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Evaluation of Antibacterial, Antioxidant and Phytochemical Analysis of *Amomum subulatum* Roxb. Seed Extract

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A B S T R A C T

Large Cardamom, also known as *Amomum subulatum* Roxb., is a perennial herbaceous crop that is grown in marshy areas on hillsides near streams of water. Since the beginning of time, it has been a well-known spice that is used to flavour many meals that are native to the Eastern Himalayan region, mainly Nepal, Bhutan, and India. In current investigation to evaluate Phytochemical, antibacterial, and antioxidant activities of ethanolic extracts of *Amomum subulatum* Roxb. Seeds. the phytochemical analysis of the ethanolic extract revealed that steroids, glycosides, carbohydrates, proteins, alkaloids, saponins, oils, amino acid, phytosterols, phenolic, terpenoids are present. *Amomum subulatum* (Roxb) seed extract were found to possess antioxidant and in-vitro antibiotic activity against *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus aerogenes*, *Pseudomonas sp*. at high doses, in conclusion, this study has shown that *Amomum subulatum* (Roxb) seed extract contain primary and secondary metabolites that can be pharmacologically useful as well as posses some antibacterial and antioxidant properties.

***Key words*:** *Amomum subulatum* Roxb, Phytochemical, Antibacterial, Ethanolic extracts, Antioxidant properties

Cardamom (Amomum subulatum Roxb.) is a genus of terrestrial, rhizomatous herb that is mostly found in Nepal and India [1]. It is also grown in Nepal and can be found in the hills of Northern West Bengal, Sikkim, and Assam. Cardamom is a priceless spice that is made from the seeds of the Elettaria cardamom perennial plant. Indian coastal areas are where cardamom first appeared. The countries of Nepal, Tanzania, Vietnam, Laos, Sri Lanka, and Bhutan are already growing it [2]. Cardamom comes in two primary varieties: small green cardamom (E. cardamom) and large red/black cardamom (*Amomum subulatum*).

The little green cardamom variety is the most popular, whereas huge cardamom is mostly grown in India, Nepal, and Bhutan. They both belong to the plant family *Zingiberaceae* [3]. The *Amomum subulatum* black cardamom shrub can reach a height of about 3 metres. It thrives in warm, humid climates with lots of rain and fertile soil. It can flourish up to 1370 metres above sea level. The bush needs shade and typically grows in a natural forest environment. After two to three years of age, the tree begins to bloom [4]. Cardamom is rated based on size and colour. The grade increases with the depth of the green and the size of the capsule. Every grade is carefully assigned [5]. The seeds are used in folk medicine to cure indigestion, vomiting, biliousness, abdominal pains, and rectal disorders. They are said to have stimulant, stomachic, alexipharmic, and astringent effects [6]. The seeds are used to cure congestive jaundice, headaches, and gonorrhoea. It has been shown that they help the body eliminate bile. It is a component of Nepali Garam masala spice blends and is also used in pickles, rice dishes, meat curries, and sweet seeds. It has smokey smell and delicious seeds [7].

The current study involves the collection of *Amomum subulatum* Roxb. seed extract from Tiruchirappalli. Phytochemical analysis was then completed using ethanolic solvents, and the effects of *Amomum subulatum* Roxb. seed extract on various pathogenic microorganisms were then assessed, including *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus aerogenes*, *Pseudomonas sp*. The bacteria chosen for this inquiry are prevalent in our environment and are the primary initiators of the majority of routine contaminations. The ethanolic solvents from *Amomum subulatum* Roxb. seed extract also performed in vitro scavenging action.

MATERIALS AND METHODS

Plant collection

In Tiruchirappalli, Tamil Nadu, India, *Amomum subulatum* Roxb. seed were collected, and the taxonomic identification of the collected plant material was confirmed at St. Joseph's College, Tiruchirappalli. Following a double-distilled water rinse, the leaves were exposed to a 0.1 percent mercuric chloride solution. The leaves were then dried for two

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weeks in the shade. The powdered dry leaves were kept for further analysis.

Preparation of extracts

The plant components were extracted using the Soxhlet method using ethanolic solvents. Using 300 cc of the solvents, 50 gram of the powdered seed material were extracted separately over a 48-hour period using Whatman No.1 filter paper. The extracts were subsequently concentrated at room temperature and kept for later use in freezers at 4ºC.

Phytochemical screening of the extracts

Using a common technique developed by Evans *et al.* [8], the selected extracts were qualitatively tested for the presence of several bioactive chemicals.

Antimicrobial activity

Antibacterial activity

The standard well diffusion method [9] using Muller Hinton agar was used to test the extracts' antibacterial activity against four bacterial strains: Escherichia coli, Staphylococcus aureus, Enterococcus aerogenes, and Pseudomonas. The plates were incubated at 37°C for 24 hours.

