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Research Article

Study on Trace Element and Phytochemical Profiling of *Alpinia galanga* Rhizome and *Clerodendrum colebrookianum* Leaves Extracts and their *In-vitro* Bioactivity

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ABSTRACT

Alpinia galanga and *Clerodendrum colebrookianum* belonging to the Zingiberaceae and Verbenaceae family, respectively is known to have many physiological properties like anti-bacterial, anti-oxidant, anti-tumor, etc. Both the plant samples possess many admirable curative properties as traditional medicine for the treatment of various diseases and disorders. The aim of this work is to identify the phytochemical and elemental components of both plant extracts which will support the therapeutic and traditional use of both plant samples. The presence or absence of micro- and macro-elements in the extracts was determined using atomic absorption spectrometry (AAS). Phytochemical analysis of the acetone and ethanol extract of both the plant samples also showed the presence of some necessary phytochemicals. Two *in-vitro* techniques (2,2-diphenylpicrylhydrazyl (DPPH) and ferric reducing antioxidant capacity (FRAP) assay where the absorbance was measured at 517 and 700 nm, respectively) were used to assess antioxidant activity, and ascorbic acid and trolox were used as the reference standard. For the DPPH assay, the highest activity (IC_{50} = 54.82 µg/mL) was shown by the acetone extract of *C. colebrookianum*, and the lowest (IC_{50} = 67.41 µg/mL) was shown by the acetone extract of *A. galanga*. The anti-microbial studies also revealed that the best activity was shown by the acetone extract of *C. colebrookianum* leaves against *Escherichia coli* i.e., 12 mm with minimal inhibitory concentration (MIC) value of 0.117 mg/mL.

Introduction

Since time immemorial, human beings have used plants not only as a source of food but also as a remedy for curing and wellness purposes. The medicinal properties shown by plants are attributed to the organic and inorganic compositions present in them[1,2] Multiple studies have also confirmed that the presence of certain elements in plants is likely to be responsible for their curative properties.[3] These elements, known as trace elements, play a chief role in maintaining normal metabolism and health in the human body but also show toxic properties. While toxic elements like Pb, Cd, Al, Hg, and Cr causes health risk in the human body, elements like K, Na, Ca, Mg, Fe, Mn, and Zn contribute a positive effect in maintaining and regulating the human's body normal functioning.[4,5] Therefore, a proper estimation of regulating the concentration of trace elements in medicinal plant consumption is a necessity for the well-being of the consumers and also for quality control.[6]

The organic compound, on the other hand, is the major constituent that contributes to the therapeutic property of these medicinal plants.[6,7] This includes the plant fragments like its essential oil and bioactive compounds like glycosides, alkaloids, vitamins, phenolic compounds, etc.[8] where more than 10,000 such types of compounds have been isolated and characterized.[9] These compounds are the basis for the pharmacological effect of such medicinal plants, contributing to properties like anti-

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microbial, anti-inflammatory, antioxidant, etc.[10] In a study, it has been reported that the essential oil also contributes to the anti-microbial activity which is due to the composition of 1,8- cineole, 1-acetoxychavicol acetate, etc., in such kinds of plants.[11] In the same context, World Health Organization (WHO) also stated the importance of medicinal plants in achieving extensive routes in discovering potent drugs that possess such anti-microbial properties.[12] For instance, the roots of *G. tapis* and *G. giganteus* are used to induce abortion in the first few months of pregnancy, whereas decoctions of *G. scortechinii* and *G. macrophyllus* are used as a postpartum preventive medicine. To cure scabies, use *G. amuyon*. Acetogenins, styryl lactones, and alkaloids with considerable cytotoxic, insecticidal, and antibacterial properties have been isolated by phytochemical studies of *Goniothalamus* spp.[13] Antioxidants have a key role in lowering oxidative stress, which can harm biological molecules.[14] High doses of synthetic antioxidants that have been used to prevent oxidation, such as butylated hydroxyl anisole (BHA), propyl gallate (PG), and butylated hydroxyl toluene (BHT), have been reported to cause internal and external bleeding in rats and guinea pigs.[15] Due to their native origin and potent ability to trap or scavenge free radicals, natural antioxidants such as bioactive flavonoids are of tremendous value and are now the focus of attention. Tea (black and green) is one such example, as it is consumed regularly over the world and is a significant source of polyphenolic chemicals.[15,16]

