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Research Article

Antibacterial Activity of *Alpinia* L. (Zingiberaceae) from Santal and Lodha Tribal Areas of Paschim Medinipur District in Eastern India

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ABSTRACT
The genus Alpinia is an important medicinal plant used by the tribal people of Paschim Medinipur district in Eastern India in various domestic and different medicinal purposes. The rhizome is an important antimicrobial agent and a digestive stimulant. Three most important species are like A. galanga L., Alpinia calcarata Rosc. and A. allughas Rosc., contain various important chemical constituents such as cinol, methyl cinnamate, cyanidin, amino acids, flavones: alpinin, galangin etc. These the species more active against four selected bacterial pathogens are Bacillus cereus, Staphylococcus aureus, Escherichia coli and Salmonella typhi by using agar well diffusion method. The diameter of inhibition zone highest (15mm) in rhizome of A. galanga L. and A. allughas Rosc. All the selected species are display significant role against Staphylococcus aureus.
Key Words: *A. galanga L., Alpinia calcarata Rosc. A. allughas Rosc.; antimicrobial agent, vunerable , biodiversity, conservation, strategies.*

INTRODUCTION

Infectious diseases have been one of the major public health concerns worldwide and account for consdiderably high cases of illness. Recent studies report that *Bacillus cereus*, *Campylobacter jejuni*, *Escherichia coli*, *Salmonella*, *Shigella* and *Staphylococcus aureus* are considered to be the most frequent pathogens [1, 2, 3, 4, 5, 6, 7].

Recently, a number of new drugs were discovered from ethnobotanical leads such as benzoin isolated from *Styrax tonkinensis* used for oral disinfectant and emetine from *Psychotria ipecacuanha* for amoebic dysentery [8]. Treatment failures arising from antibiotic-resistant bacteria [9, 10], stimulation of toxin production [11], together with the recent upturn in consumer mistrust of synthetic additives, it is therefore necessary to search for natural compounds from plants to replace antibiotics or artificial antimicrobials.

In India native herbs are becoming more widely used at a commercial scale in the food industry, mainly for their flavoring properties. Even though certain plants have been demonstrated of their effects against pathogenic bacteria [12] and a number of them have not yet been investigated for their antibacterial activities. Hence, it is essential to establish the scientific basis for their therapeutic actions as these may serve as the source for the development of effective drugs.

Many secondary metabolites of plants are commercially important and find use in a number of pharmaceutical compounds. Human beings have been dependent on plants for their health care needs since the beginning of civilization. Of the 2, 50,000 higher plant species on earth [13], more than 80,000 are medicinal in nature. About 5000 species are extensively used in traditional systems of medicine and are studied in some detail. "*The Red Data Book of India*" has 427 entries of endangered species of which 28 are considered extinct, 124 threatened, 81 vulnerable, 100 rare and 34 insufficiently known species [14].

This plant is cultivated for its rhizome in tropical areas of South and East India. Because of the presence of essential oil, the rhizomes are used in bronchial troubles and as a carminative. It is one of the ingredients of medicated "*Pan*" used for removing the foul smell of the mouth and getting relief in throat inflammation. In Ayurveda, "*Rasna-saptak-kwath*" and "*Rasna-adikamath*" are used as antiinflammatory decoctions. In Unani, it is an ingredient of aphrodisiac preparations, "*Majun*

Mugawivi ma Mumsik", "*Majun Samagh*", and antispasmodic nervine tonic "*Majun Chobchine*" and "*Lubab Motadil*". It is also used in "*Arq Pan*" as a cardiac stimulant carminative [15]. They are also useful in vitiated conditions of *vata* and *kapha*, rheumatoid arthritis, inflammations, stomatopathy, pharyngopathy, cough, asthma, hiccough, dyspepsia, stomachalgia, obesity, diabetes, cephalalgia, tubercular glands and intermittent fevers [16]. Charaka includes rasna (Alpinia) in the *Vayasthapana Varya*, the group of drugs that are capable of maintaining the youthful vigour and strength. The impoprtant part is the rhizome which forms a major ingredient of preparations like *Rasnadi Kasaya, Rasnadi churna, Rasnadi taila, Asvagandharishta, etc* [17]. The drug stimulates digestion, purifies blood and improves voice [18]. The present study was undertaken to assess the potential of medicinal plants as antimicrobial agents against common common pathogenic bacteria. A total of 6 extracts from 3 plant species of *Alpinia* L belonging to the family Zingiberaceae were commonly used in Eastern India to cure bacterial infections. These included *A. galanga* L., *Alpinia calcarata* Rosc. and *A. allughas* Rosc. They were primarily screened for attributed antibiotic properties against selected important group of both gram-positive and gram-negative bacteria. This paper also reports the ethnomedicinal uses of these plants.

MATERIALS AND METHODS

Field surveys

Extensive surveys were conducted in the different rural areas of the West Midnapore. The places included Kharagpur(22.02°N latitude and 87.11°E longitude), Jhargram (22° 26' 59" N latitude and 87° 00' 4" E longitude) and Ghatal (22.67°N and 87.72°E) sub-divisions specially Dantan, Narayangarh, Kashiary, Pingla (22° 16' 1" N latitude and 87° 37' 36" E longitude), Sabang (22° 8' 15" N latitude and 87° 38' 5" E longitude), Amlachati (22° 22' 36" N latitude and 87° 02' 33" E longitude), Jamboni, Gopiballavpur, Nayagram and Belda blocks. Collected plant specimens were identified with the help of available literature and a voucher specimen of each has been deposited in the herbarium (VUH) of the Department of Botany and Forestry of Vidyasagar University, Midnapore, West Bengal, India for further reference. Field information regarding their habit, habitat, dominance, local uses and their ethno-medicinal uses are based on personal observation and detailed discussions with the tribes inhabiting these areas, particularly the aged people and the ojhas (quack doctors) during the regular field surveys.