Antioxidant activity

The described approach was used to evaluate the ability of *Amomum subulatum* Roxb. Seed extracts to scavenge 1, 1-diphenyl-2 picrylhydrazyl (DPPH) [10]. The solvent served as a blank while ascorbic acid served as the standard. At various concentrations (100, 200, 300, 400, and 500 g/ml), test samples were collected. The test tubes were filled with DPPH reagent, and the absorbance was measured at 517 nm.

Calculating percent antioxidant activity is done by multiplying (absorbance at blank) by 100 and subtracting (absorbance at test) from (absorbance at blank).

RESULTS AND DISCUSSION

Collection of Amomum subulatum Roxb. seed extracts and extract preparation

The plant material for large cardamom, *Amomum subulatum* Roxb., was gathered in the Indian district of Tiruchirappalli. The plants were identified at St. Joseph's College in Tiruchirappalli's Department of Botany. The plant seeds were pulverized after drying in the shade. A sample of 50 gm powder was dissolved in 300 ml of an ethanol-based solvent and stored.

Seed extracts were subjected to the standard technique developed by Evans *et al.* [9], for phytochemical screening. Compounds are indicated by + symbols when present, and they are indicated by - symbols when absent.

Phytochemical screening

Amomum subulatum Roxb. seed extract

Experiment	Ethanolic extracts
Alkaloids detection	
a) Mayer's test	+
b) Wagner's test	+
c) Dragendorff's test	+
Carbohydrates detection	
a) Molish's test	+
b) Fehling's test	+
c) Barfoed's test	+
d) Benedict's test	+
Glycosides detection	
Borntrager's test	+
Legal's test	+
Saponins detection	
Glycosides Foam test	+
Proteins detection	
Millon's test	+
Biuret test	+
Amino acids detection	
Ninhydrin test	+
Phytosterols detection	
Libermann-Burchard's	+
Fixed oils and fats detection	
Spot test	+
Saponification test	+
Phenolic compounds detection	
Ferric chloride test	+
Gelatin test	+
Lead acetate test	+
Flavonoids compounds detection	
Alkaline reagent test	+
Magnesium and hydrochloric acid reduction	+
Gum and Mucilages detection	+
Volatile oil detection	+
Terpenoids detection	
Salkowshi Test	+
Phlobatannins detection	-
Ascorbic acid detection	-
Anthraquinone detection	
Lignins detection	-
Resins detection	-
Lactones detection	-
Anthocyanin detection	-
Betacyanin detection	-

Antimicrobial activity

Antibacterial activity

By using a disc diffusion experiment, the in vitro antibacterial activity of ethanolic extracts of various doses of *Amomum subulatum* Roxb was assessed against five bacterial pathogens. Both gram-negative and gram-positive bacteria are present. The presence or absence of the inhibitory zone and the zone of diameter in (mm) correspondingly provided in (Table 2) were used to qualitatively evaluate the antibacterial activity of the plant extracts and their potency.

Disc diffusion method

The in vitro results were seen in the inhibitory zone that formed around each disc as a result of the antibacterial characteristics diffusing into the surrounding media from the plant extract-impregnated discs. Additionally, (Table 2) lists the inhibitory zones created by filter paper discs loaded with ethanol (a negative control) and regular antibiotic discs (Amoxicilin 30 mg/disc) as well. Each sample's inhibition zone diameter was compared to that of a typical antibiotic.

As shown in (Table 1), different concentrations of *Amomum subulatum* Roxb's ethanolic extracts exhibited varying degrees of inhibition against *staphylococcus aureus* (25mm), *E. coli* (24mm), and *pseudomonas aeruginosa* (20mm), while 20g/ml concentrations of *pseudomonas aeruginosa* (15mm), *E. coli* (17mm), and *staphylococcus aureus* showed the least amount of inhibition (18mm). The results of the current investigation showed that seed extract of *A. subulatum* Roxb was effective against gram positive and

gram-negative bacterial pathogens. Each sample's inhibitory zone diameter was compared to that of conventional antibiotics. It was observed that the samples' inhibition zones were either smaller, larger, or equal to those of conventional antibiotics.

