One part of decoction was mixed with five parts of simple syrup (1:5).

**Organoleptic evaluation** The organoleptic characters such as colour, odour, pH, taste and texture were evaluated based on the method described earlier [16].

### Antimicrobial activity:

Antimicrobial activity of the extracts was determined by well diffusion method. 8 hour old cultures were swabbed on nutrient agar plates and using sterile borer, wells of 3 mm diameter and about 2 cm apart were made on each plates. About 100  $\mu$ l of the plant extracts and prepared syrup were added into the wells was incubated at 37°C for 24 hours. The development of the zone was noticed, whose diameter was measured. The experiment was repeated thrice to confirm the accuracy of the result.

## RESULTS AND DISCUSSION

The aqueous extracts of the leaves were studied for their total protein, free phenolic and hydroxyl free radical scavenging activity. The results obtained are tabulated in table 1.

Table 1: Antioxidant Activity

	Total protein (mg BSAE)	Total free phenol (mg GAE)	% Inhibition of Hydroxyl free radical
<i>A.calamus</i> extract	1.221 $\pm$ 0.418	6.758 $\pm$ 0.824	37.24 $\pm$ 3.42
<i>O.kilimand scharicum</i> extract	1.571 $\pm$ 0.425	6.912 $\pm$ 0.923	41.54 $\pm$ 5.82

*Ocimum kilimandscharicum* was reported for its high free radical scavenging capacity and efficient antioxidant activity using thiobarbituric acid assay in liver and muscle assay systems of ovarian models. This activity is due to abundant presence of flavanoids have great potential to be exploited as antioxidant [17]. The *Acorus calamus* extract was reported to have antioxidant activity and render the protection against  $\gamma$ -radiation induced oxidative stress [18,19].

The extracts and prepared syrup were tested for their antimicrobial activity against *P.aragenosa*, *B.cereus*, *E.coli* and *N.meningitis*. The results obtained are tabulated in Table 2.

Table 2: Antimicrobial Activity

	ANTIMICROBIAL ACTIVITY (mm dia)			
	<i>P.aragenosa</i>	<i>B.cereus</i>	<i>E.coli</i>	<i>N.meningitis</i>
<i>A.calamus</i> extract	2.5	2.7	2.4	2.34
<i>O.kilimand scharicum</i> extract	2.2	3.4	3.1	5.2
<i>A.calamus</i> syrup	5.6	4.4	4.7	5.2
<i>O.kilimand scharicum</i> syrup	4.1	5.5	4.4	3.7

*Ocimum kilimandscharicumis* reported to be active against a number of bacteria i.e. *bacillus subtilis*, *lactobacillus casei*, *staphylococcus aureus*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, and *Pseudomonas aeruginosa* [8,9]. The oil extracted from the plant showed the presence of many antibacterial compounds observed in essential oil such as thymol,  $\alpha$ -cadinene,  $\alpha$ -cedrene, terpinolene linalool, transmethyloisoeugenol, zingiberene, limonene, eugenol, scarvone, cinnamaldehyde,  $\alpha$ -cedrene and terpinenol-4-ol. [20] whereas the leaf and rhizome part of *Acorus Calamus* possess antibacterial activity against strains of *Salmonella typhi*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus* [21]. The activity of *Acorus Calamus* was proposed to be due to the presence of  $\beta$ -asarone [3,22,23]

The formulated syrup was studied for their Organoleptic characters which are tabulated in table 3.

Table 3: Organoleptic Evaluation of Formulation

	Appearance	Colour	Odour	Taste	pH
<i>A.calamus</i> syrup	Translucent liquid	Greenish	Pleasant	Bitter Sweet	6.4
<i>O.kilimand scharicum</i> syrup	Translucent liquid	Greenish brown	Pleasant	Bitter sweet	6.5

All the prepared formulations were evaluated for various parameters such as pH, appearance, colour, odour and taste and further studies are suggested to evaluate the antitussive properties of the formulation.

We found the plant extracts were active against clinical pathogen. Our study also confirmed the reported properties of the sample under study.

### CONCLUSION

The study was done to determine the antioxidant and antimicrobial activity of aqueous extract of *Acorus calamus* and *Ocimum kilimandscharicum*. We found that both the extracts possessed excellent antioxidant and antibacterial activity. The formulated syrup too was found to be effective on clinical pathogens.

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