Antimicrobial activity determination

Collection and preparation of plant material for extraction

Plant parts were washed with 70% alcohol and then rinsed with sterilized distilled water and air dried. Clean dry plant samples were stored in cotton bags. The materials were homogenized to a fine powder with the help of a mixer grinder. These powered materials were then used for extraction of dyes.

Preparation of methanolic extracts

10 g of powdered material of each sample was soaked in 30 ml of 70% methanol and kept at 37°C for 24 h on a rotary shaker. After 24 h the previous portion of added methanol was evaporated and the same volume of methanol was again added and placed on a rotary shaker for another 24 h at 37°C. It was then filtered with the help of a Whatman No. 1 filter paper. The filtrate was centrifuged at 2000 rpm for 10 min. The supernatant was then collected and allowed to evaporate until it was completely dry. The extracts were kept in sterile air tight bottles at 4°C until further use. Before use 30 mg of dry extract was re-suspended in 1 ml of 70% methanol so that the final concentration of the extract was 30 mg/ml [19].

Bacterial strains

The antibacterial activities of the plant extracts were studied against two pure cultures of gram positive bacteria [*Bacillus cereus* (ATCC 1778) and *Staphylococcus aureus* (ATCC 25923)] and two gram negative bacteria [*Escherichia coli* (RIMD 059952) and *Salmonella typhi* (PSSCMI 0034)]. Bacterial strains were obtained from Department of Microbiology, Vidyasagar University, Midnapore and Department of Microbiology, Lady Brabourne College, Kolkata, West Bengal, India.

Agar well diffusion

Antimicrobial activity was determined by the agar-well diffusion method. Mueller Hinton Agar was used as media. To standardize the inoculum density for sensitivity test, a Barium Sulphate (BaSO4) turbidity standard, equivalent to 0.5 Mac Farland standards was used and was cultivated on agar medium. Thereafter 6 mm diameter wells were punched in the agar plates. Methanolic extracts (100 µl) of the different dyes were added to the wells. Streptomycin sulphate was used as positive control (30µg/ml). The plates were then incubated at 37°C for 24 h. After incubation the antimicrobial activity was evaluated by measuring the inhibition zone diameter observed [20]. Each test was performed twice and the average of the results was taken.

RESULTS AND DISCUSSION

All the selected plants were showed antibacterial activity against experimented pathogens and exhibited different diameters inhibition zone, which have been tabulated in Table 1, 2 and shown in [Figures 1 (a-f), 2 (a-e), 3(a-e) and Figures no. 4,5,6]. The availability of these plants in this region, their habit, habitat, common names as well as local names and plant parts for various medicinal purposes by the regional tribal people have been documented in Table 3.

***Alpinia galanga*(Linn.) Willd.**

Alpinia galanga is a perennial herb, about 2m high with lower portion covered with smooth leaf sheaths. The leaves are broadly lanceolate, 30-60cm long and 10-15cm broad. The flowers are arranged in erect, terminal panicles composed of numerous spreading dichotomous branches each with two to six, pale greenish-white faintly fragrant flowers. Fruits are 1.25 cm long, oblong, constricted in the middle or even pear shaped, three sided and deep orange red in colour. Seeds are ash coloured, three angled, finely striated towards the hilum. Both the seeds and rhizomes have pungent aroma [15, 21, 17, 16, 22]. Crude methanolic extracts of leaf showed moderate antimicrobial activity against *Staphylococcus aureus* with the greatest diameter of inhibition zone of 12 mm (including the 6 mm diameter of the wells) and 11 mm inhibition zones in case of the *Bacillus cereus*. The methanolic extract of rhizome of the *Alpinia galanga* showed moderate antibacterial activities against all experimented bacteria except *Escherichia coli*. The highest diameter of inhibition zone of rhizome 15mm was observed against *Bacillus cereus* and followed by 13mm, 9mm against *Salmonella typhi* and *Staphylococcus aureus* in methanolic extract. Diameter of inhibition zone of leaf aqueous extract was observed 13mm against only *Staphylococcus aureus* and inhibition zone of rhizome aqueous extract was highest 15mm against *Salmonella typhi*, followed by 12mm, 8mm against *Bacillus cereus* and *Staphylcoccus aureus.*

The rhizome contains tannins and flavonoids, some of which have been identified as kaempferide, galangin and alpinin [23]. From roots, kaempferide, galangin and alpinin were isolated. From green rhizomes, pale yellow oil with a pleasant odour can be obtained on distillation. The oil contains 48% methyl cinnamate, 20-30% cineole, camphor and probably a-pinene [24]. Itokawa *et al* [25] isolated two anti tumour principles from *A. galanga*. Twelve compounds have been characterized by GC/MS in *A. galanga*. The major compound is myrcene; 94.51% in rhizome and 52.34% in leaves [26]. Toxicity studies on *A. galanga* were carried out by Qureshi *et al* [26]. The steam volatile oil stimulates bronchial glands when exposed to its vapours [24].