Alpinia galanga, an *Alpinia* genus of the Zingiberaceae family, which is also commonly known as greater *galanga* [17] has been and is still being used as a natural remedy for curing and treating a variety of diseases.[18] Reports on the cure and treatment of diseases like heart burns, renal calculus, bronchitis, diabetes mellitus, etc. by *A. galanga* have been given by many workers.[19,20] It is not only used as a spice but also it is widely practiced as a local medicine all over the world thus, this very plant has been proving itself to be an important remedy since time immemorial.[21] In Nagaland, *A. galanga* is being used as a remedy to cure and treat certain diseases like rheumatism, respiratory complaints, stomach complaints, etc. It is also used as an antiseptic or as a stimulant/tonic.[22] But very little is known about its scientific knowledge on its medicinal property, which needs to be studied extensively.

Clerodendrum colebrookianum has shown to possess admirable curative properties as a traditional medicine for the treatment of various diseases and disorders. In Nagaland, the plant is mainly used for the treatment of blood pressure. It is also used as a remedy for malaria, and heart troubles and also as an appetizer.[22] The plant sample has also been reported for its therapeutic and pharmacological uses such as hypolipidemic,[23] antioxidant,[24] anti-inflammatory,[25] analgesic,[26] antibacterial[27] etc.

By screening for their phytochemicals and the essential trace elements that are present in them and also studying their *in-vitro* bioactivity, this work has been undertaken to validate further information on the selected medicinal plants that are found in Nagaland. It may also be possible to quantify some of the phytochemicals and trace elements, which may help us in further understanding the chemical constituent of the selected plant samples as it will give us a better insight into the phytochemicals and trace elements and possibly use as a potent drug.

Materials and Method

Chemicals and Reagents

All the chemicals and reagents used in this study were obtained from HiMedia, SDFCL and Merck and were used without further purification.

Collection and Preparation of Plant Material for Extraction

The rhizome of *A. galanga* and leaves of *C. colebrookianum* were collected from the local area of Zunheboto district, Nagaland, India. The collected samples were washed thoroughly in running water then with distilled water and oven dried at 40°C for 72 hours or until a constant desired weight is achieved. About 20 gms of both the dried samples were individually extracted at soxhlet apparatus using 500 mL of acetone and ethanol and the collected filtrate was centrifuged at 5000 rpm for 20–30 minutes. The collected filtrate was then evaporated to obtain the crude product of the samples using a rotary vacuum evaporator and was stored in an airtight container for further use.

Phytochemical Profiling for Both the Plant Samples

The preliminary screening of phytochemical content for both the plant samples was demonstrated through known procedures as reported by Harborne and Trease *et al.*[28,29]

Estimation of Minerals and Trace Elements

Inductive coupled plasma optical emission spectroscopy (ICP-OES) was used for the determination of elements in both the plant samples. For this study, oven-dried samples were used which were digested using a mixture of concentrated HNO_3:HCl (3:1) and the analysis was performed as described by Jasha Momo H. Anal *et al.*[30]

Antibacterial Activity Assay

For the *in-vitro* antibacterial studies, the disc diffusion method[31] was employed where the activity of the samples was determined by measuring the ring of the inhibited zone (mm). The study was evaluated against two strains of gram-positive bacteria i.e., *Bacillus subtilis* and *Staphylococcus aureus*, and two gram-negative bacteria i.e., *Escherichia coli* and *Klebsiella pneumonia*. To further quantify the sample's antibacterial potency, minimal inhibitory concentration (MIC) was determined by means

of a two-fold serial broth dilution method.[32] All the samples including the reference standard, streptomycin were prepared in a concentration of 10 mg/mL DMSO and the test was performed in triplicates.

