



**Antimicrobial activity of essential oil from the fruits of
*Amomum subulatum***

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Abstract

The efficacy of seed extracts of *Amomum subulatum* Roxb. activity against bacteria such *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia Coli*. were studied. The minimum inhibitory concentration (MIC) of the extracts was determined using standard methods. Results obtained showed considerable inhibition against the bacteria tested showed considerable resistance at all concentrations of the extract. However the standard antibacterial drug *Erythromycin* exhibit superior activity than the extract. This activity was indicative of the possible means of finding pure active principles from natural source with possible high potency that could serve as a lead to the pharmaceuticals. The low concentration (MIC) activity of the extracts gives credence and scientific base for the claim therapeutic capabilities of *Amomum subulatum* as an anti-bacterial agent. The oil extract yield of the seed extracts was estimated to be 1.2 mL which is pale yellow in colour. The study provides the basis of use of this plant *Amomum subulatum* in treatment of infections caused by pathogens (bacteria) and the phytochemical found are implicated in having anti-bacterial properties.

Key-Words: Anti-bacterial, *Amomum subulatum*, Oil extract

Introduction

Zingiberaceae is one of the essential oil bearing plant families. The genus *Amomum* in this family has over ninety species distributed in Africa, tropical Asia, Australia and the Pacific Islands [1, 2]. These plants are mostly terrestrial, rhizomatous herbs. *Amomum* seeds are used as spices and their plant parts are used in traditional medicine for curing toothache, dysentery, diarrhoea, rheumatism, vomiting, and dyspepsia and lung diseases [3, 4]. *Amomum subulatum* or 'large cardamom' distributed in the eastern Himalayas is the most investigated *Amomum* species. The chemical composition of essential oils from the seeds of *A. subulatum* has been studied by various groups and the major constituent, 1, 8-cineole, has been found to vary from 61 to 86% [5, 6]. Also, oils from different parts of other *Amomum* species such as *A. thyrsoideum* [7], *A. longiligulare* [7], *A. villosum* [8], *A. muricarpum* [9], *A. kwangsiense* [10], *A. schmidti* [11], *A. xanthiooides* [12], *A. tsao-ko* [12], *A. linguiforme* [4, 13] and *A. testaceum* [14] were studied previously.

The cardamom is a native of southern India and grows abundantly in forest 2,500 to 5000 feet above sea level in north canara and wynnaad where it's largely cultivated. Flowering May-April and the fruit gathering in dry weather for the months starting in October [15]. The genus *Amomum* is the second largest genus and comes under the family Zingiberaceae. Large cardamom or Nepal cardamom (*Amomum subulatum* Roxb.) is a large perennial spice crop cultivated in the swampy places in north-eastern and the central Himalayan region of India [16]. The natural colour of cardamom without processing is green or pale buff. If treated with sulphur dioxide it changes to white. Its odour is aromatic, agreeable and pleasant. Strongly aromatic in taste. The cardamom fruits i.e. capsules are about 2cm in length. They are ovoid or oblong, plump, 3-sided sharply beaked at the top with smooth or longitudinally striated surface. Each capsule of the fruit contains three chambers. Each chamber consists of two rows of seeds, about 5 to 10 in number. The seeds are 3 to 4 mm in size, very hard to touch, reddish-brown in colour, and are covered by membranous arid. Seeds are derived from anatropous ovules. They are irregularly triangular in shape,