Intravenous injection of a small dose of tincture or an infusion of *A. galanga* produces a sharp fall in blood pressure in experimental animals. The blood pressure, however, comes to normal in a short time. The fall in blood pressure is accompanied by a rise in volume of the intra-abdominal organs like the spleen and the intestine showing that dialation of the blood vessels is one of the important constituents of the drug, and its use as a carminative is suggested [27]. The pharmacognosy and toxicology of anti-carcinogenic natural products from galanga root oil has been studied by Zheng *et al* [28]. The effect of *A. galanga* treatment on cytological and biochemical changes induced by cyclophosphamide in mice has been studied by Qureshi *et al* [26].

***Alpinia calcarata* Rosc.**

Alpinia calcarata is another species of the genus with much medicinal importance. It is shorter in stature but stronger in aroma than *Alpinia galanga*. It is a perennial herb with non- tuberous root-stock. Stem is slender and 0.6-1.2m high. Leaves are 15-30 x 2.5-5cm; lanceolate, acuminate, green and glossy. Flowers are numerous, large, arranged in dense panicles, 7.5-10cm long, with pubescent rachis and small ovate bracts. Calyx tube is funnel-shaped and 6-8mm long. Corolla segments are

13mm long. Lip is 2.5-3.8cm long, ovate-oblong, sessile, yellow, streaked with purple veins and emarginate. Ovary is densely pubescent with many ovules in each cell. Capsules are globose and red [22]. Developmental studies in *A. calcarata* show that the placentae intrude into the ovary chamber and fuse at the lower portion of the ovary, leaving the upper portions of the ovary chambers confluent [29, 17, 21].

Crude methanolic extracts of the leaf of *Alpinia calcarata* showed moderate antimicrobial activity against only *Escherichia coli* with 8mm diameter of inhibition zone (including the 6 mm diameter of the wells). The methanolic extract of rhizome of the *Alpinia calcarata* showed moderate antibacterial activities against *Escherichia coli.* The highest diameter of inhibition zone of rhizome 13mm was observed against *Escherichia coli* and 12mm against *Staphylococcus aureus* in methanolic extract. Diameter of inhibition zone of leaf aqueous extract was observed 12mm against only *Escherichia coli* and inhibition zone of rhizome aqueous extract was highest 10mm against *Escherichia coli* and was 9mm against *Staphylcoccus aureus.*

The rhizomes of *A. calcarata* are anti- inflammatory [30]. The physiochemical characteristics of the essential oils from leaves and roots of *A. calcarata* have been described by Rath *et al* [31]. The rhizomes are bitter, acrid, thermogenic, aromatic, nervine tonic, stimulant, revulsive, carminative, stomachic, disinfectant, aphrodisiac, expectorant, broncho-dilator, antifungal, febrifuge, antiinflammatory and tonic [16]. Rhizome is CVS and CNS active, diuretic, hypothermic. Seed is antiulcerative [32].

***Alpinia allughas* Rosc.**

Another species of *Alpinia*, which has the therapeutic properties, is *A. allughas* It is a stout perennial herb with tuberous, aromatic roots. Leaves are sessile, oblong,-lanceolate, acuminate, cuspidate, glabrate, striate and compressed. Flowers are inodorous, pink, inerect, arranged in dense panicles with pubescent rachis and small ovate cupular bracts. Lip more than 2-5cm long, pink, obovate-cuneate, or suborbicular. Fruits are black, thin, globose and irregularly rupturing. Seeds are many, small, black and angular [22].

Crude methanolic extracts of the leaf of *Alpinia allughas* showed moderate antimicrobial activity against only *Staphylococcus aureus* with 9mm diameter of inhibition zone (including the 6 mm diameter of the wells). The methanolic extracts of rhizome of the *Alpinia allughas* are showed moderate antibacterial activities against *Staphylococcus aureus* with the highest diameter of inhibition zone of 13mm and 12mm against *Bacillus cereus* in methanolic rhizome extract. Leaf aqueous extract was showed no diameter of inhibition zone against all selected bacteria and inhibition zone of rhizome aqueous extract was highest 12mm against *Staphylococcus aureus* and was followed by 11mm, 10mm against *Escherichia coli* and *Bacillus cereus.*

Rhizome spray in ether, over a space showed high knock down values against houseflies. Alcohol (50%) extract of rhizome is anti-amphetaminic. Unani physicians consider it good for impotence (Asolkar *et al*, 1992. In case of *A. allughas*, rhizomes yielded essential oil (0.05%) which contained caryophyllene oxide (23.07%), geraniol (19.93%), eudesmol (19.93%), citronellyl acetate (16.5%), citronellol (6.8%), b- caryophyllene (5.45%), a-pinene (3.84%), linalool (2.86%) (-)a-phellandrene (1.6%) and geranyl acetate (0.16%) (33).

The *A. galangal* L, *Alpinia calcarata* and *A. allughas* are important medicinal plants and extremely used by tribal people of Medinipur district in Eastern India in their day to day life with potential source of drugs for pharmaceutical industry. These plants are not only propagated by rhizome but also propagated by seed on a wide range of climate and soils. Well drained hilly areas and places of 1400m high altitude are good for its cultivation. This is commercially propagated vegetative by rhizomes. The field should be ploughed to a good tilts. All the stones and pebbles should be removed. Organic manures at 10t/ha are applied during land preparation. Seedbeds are prepared with 1m breadth, 2m length and 15cm height. Small pits are made at 25cm spacing above the seedbeds and 5cm long rhizomes are planted. Seedbeds are covered with dried leaves. It is irrigated immediately after planting. Regular weeding is needed during the initial stages of crop growth. This is cultivated also as an intercrop in coconut or rubber plantations. Rhizomes are dug out after cutting the top portions when the crop reaches 1.5-2 years of maturity. The average yield is 10-15 tonnes of fresh rhizomes/hector and the driage is 25- 30%. The collected rhizomes are washed and cut into pieces of 5cm long and dried in sun for 4 days before sale [34]. The antibacterial compounds from these

plants may inhibit growth by different mechanisms than those presently used antimicrobials and may provide a significant clinical value in treatment of resistant pathogenic bacteria.