Antioxidant Capacity Estimation

The antioxidant capacity of both the plant samples was ascertained by 2,2-diphenylpicrylhydrazyl (DPPH) and ferric reducing antioxidant capacity (FRAP) assay[33,34] and compared with the antioxidant potency of reference standards ascorbic acid and trolox. DPPH radical scavenging capacity for the plant samples was determined by measuring the absorbance of different concentrations of the samples at 517 nm and then calculating their IC_{50} value. As for the FRAP assay, antioxidant capacity was determined by measuring the reducing power of the sample optical density (OD) of 700 nm. It was expressed as an increase in A_{700} after the subtraction of the blank solution where A_{700} is defined as the absorbance that is recorded at OD 700 nm against the blank solution.

Results

Phytochemical Profiling

The phytochemical profiling from both the plant samples extracts revealed the presence of alkaloids, carbohydrates, flavonoids, protein, phenol, and tannin (Table 1). On the other hand, amino acids were not present in both extracts of the plant samples. Also, carbohydrates were absent in both the acts of *A. galanga* rhizome (Table 1).

The presence of such phytochemicals in both plant samples is capable of pharmacological properties. This study supports the therapeutic properties and traditional use of both samples for various treatments and curing purposes.

Estimation of Minerals and Trace Elements

The relative concentration of minerals and trace elements of both samples were determined through inductive coupled plasma optical emission spectroscopy and a total of 13 elements were analyzed for this study. The analytical data for elemental and mineral determination are shown in Table 2. From the analytical studies, it was revealed that the concentrations of the elements were either in acceptable concentration or below the acceptable range except Cd for *C. colebrookianum*, which was not detected. Pd and Al for *C. colebrookianum* showed the least concentration i.e., -0.010 ± 0.003 and -0.129 ± 0.003.

Table 1: Phytochemical screening of both the solvent extracts of *A. galanga* rhizome and *C. colebrookianum* leaves

Test	Ag(A)	Ag (E)	Cc(A)	Cc(E)
Alkaloid	+	+	-	+
Amino acids	-	-	-	-
Carbohydrates	-	-	+	+
Flavonoids	+	+	+	+
Protein	+	+	+	+
Phenol	-	+	-	-
Tannin	+	+	+	+

N.B.: + = present and – = absent; Ag (A) = Acetone extract of *A. galanga* rhizome; Ag (E) = Ethanol extract of *A. galanga* rhizome; Cc (A) = Acetone extract of *C. colebrookianum* leaves; Cc (E) = Ethanol extracts of *C. colebrookianum* leaves.

Table 2: Mean concentrations of elements present in the oven-dried rhizome of Alpinia galanga and leaves *Clerodendrum colebrookianum*

S. No.	*Elements*	*Concentration (in ppm)*	
		A. galanga	*C. colebrookianum*
1.	Cu	0.098 ± 0.002	0.300 ± 0.009
2.	Cr	0.319 ± 0.056	0.021 ± 0.701
3.	Ca	0.029 ± 0.006	0.183 ± 0.054
4.	Mn	0.352 ± 0.098	2.708 ± 0.043
5.	Mg	5.628 ± 0.162	2.435 ± 0.014
6.	Fe	16.08 ± 0.721	1.523 ± 0.034
7.	Zn	0.554 ± 0.068	0.296 ± 0.003
8.	Ni	0.012 ± 0.005	0.152 ± 0.019
9.	Co	0.104 ± 0.042	0.003 ± 0.011
10.	Cd	0.001 ± 0.024	ND
11.	Pb	0.020 ± 0.003	-0.010 ± 0.003
12.	Na	0.895 ± 0.023	0.438 ± 0.01
13.	Al	5.053 ± 0.005	-0.129 ± 0.003