Table 1 Antibacterial activity of a sample

Sample	Concentrations (µg/ml)	Organisms/Zone of inhibition (mm)				
		AS-70% ethanol				
		Escherichia coli	*Staphylococcus aureus*	*Enterococcus aerogenes*	*Pseudomonas aeruginosa*	*Proteus vulgaris*
AS-70% ethanol	20	17	18	0	15	0
	40	19	20	0	17	0
	60	21	23	0	19	0
	80	24	25	0	20	0
Ethanol	10 µl/disc	0	0	0	0	0

Table 2 Antibacterial activity of standard

Sample	Concentrations (µg/ml)	Organisms/Zone of inhibition (mm)				
		Amoxicillin				
		Escherichia coli	*Staphylococcus aureus*	*Enterococcus aerogenes*	*Pseudomonas aeruginosa*	*Proteus vulgaris*
Amoxicillin	20	14	16	10	13	15
	40	12	13	12	11	18
	60	10	10	14	9	18
	80	9	8	17	7	16
Ethanol	10 µl/disc	0	0	0	0	0

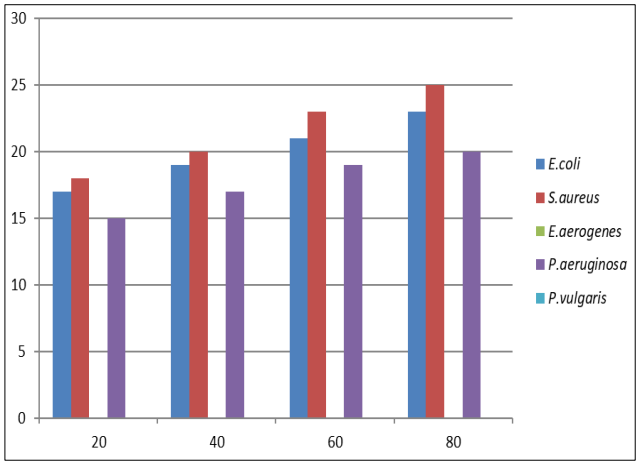

Fig 1 Antibacterial activity of *Amomum subulatum* (Roxb)

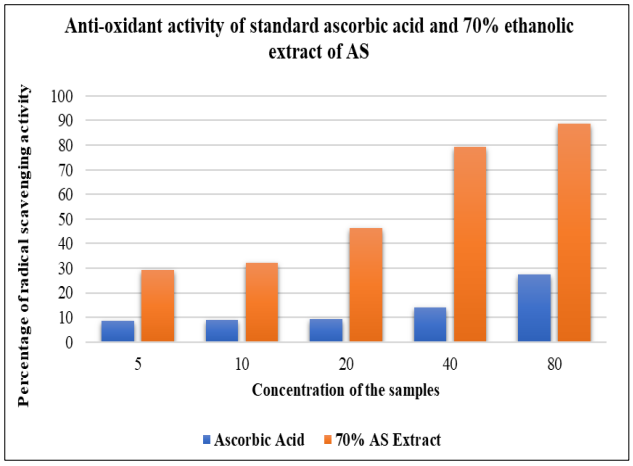

Fig 2 Antioxidant activity of *Amomum subulatum* (Roxb)

Table 3 Antioxidant activity of *Amomum subulatum* (Roxb)

Concentration of the samples (µl/ml)	Initial absorbance (Blank)	Sample absorbance		Percentage of scavenging activity	
		Standard ascorbic acid	70% AS extract	Standard ascorbic acid	70% AS extract
5	1.345	1.227	0.95	8.77	29.36
10	1.345	1.226	0.912	8.84	32.19
20	1.345	1.218	0.724	9.44	46.17
40	1.345	1.154	0.28	14.2	79.18
80	1.345	0.974	0.15	27.58	88.84
IC50 Values				171.173 µl/ml	20.981 µl/ml

Antioxidant activity

Cells are shielded by antioxidants from harm brought on by free radicals, or reactive oxygen species. Plant extracts' anti-oxidant properties are primarily brought about by the presence of phenolic components such flavonoids, phenolic acids, and tannins. Numerous studies have shown that plant

phytochemicals with antioxidant activity can improve health and lower the risk of cancer.

Using the DPPH assay method, *Amomum subulatum* Roxb's methanolic extract and common ascorbic acid were examined for in vitro antioxidant activity. The graph produced by graphing the % scavenging against the applied concentrations was used to calculate the IC50. The outcome is displayed in (Fig 2). Ascorbic acid >seed extracts were shown to have the greatest free radical scavenging action on the DPPH, which was concentration dependent. The IC50 value for the antioxidant activity in the sample was 20.981 g/ml. Ascorbic acid's IC50 value was 171.173 g/ml. The activity of the sample is greater than that of ascorbic acid, the industry standard.

CONCLUSION

The first findings from this study appear to support the use of *Amomum subulatum* (Roxb) in treating a variety of health issues due to its affordability and accessibility, particularly for those living in remote areas. According to the findings of the current investigation, *Amomum subulatum* (Roxb) exhibited potent antibacterial and antioxidant activity. The current study may offer scientific justification for the traditional usage of this plant and may give rise to a useful suggestion for a thorough investigation of the bioactive chemical responsible for these biological features, as well as a potential mechanism of action.

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