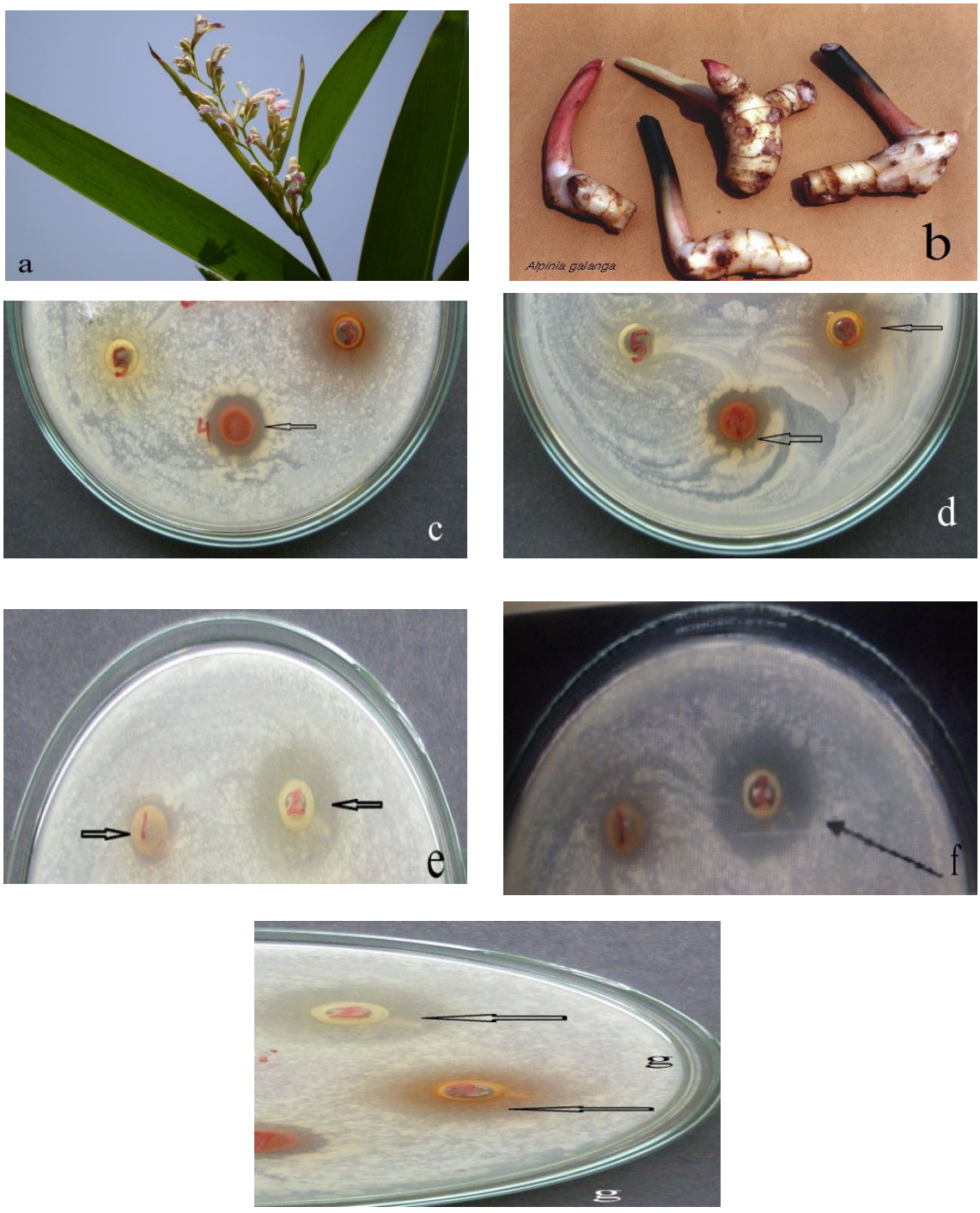


Figure 1.a) Flowering twig, b) rhizomes of *Alpiniagalanga* L., c) Arrow showing antimicrobial activity of aquatic extract of leaf against *Staphylococcus aures*, d) Arrows showing antimicrobial activity of methanol extract of leaf and rhizome against *Staphylococcus aureus,* e) Arrow showing antimicrobial activity of aqueous extracts of rhizome against *Staphylococcus aureus.* f) Arrows showing antimicrobial activity of methanol extract of rhizome against *Bacillus cereus,* g) Arrows showing antimicrobial activity of methanol extract of rhizome and leaf against *Bacillus cereus.*