All concentrations in ppm (parts per million); ND- Not Detectable

Antioxidant Capacity Estimation

The antioxidant activity of both the plant sample extract was evaluated using the DPPH and FRAP assay. The antioxidant activity of both the plant extracts and the standards by DPPH assay are shown in Tables 3 and 4. The IC_{50} value for the plant samples extract was obtained through a linear regression equation by plotting a graph of concentration against the percentage of radical scavenging activity. For the DPPH assay, the highest activity (54.82 µg/mL) was shown by the acetone extract of *C. colebrookianum* and the lowest (67.41 µg/mL) was shown by the acetone extract of *A. galanga*. It was also observed that both the selected plant samples showed better antioxidant activity than the standard Trolox.

Fig. 1 illustrates the FRAP activity of both the plant sample extracts compared to the standards, ascorbic acid, and trolox, where the antioxidant power was determined as the greater the value of absorbance as shown by the sample, the greater is its antioxidant potency. Both the plant samples showed better antioxidant potency than the standard trolox. Of the two plant samples, *A. galanga* showed better antioxidant activity than *C. colebrookianum*.

Table 3: DPPH radical scavenging activity, IC_{50} values of acetone and ethanol extracts of *Alpinia galanga* rhizome and *C. colebrookianum* leaves.

Concentration (µg/mL)	*Alpinia galanga*				*Clerodendrum colebrookianum*			
	Acetone Extract		*Ethanol Extract*		*Acetone Extract*		*Ethanol Extract*	
	%Inhibition	*IC_{50} µg/mL*	*%Inhibition*	*IC_{50} µg/mL*	*%Inhibition*	*IC_{50} µg/mL*	*%Inhibition*	*IC_{50} µg/mL*
20	22 ± 0.32		28 ± 0.55		35 ± 0.66		34 ± 0.13	
40	37 ± 0.24		39 ± 0.87		41 ± 0.56		40 ± 0.97	
60	48 ± 0.53	67.41	48 ± 0.33	64.05	56 ± 0.38	54.82	51 ± 0.93	60.46
80	56 ± 0.31		59 ± 0.68		67 ± 0.38		62 ± 0.42	
100	65 ± 0.67		69 ± 0.57		78 ± 0.45		70 ± 0.49	

N.B.: Values are presented as ± S.E. (n=3)

Table 4: DPPH radical scavenging activity, IC_{50} values of the standards trolox and ascorbic acid.

Concentration (µg/mL)	*Ascorbic acid*		*Trolox*	
	%Inhibition	*IC_{50} µg/mL*	*%Inhibition*	*IC_{50} µg/mL*
20	29 ± 0.32		20 ± 0.09	
40	50 ± 0.18		31 ± 0.55	
60	62 ± 0.68	48.149	40 ± 0.02	71.72
80	78 ± 0.42		58 ± 0.29	
100	89 ± 0.28		66 ± 0.25	

N.B.: Values are presented as ± S.E. (n=3)

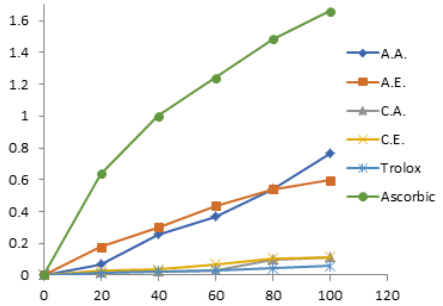

Fig. 1: Antioxidant power of the extracts of *A. galanga* rhizome and *C. colebrookianum* leaves as compared to standard Trolox and Ascorbic acid at a concentration of 1-mg/mL. A.A.= Acetone extract of *A. galanga* rhizome; A.E. = Ethanol extract of *A. galanga* rhizome; C.A. = Acetone extract of *C. colebrookianum* leaves; C.E. = Ethanol extract of *C. colebrookianum* leaves.