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covered with transverse wrinkle, known as rugae. The seeds are strongly aromatic in taste and colour. The proportion of pericarp to seeds is 1:3 w/w [17]. Cardamom is a herbaceous perennial with branched underground rhizome, which gives off several erect leafy shoots. The leaves are dark green, lanceolate with an acuminate apex (25-90 * 5-15 cm) and have sheathing leaf bases. The flowers are said to be self-sterile and are borne on long panicles that emerge directly from the rootstock [18]. Large cardamom is essentially a cross pollinated crop due to the heterocyclic nature of its flowers, though they are self-fertile. Each spike bears 40–50 flowers, which open in an acropetal sequence, but only 10–15 capsules are formed per spike. The flowers remain viable for 14 h after opening. They are borne on shortly peduncled spikes of about 5–6 cm in diameter. The number of inflorescences produced on each clump ranges from 20 to 45, depending on the age of the clump. Each inflorescence produces 30–50 flowers. The flowers are yellowish and measure 7.03 cm in length [19]. Cardamom is a humid tropical plant. It is grown under natural conditions of ever green forests at an elevation from 600 to 1500 m above MSL. Optimum elevation is 900 to 1200 m. The plant prefers temperature of 10 to 35°C and a well distributed rainfall of 1500 mm per annum. Under exposed conditions, the plant does not attain its full vegetative growth because of sun scorching. It grows luxuriantly under shade [20]. The plant matures during the third year of its growth. Leaves are green, distichous, simple, linear and lanceolate, glabrous on both side with prominent mid rib. Inflorescence is a condensed spike on a short peduncle bearing 40 to 50 flower buds in an acropetal sequence [21].

Material and Methods

Plant collection and oil extraction

The plant *A. subulatum* were collected from local herbal garden in Dehradun, uttrakhand (India) in june 2013. The sample was authenticated by Botanical survey of India, Northern region centre, Dehradun. The accession no. of the specimen at B.S.I Herbarium is BSD-112741. Hydro distillation of the fresh fruits (600 g) using a Clevenger-type apparatus for three hours yielded 0.8 mL of a pale yellow colour oil, pleasant smelling oil.

Test Organisms

The bacterial microbes used include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Escherichia Coli*. These organisms were clinical isolates obtained from Department of Microbiology, SGRRITS, Dehradun Standard and susceptibility antibiotic discs used was Erythromycin .

Anti-bacterial Activity (Disc –diffusion technique)

The plate whole diffusion assay as described by [22] was used to determine the growth inhibition of bacteria by the seed extract. Nutrient agar was prepared and 25ml each was poured into sterile Petri dish. This was allowed to solidify and dry. The dilution ratio for grams positive and gram negative bacteria were 1:1000 and 1:5000 respectively using peptone water[23]. Using a sterile cock-borer of 9mm diameter three equidistant holes per plate were made in the set agar and were inoculated with 0.5ml overnight suspension of the bacteria. Thereafter, the holes were filled with the oil of seed extract solution, This was done in triplicate and the plates were incubated at 37°C for 18 hours.The antibacterial activities were observed and measured using a transparent meter rule and recorded the zone of inhibition [24].

Minimum Inhibitory Concentration (MIC)

MIC is defined as the lowest concentration where no visible turbidity is observed in the test tube (bacteriostatic concentration). The [25] method as modified [24] was employed. In this method, the broth dilution technique was utilized where the plant extract was prepared to the highest concentration of 500mg/ml (stock concentration) in sterile distilled water and serially diluted(twofold) to a working concentration ranging from 3.12mg/ml to 200mg/ml using nutrient broth and later inoculated with 0.2ml suspension of the test organisms. After 18 hours of incubation at 37°C, the test tubes were observed for turbidity. The least concentration where no turbidity was observed was determined and noted as the minimum inhibitory concentration (MIC) value.

Results and Discussion

Table 1 shows results for antimicrobial activity. No zone of inhibition was seen in control plates and results of the minimum inhibitory concentration of the oil which inhibit the growth of the bacteria under test. The antibacterial screening of the oil showed considerable inhibition against the bacteria *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia Coli*.

Table 1: Antimicrobial study of essential oil of *Amomum subulatum* seeds

Microorganism	ZOI of STD (mm)	ZOI of ASO (mm)
<i>Bacillus subtilis</i>	18	16
<i>Staphylococcus aureus</i>	16	14
<i>Escherichia coli</i>	20	18
<i>Pseudomonas aeruginosa</i>	16	13

ZOI = Zone of inhibition, ASO = *Amomum subulatum* essential oil, STD = standard drug erythromycin

It was observed that the oil exhibited greater inhibition on *Escherichia coli* and *Bacillus subtilis* in comparison with the standard antibacterial drug erythromycin.

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