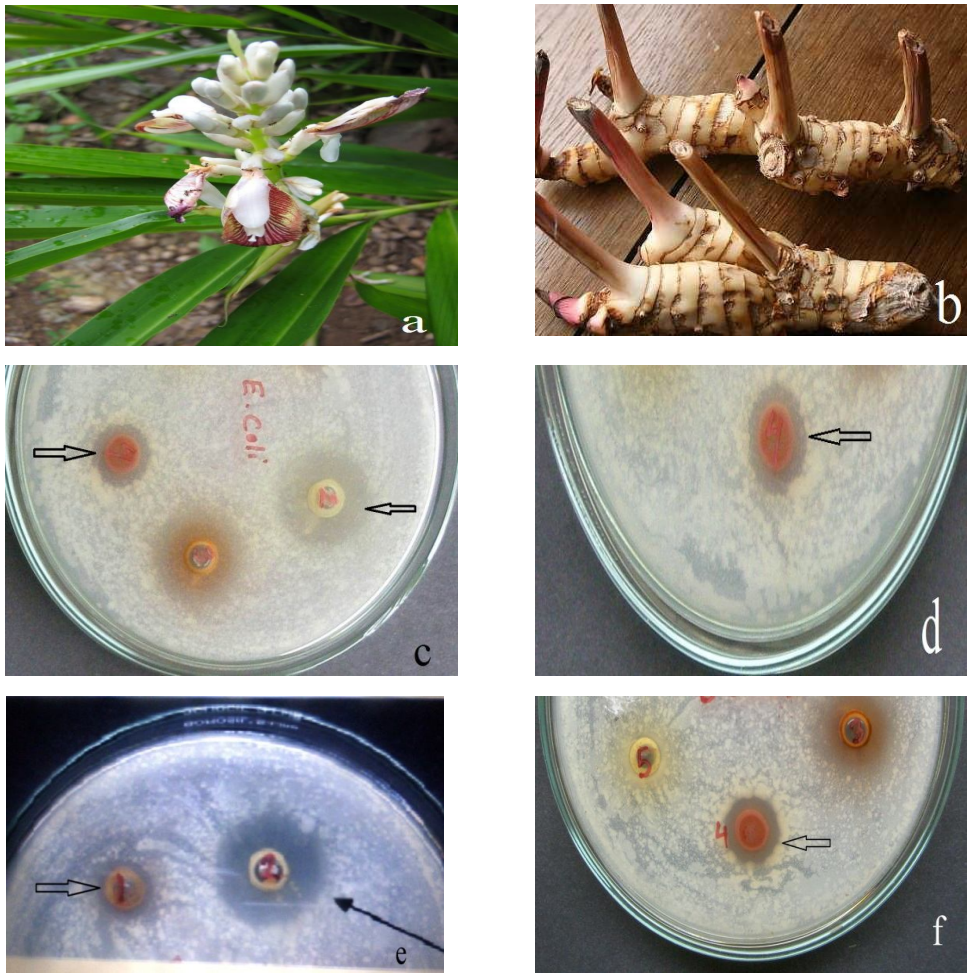


Figure 2.a) A flowering twig, b) *Alpiniacalcarata* Rosc.with rhizomes, c) Arrows showing antimicrobial activity of aqueous and methanolic extracts of leaf and rhizome against *Escherichia coli.,* d) Arrow showing antimicrobial activity of aquatic extract of rhizome against *Staphylococcus aures,* e) Arrow showing antimicrobial activity of aqueous extracts of rhizome against *Bacillus cereus,* f) Arrows showing antimicrobial activity of methanolic extract of rhizome against *Staphylococcus aures.*

Table-1: Determination of the zone of inhibition for antibacterial activities of the aqueous extract of the parts of three plant species of the genus *Alpinia* L.

Plant species	Parts(s) used in test	**Diameter of the inhibition zone (mm)**			
		Bacillus cereus (ATCC 1778)	*Staphylococcus aureus* (ATCC 25923)	*Escherichia coli* (RIMD 059952)	*Salmonella typhi* (PSSCMI 0034)
A. galanga L	Leaf	-	13	-	-
	Rhizome	12	8	-	15
A. calcarata Rosc.	Leaf	-	-	12	-
	Rhizome	11	9	10	-
A. allughas Rosc.	Leaf	-	-	-	-
	Rhizome	10	12	11	-