Antibacterial Activity Assay

The antibacterial activity of the plant sample extracts determined through the disc diffusion method and MIC are as shown in Tables 5 and 6, respectively. These properties of the plant extracts were assessed against two strains of gram-positive bacteria i.e., *Bacillus subtilis* and *S. aureus*, and two gram-negative bacteria i.e., *E. coli* and *K. pneumonia*. The study revealed that almost all the extracts showed a potential effect in suppressing the bacterial growth with a concentration of 10 mg/mL DMSO except for Cc (A) and Cc (E), which showed no inhibition against *Bacillus subtilis* and *E. coli,* respectively. The highest activity was recorded for Cc (A) against *E. coli* whereas, other plant extracts showed variable antimicrobial activity. Since the antibacterial study gave almost positive results, MIC against all the bacterial strains was also conducted and the results are recorded in Table 6.

Discussion

The presence of phytochemicals in plants is proven to possess therapeutic properties like antimicrobial, antioxidant, anti-inflammatory, etc. Alkaloids are an important class of phytochemicals that shows potency towards physiological activity like anti-inflammatory, analgesic, antimicrobial, and antispasmodic actions.[35] Steroidal alkaloids are also reported to exhibit medicinal properties due to their biological activities like cardiotonic, insecticidal, and antibacterial activities.[36] Flavonoids, on the other hand, have gained a lot of attention over the years as they have shown to be beneficial to health attributing to their pharmacological actions as anti-inflammatory, antimicrobial, antioxidant, and anti-tumor which are all allied with radical scavenging properties. Flavonoids have also been demonstrated to possess anti-diabetic and hypoglycemic effects.[37] Although no therapeutic properties have been reported for carbohydrates, they have been exploited for producing polysaccharide immunomodulators with curative implications and are also considered to be possibly responsible for the therapeutic effect of other important components.[38] Phenols and tannins are considered good sources of antioxidants because of their free radical-scavenging ability. The hydroxyl group present in such compounds is capable to react with active oxygen radicals and is thus, responsible for the therapeutic property of plants.[39] Minerals and trace elements present in plants have also been proven to be an essential source of nutritional benefits and also serve as an important component in different cellular processes.[40] These essential and heavy elements greatly influence numerous functions in our body depending on their concentrations. For example, elements like K plays a vital role in many basic cellular enzymatic reactions like the transfer of phosphate ATP to pyruvic acid; Ca plays a role in the activation of enzymes, regulations of muscle functions and nerve and

Table 5: Zone of inhibition against the four bacterial strains shown by the two extracts of *A. galanga* rhizome and *C. colebrookianum* leaves as compared to the standard streptomycin (in mm)

Test Sample	Zone of Inhibition (mm)			
	Bacillus subtilis	*Escherichia coli*	*Klebsiella pneumonia*	*Staphylococcus aureus*
Ag (A)	10	11	11	10
Ag (E)	11	11	10	11
Cc (A)	NI	12	>10	>10
Cc (E)	>10	NI	>10	>10
Streptomycin	23	22	22	22

N.B.: Ag (A) = Acetone extract of *A. galanga* rhizome; Ag (E) = Ethanol extract of *A. galanga* rhizome; Cc (A) = Acetone extract of *C. colebrookianum* leaves; Cc (E) = Ethanol extract of *C. colebrookianum* leaves; NI = No Inhibition.

Table 6: MIC of the two solvent extracts of *A. galanga* rhizome and *C. colebrookianum* leaves as compared to the standard streptomycin against the four bacterial strains (in mg/mL)

Test Sample	Minimum Inhibitory Concentration (mg/mL)			
	Bacillus subtilis	*Escherichia coli*	*Klebsiella pneumonia*	*Staphylococcus aureus*
Ag (A)	0.93	1.87	1.87	0.93
Ag (E)	1.87	1.87	3.75	1.87
Cc (A)	---	0.117	3.75	3.75
Cc (E)	3.75	---	3.75	3.75
Streptomycin	0.0072	0.0072	0.0029	0.0058

N.B.: Ag (A) = Acetone extract of *A. galanga* rhizome; Ag (E) = Ethanol extract of *A. galanga* rhizome; Cc (A) = Acetone extract of *C. colebrookianum* leaves; Cc (E) = Ethanol extract of *C. colebrookianum* leaves.