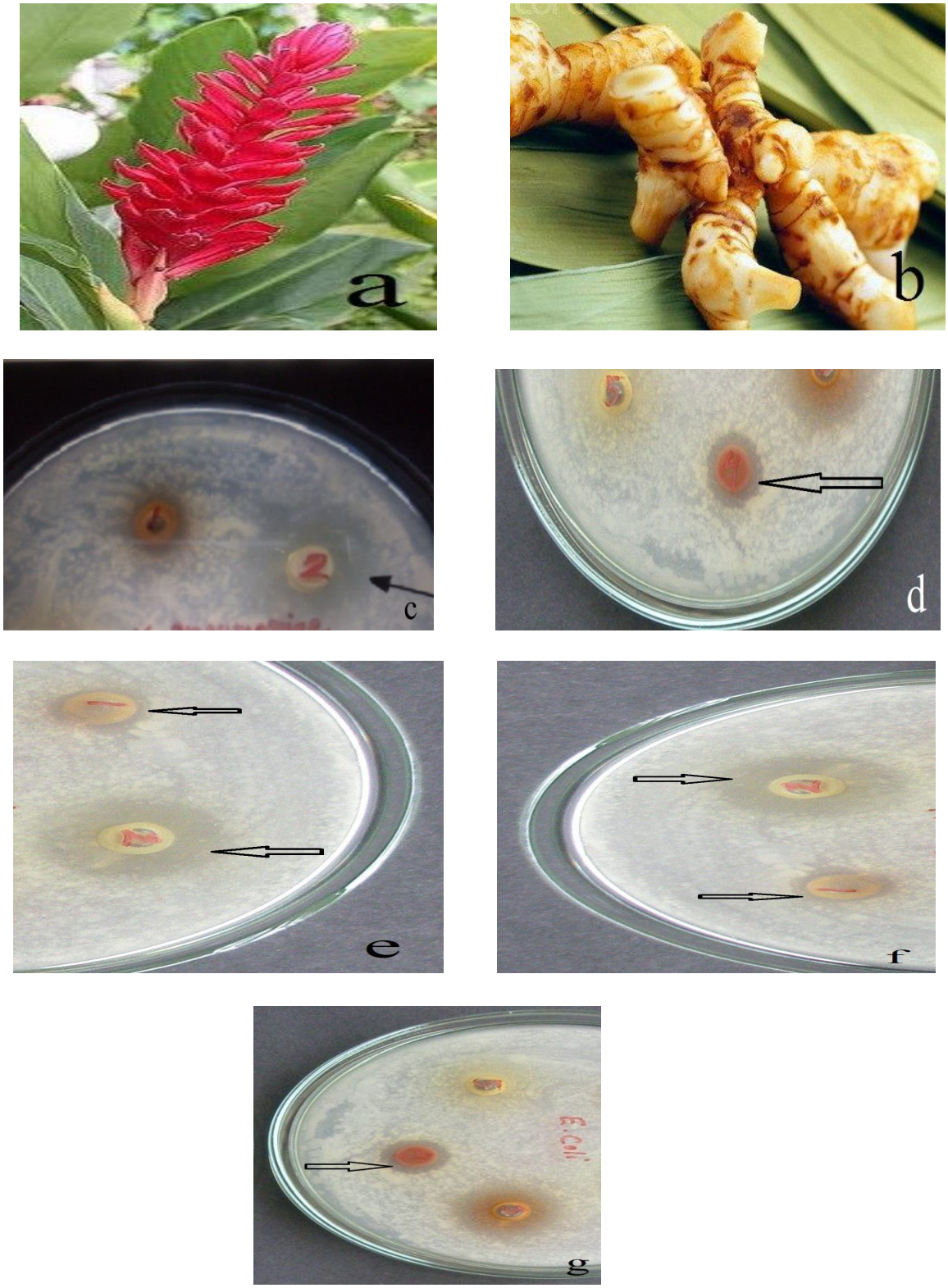
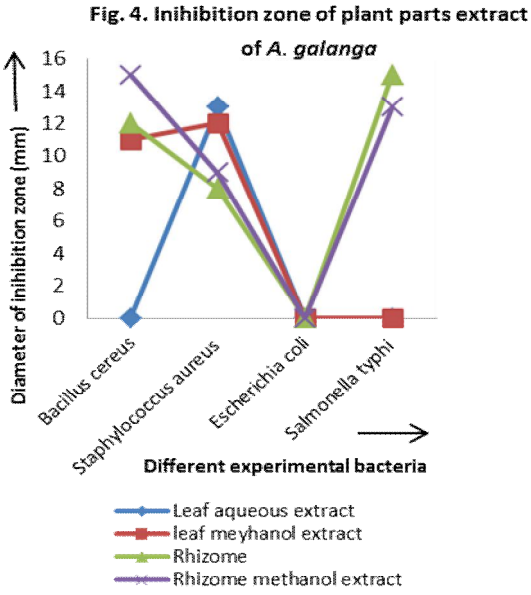
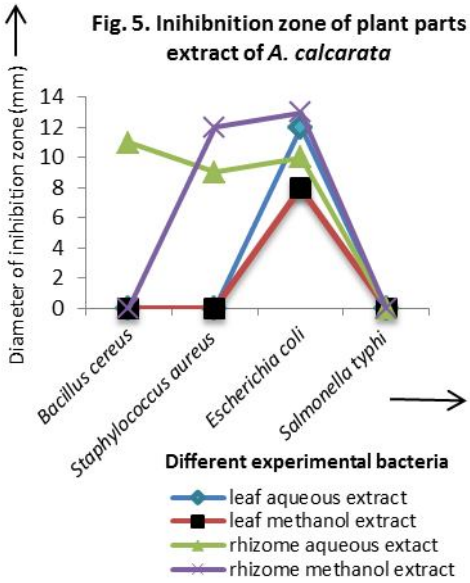


Figure 3.a) A flowering twig, b) rhizomes of *Alpiniaallughas* Rosc., c) Arrow showing antimicrobial activity of aquatic extract of rhizome against *Staphylococcus aures*, d) Arrow showing antimicrobial activity of aqueous extracts of rhizome against *Bacillus cereus,*e) Arrows showing antimicrobial activity of methanol extract of leaf and rhizome against *Staphylococcus aureus.*f) Arrows showing antimicrobial activity of methanol extract of rhizome against *Bacillus cereus* g) Arrows showing antimicrobial activity of aqueous extract of rhizome *Escherichia coli.*

Table-2: Determination of the inhibition zone for antibacterial activities of the methanol extract of the parts of three plant species of the genus *Alpinia* L.

Plant species	Parts(s) used in test	Diameter of the inhibition zone (mm)			
		Bacillus cereus (ATCC 1778)	*Staphylococcus aureus* (ATCC 25923)	*Escherichia coli* (RIMD 059952)	*Salmonella typhi* (PSSCMI 0034)
A. galanga L	Leaf	11	12	-	-
	Rhizome	15	9	-	13
A. calcarata Rosc.	Leaf	-	-	8	-
	Rhizome	-	12	13	-
A. allughas Rosc.	Leaf	-	9	-	-
	Rhizome	12	13	-	-