is also one of the main constituents of teeth and bones; Na normalizes plasma volume in the human body and also acts as the principal cation in extracellular fluids; Mg is also a constituent of teeth and bones, and acts as an activator for phosphate transferring enzymes; Fe plays a key role in the transport of oxygen throughout the human body by functioning as hemoglobin. It also helps in cellular respiration by acting as an essential constituent of enzymes that are involved in biological oxidation; Mn plays as a coenzyme of decarboxylase, hydrolase, phosphohydrolase, etc. It also takes part as an enzyme in the formation of urea.[41]

Understanding the alternatives, constraints, and potential improvements in the selection of assays used for the *in-vitro* evaluation of antioxidant and antibacterial activities is essential given the significance and sheer volume of natural product research. Given the wide variety of chemical substances available, evaluating plant bioactivity is challenging because no single assay or set of assays is definitely the best option.[42]

The ability of plants to act as an antioxidant and to scavenge radicals are all associated with their therapeutic values. This property of plants to scavenge radicals significantly plays an important role in eliminating the free radicals before they target and attack the biological cells and prevent any further cause of diseases.[43] DPPH and FRAP assays are some commonly employed methods for determining antioxidant activity where both the assays have simple procedures. DPPH radicals are found to be stable and very well known that are employed to detect the molecule's antioxidant activity. This radical upon accepting an electron from the given molecule changes its color from purple to yellow during the course of the reaction and gives a strong absorption at 517 nm. FRAP, on the other hand, is the ability of the given molecule to reduce the ferric tripyridyltriazine complex to a ferrous complex by electron donation. The color of the solution changes from yellow to dark blue during the process giving a strong absorption at 700 nm.[44,45]

The emergence of microbes that are resistant to antibiotics has increased the urgency of the hunt for new antibacterial substances. Numerous compounds with plant origins, including alkaloids, flavonoids, glycosides, terpenes, tannins, and polyphenols, have been found to have antibacterial activity. Many have also demonstrated synergistic benefits with already-available antibacterial medications.[42] In a study, the effectiveness of various plant extracts (aqueous and 40% hydroalcoholic) against canine oral bacteria was assessed. It was discovered that extracts from the leaves of the guava tree, garlic (*Allium sativum*), and "espinheira santa" (*Maytenus ilicifolia*) were effective at killing isolated strains of *Streptococcus oralis, Streptococcus mitis*, and the standard *S. aureus* strain (*Psidium guajava*). In a similar vein, chamomile was discovered to have antibacterial activities against *S. aureus*. This activity is caused by the phenolic chemicals in its ethanol extract. The growth of *Bacillus cereus, B. subtilis, Pseudomonas aeruginosa,* and *S. aureus* was also reported to be suppressed by the aqueous extract from the artichoke (*Cynara scolymus*) and the ethanol extracts (80%) from both the artichoke and "macela" (*Achyrocline satureioides*).[46] From this very study, we may conclude that both the plant samples possess almost all of the essential and important phytochemicals constituents as well as the necessary minerals and elements in appreciable concentration. The extracted fractions of both the plant samples also showed good biological properties i.e., proved to possess a good antioxidant property which might contribute to preventing various oxidative stress caused by free radicals; showed potential effectiveness as an antibacterial agent against *B. subtilis*, *S. aureus*, *E. coli* and *K. pneumonia* confirming its potential use as an alternative natural preservative for food or as an antibacterial agent in other pharmaceutical fields. The findings from this study thus provide an overview of the chemical and elemental concentrations and the biological properties of *A. galanga* rhizome and *C.colebrookianum* leaves with possible use of both the plant samples in treating various ailments, in clinical use and

can also be continued using as a conventional crop. The plant samples prove to be a potential agent to be employed in pharmaceutical, food, and cosmetic industries, hence, further analysis and isolations of bioactive compounds ought to be done.

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