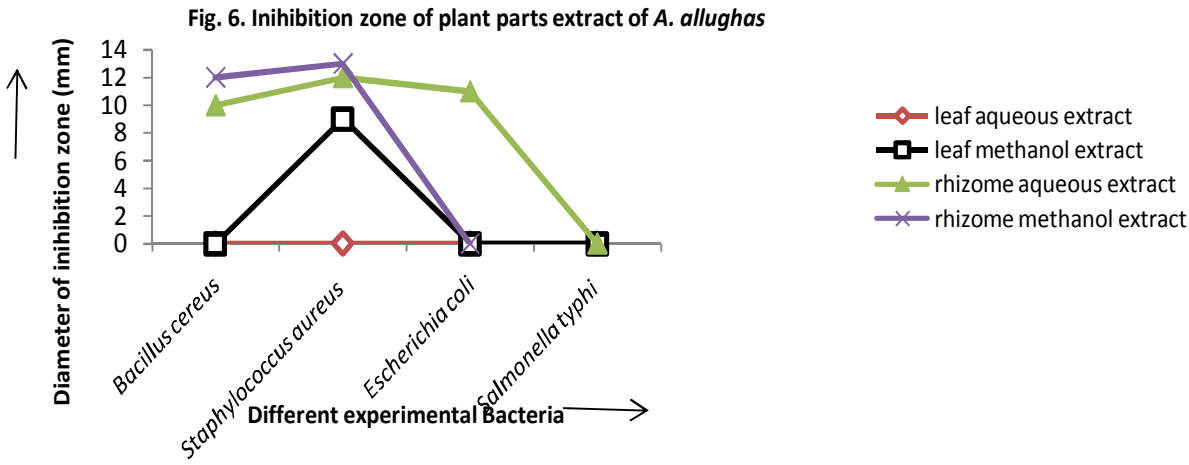


Table- 3: Medicinal uses of the selected plants of the family Zingiberaceae

Plant name	Synonym	Common name and Local name	Status	Habitat	Habit	Flowering period	Useable parts	Local medicinal uses
A. galangal L.	*Alpinia speciosa* Schum	Greater galangal, Java galangal, Siamese galangal, Sugandhamula, Rasna; Kulainjan,	Less frequent	Found near in shallow water pond and ditches	Marshy herbs	April-September	Leaf and Rhizome extract	Use in flatulence, dyspepsia, vomiting and sickness of stomach, being recommended as a remedy for sea-sickness, bronchial catarrh, bad breath and ulcer.
A. calcarata Rosc.	*Languas calcarata* Gaertn.) B. L. Burtt	Kolinchi.	Rare	Found near in ditches and marsh y land	Marshy herbs	March-September	Leaf, rhizome and root extract	uses as veterinary and homeopathic medicine, checks hemorrhage or secretions by coagulation of proteins on the soft surface, Used in lung diseases, rheumatism, indigestion, stomach ailments, fever, malaria, cholera and diarrhea
A. allughas Rosc.	*Heritiera allughas* Retz. *Alpinia nigra*	Taraka, Tara, Malayinjikkuva	Rare	Found in marsh y land	Herbs	April-October	Leaf, rhizome and root extract	Used in the treatment of heart diseases, wounds, eye diseases, stomach ulcer, body pain, rheumatism, dyspepsia, whooping colds in children, throat infection and fever

CONCLUSION

These plants are rare and less frequent in floral diversity of the region of eastern India from the ethno-medicinal point of view. Extreme increase of agricultural crops field has decrease the propagation rate of the genus *Alpinia* L. But at the same time these the plants are being over-exploited for pharmaceutical uses and found as less frequent to the species from its natural habitat. Which are undergoes as endangered and will be extinct soon due to exploitation. Hence strict measures need to taken to save this genus by proper conservation and large scale multiplication by *in vitro* propagation and *ex situ* plantations.

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REFERENCES

1. Gaulin, C., Viger, Y.B. & Filklion, L. (2002). An outbreak of Bacillus cereus implicating a part-time banquet caterer. Can. J. Public Health 93, 353-355.
2. Prado, V., Solari, V., Alvarez, I.M., Arellano, C., Vidal, R., Carreno, M., Mamani, N., Fuentes, D. & Munoz, V. (2002). Epidemiological situation of foodborne diseases in Santiago, Chile in 1999-2000. Rev. Med. Chil. 130, 495-501.
3. Di pietro, S., Haritchabalet, K., Cantoni, G., Iglesias, L., Manchi, S., Temperoni, A., Labanchi, J.L., Barbarossa, N., Garcia, M.T., Cofre, M. et al. (2004). Surveillance of foodborne diseases in the province of Rio Negro, Argentina, 1993-2001. Medicina (B Arires) 64, 120-124.

4. Branham, L.A., Carr, M.A., Scott, C.B. & Callaway, T.R. (2005). E. coli o157 and Salmonella spp. in white-tailed deer and liverstock. Curr. Issues Intest. Microbial.
5. Graham, C., Whyte, R., Gilpin, B., Corneulius, A., Hudson, J.A., Morrison, D., Graham, H. & Nicol, C. (2005). Outbreak of campylobacteriosis following pre-coojed sausage consumption. Aust. N. Z.J. Public Health 29, 507-510.
6. Hall, G., Kirk, M.D., Becker, N., Gregory, J.E., Unicomb, L., Millard, G., Stafford, R., Lalor, K. and Ozfoodnet working group. (2005). Estimating foodborne hastroenteritis, Australia. Emerg. Infect. Dis. 11, 1257-1264.
7. Jimenez, M., Soler, P., Venanzi, J.D., Cante, P., varela, C. and Martinez-Navarro, F. (2005). An outbreak of Campylobacter jejuni enteritis in a school of Madrid, Spain. Euro Surveill. 10, 118-121.
8. Cox, P.A. (1994). The ethnobotanical approach to drug discovery: Strengths and limitations. In Ethnobotany and the Search for New Drugs (G.T. prance, ed.)pp. 25-41, Wiley, Chichester, U.K.
9. Boyce, J.M. (1992). Methicillin-resistant Staphylococcus aureus in hospitals and long-term care facilities: Microbiology, epiderminology and preventive measures, Infect. Control Hosp. Epidemiol. 13, 725-737
10. Berns, J.S. (2003). Infection with antimicrobial-resistant microorganism in dylis patients. Semin. Dial. 16, 30-37.
11. Yoh, M., Frimpong, E.K., Voravuthikunchai, S.P. & Honda, T. (1999). Effect of sub inhibitory concentrations of antimicrobial agents (quinolones and macrolide) on the production and release of Vero toxin by enterohaemorrhagic Escherichia coli o157:H7. Can. J. Microbial. 45, 732-739.
12. Voravuthikunchai, S.P., Popaya, W. & Supavita, T. (2004). Antibacterial activity of crude extracts of medicinal plants used in Thailand against pathogenic bacteria. Ethnopharmacologia 33, 60-65.
13. Borris, R.P. (1996). Natural productions research: perspectives from a major pharmaceutical company, J. Ethnopharmacol. 51: 29-38.
14. Thomas, J. (1997). Medicinal and aromatic plants research in India. In UNDP. 1997. Proc. Training course on Industrial Exploitation of Indigenous Medicinal and Aromatic Plants. Beijing, China. 17-27 June, 1997.
15. Thakur, R. S., Puri, H. S. & Husain, A. (1989). Major Medicinal Plants of India, CIMAP, Lucknow, India.pp.50-52.
16. Warrier, P. K., Nambiar, V. P. K. and Ramankutty, C. (1993-1995). Indian Medicinal Plants. Vol.1-5. Orient Longman Ltd., Madras.
17. Sivarajan, V. V. & Balachandran, I. (1994). Ayurvedic drugs and their Plant Sources. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi. 570p.
18. Charles, D. J., Simon, J.E. & Singh, N.K. (1992). The essential oil of Alpinia galanga Willd. Journal of Essential Oil Research, 4 (1):81-82.
19. Ushimaru, P.I., Mariama, T.N., Luiz, C., Di Luciano, B., Ary, F.J. (2007). Antibacterial activity of medicinal plant extract. Braz. J. Microbiol., 38: 717-719.
20. Ulusoylu, M, Öndersev, D.V., Soyoğul, U., Gürkan, E., Tuzlaci, E. (2001). The cytotoxic and the biological (antibacterial and antifungal) activities of Centaurea iberica and Ferulago confuse. *J. Fac. Pharm. Gazi Univ.*, 18(**2**): 75-80.
21. Gamble J.S. (1987). Flora of the presidency of Madras. Vol.III. Bishen Singh Mahendra Pal Singh, Dehra Dun, India. Pp.1478-1493.
22. Kirtikar, K. R. & Basu, B.D. (1987). Indian Medicinal Plants. Internat. Book Distributors, Dehra Dun.p.2444-2449.
23. Sastry, M.S. (1961). Comparitive chemical study of two varieties of galangal. Indian J. Pharm., 23:76.
24. Chopra I.C., Khajuria, B.N. & Chopra, C.L. (1957). Antibacterial principles of Alpinia galanga and Acorus calamus. *Antibiotics Chemother*, 7:378.
25. Itokowa, H., Morita, H., Sumitomo, T., Totsuka, N. & Takeya, K. (1987). Antitumour principles from Alpinia galanga. Planta Med., 53 (1): 32-33.
26. Quereshi, S., Shah,A.H., & Ageel, M.M. (1992). Toxicity studies on Alpinia galanga and Curcuma longa. *Planta Medica*, V. 58 (**2**):124-127.
27. Inamdar, M.C., Khurana, M.L. & Rao, R.M.M. (1961). Expectorant activity of Alpinia galanga. Indian J. Physiol. Pharmacol., 6:150.
28. Zheng, G.Q. Kenney, P.M. & Lam, L.K.T. (1993). Potential anti-carcinogenic natural products from lemongrass oil and galanga root oil. *Journal of Agriculktural and Food Chemistry*,V. 41 (**2**):1530156.
29. Mangary, J.K. & Hamsa, P.V. (1991). Placentation in Zingiberaceae. Workshop, Prince of Songkla University, Hat Yai, Thailand. P.15.
30. Asolkar, L.V., Kakkar, K. K. & Chakre, O. J. (1992). Second Supplement to Glossary of Indian Medicinal Plants with Active Principles Part I (A-K). (1965-81). Publications and Informations Directorate (CSIR), New Delhi. 414p.
31. Rath, S.P., Sahoo, S.B. & Sreenivasulu, C. (1994). Analysis of cultivated Alpinia calcarata. *Journal of Natural Products*,V. 10 (**1**)_:12-13.
32. Husain, A., Virmani, O. P., Popli, S. P., Misra, L. N., Gupta, M. M., Srivastava, G. N. Abraham, Z. & Singh, A. K. (1992). Dictionary of Indian Medicinal Plants. CIMAP, Lucknow, India .546p.
33. Rastogi, R. P. & Mehrotra, B. N. (1991). Compendium of Indian Medicinal Plants. Vol. II. Central Drug Research Institute, Lucknow and Publications and Information Directorate, New Delhi. 833p.
34. Prasad, P. P. & Joseph, B. (1997). Oushada Sasyemgalum Avayude Krishireethikalum (Malayalam). Nagarjuna Research Foundation, Thodupuzha, India.