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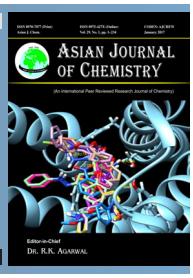

Comparative Efficacy and Potency of Various Extracts of Rhizomes and Leaves of *Alpinia allughas*

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The extract of dried rhizomes and leaves of *Alpinia allughas* was partitioned with different solvent system by increasing their polarities (petroleum ether, hexane, dichloromethane and methanol). All the extracts were tested for there phytochemical make up and revealed to the presence of secondary metabolites like steroids, terpenoids, alkaloids, glycosides, saponins, phytosterol, *etc*. The phenolic content and flavonoid content were found maximum 348.18 mg/g GAE (gallic acid equivalent) and 52.82 mg/g CNE (catechin equivalent) quantitatively, respectively in methanolic extracts of leaves, whereas *o*-dihydric phenol content was found maximum 54 mg/g CLE (catechol equivalents) in rhizomes extract. The results thus revealed that this herb is a big repository of phytochemicals. The greater correlation coefficient among these parameters in leaves as compared to rhizomes of *Alpinia allughas* indicated the potency of leaves for usage as drug in indigenous system of medicine and for the development new molecules.

Keywords: *Alpinia allughas*, Phytochemicals, Leaves, Rhizomes, Correlation.

INTRODUCTION

Medicinal plants and the active constituent present in these have been extensively used since years for the ailment of the various diseases. These are not only considered useful but also are considered as economical for humans [1]. Due to the resistance development by the various microorganism against the antibiotics there exist an urgent demand to develop natural drugs which are easily available, ecofriendly and low-cost. Thus the evaluation of the traditional medicine for the treatment of various diseases is increasing day by day [2]. Plant consists of variety of substance that can be used for the treatment of the variety of the infectious diseases [3]. Secondary metabolites such as the terpenoid and phenolics possess plant defense mechanism against a variety of the microorganism [4]. Phytochemical such as alkaloids, flavonoids, tannins and phenolic compounds are called as non-nutritive chemicals that possess disease preventive property [5].

The plants of family Zingiberaceae are well known for their medicinal values and have been used as food, spices, medicines, dyes, perfume and aesthetics [6]. The rhizomes of this family not only possess nutritional characterstics but are used as tonic, stimulant and astringent [7]. The genus *Alpinia* belonging to the family Zingiberaceae, comprised of 244 species throughout the world [8]. *Alpinia* spp. are widespread and are being cultivated for its rhizomes in tropical areas of South and East India [9]. *Alpinia* plays an important role in the treatment of stomatopathy, asthma, hiccough, stomachalgia, tubercular glands and fevers [10]. The medicinal part of the herb is rhizome which forms a major ingredient of preparations like Rasnadi, Kasaya Asvagandharishta and are used are used in the treatment of bronchial troubles, anti-inflammation decoctions and as cardiac stimulant [9,11]. In view of the above properties exhibited by the plant *Alpinia* an attempt was made to explore the phytochemicals in the leaves as well as the rhizome of *Alpinia allughas syn. Apinia nigra*.

EXPERIMENTAL

Fresh rhizome and leaves of *A. allughas* were collected from Tarai region of Kumaun hills in India. The taxonomic identity of the plant was confirmed by Sumer Chandra, Systematic Botany Division of Forest Research Institute, Dehradun, where herbarium specimens, Nos.: 9747 and 72265, dated 12 January 2004 were deposited.

Preparation of the extracts: The shade dried rhizomes and leaves of *A. allughas* were cut into small pieces, then ground to fine powder. About 2 kg of the material was extracted by successive soaking for 7 days each in organic solvents of different polarity, like petroleum ether, hexane, dichloromethane and methanol. The extracts were filtered and concentrated using rotary evaporator. After drying the extracts were stored at 4 °C for further analysis and biological activity determinations.

Rhizome of *Alpinia allughas*

Leaves of *Alpinia allughas*

Qualitative phytochemical screening: The phytochemical screening of all extracts of rhizomes and leaves of *A. allughas* was performed by the standard methods [12].

Detection of alkaloids: Extracts were dissolved individually in dilute hydrochloric acid and separated out by filtration. The filtrates were used to test for the presence of alkaloids.

Hager's test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Formation of a yellow coloured precipitate indicated the presence of alkaloids.

Detection of carbohydrates: Extracts were dissolved individually in 5 mL distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's test: Filtrates were treated with few drops of alcoholic α-naphthol solution in a test tube and 2 mL of conc. sulphuric acid was added carefully along the sides of the test tube. Formation of violet ring at the junction indicated the presence of carbohydrates.

Detection of glycosides: Extracts were hydrolyzed with dil. HCl and then subjected to test for glycosides.

Modified Borntrager's test: Different extracts were treated with ferric chloride solution and immersed in boiling water for about 5 min. The mixture was cooled and shaken with an equal volume of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicated the presence of anthranol glycosides.

Detection of saponins: The saponin were also tested for their presence.

Froth test: Extracts were diluted with distilled water and shaken in a graduated cylinder for some time. Formation of 1 cm layer of foam indicated the presence of saponins.

Detection of phytosterols: The phytosterols were tested by using following well known test.

Salkowski's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of conc. sulphuric acid, shaken and allowed to stand. The appearance of golden yellow colour indicated the presence of triterpenes.

Detection of fixed oils and fats: Following test was performed to detect the fixed oil and fats in extracts.

Stain test: Small quantities of extracts were pressed between two filter papers. An oily stain on filter paper indicated the presence of fixed oil.

Detection of resins: Following test was applied for the detection of resins in different extracts.

Acetone-water test: Extracts were treated with acetone. Small amount of water was added and shaken. The appearance of turbidity indicated the presence of resins.

Detection of phenols: To detect phenol in extracts ferric chloride test was performed.

Ferric chloride test: Extracts were treated with a few drops of ferric chloride solution. Formation of bluish black colour indicated the presence of phenols.

Detection of tannins: The tannins were detected by gelatin test.

Gelatin test: To the extract, 1 % gelatin solution containing sodium chloride were added. Formation of white precipitate indicated the presence of tannins.

Detection of flavonoids: Following test was performed for the detection of flavonoids in the extracts.

Alkaline reagent test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on the addition of dilute acid, indicated the presence of flavonoids.

Detection of proteins and amino acids

Biuret test: The extracts were treated with 1 mL of 10 % sodium hydroxide solution and heated. To this a drop of 0.7 % copper sulphate solution was added. Formation of purplish violet colour indicated the presence of proteins.

Detection of triterpenes

Copper acetate test: Extracts were dissolved in water and treated with a few drops of copper acetate solution. Formation emerald green colour indicated the presence of triterpenes

Total phenolic assay: The total phenolic content of the various extracts was estimated by using the Folin-Ciocalteu reagent with slight modification as reported earlier [13]. 0.5 mL of the extract solutions and oil were mixed with 1 mL of Folin-Ciocalteu reagent, 1.0 mL of aqueous solution of 7 % sodium carbonate and 5 mL of distilled water. The reaction mixture

was mixed thoroughly and was allowed to stand for 30 min. The absorbance was read at 765 nm. The standard curve was established using various concentrations of gallic acid. The total phenolic content was expressed as gallic acid equivalents (GAEs) in mg/g of dry material.

Estimation of flavonoids: 1 mL of plant extract was mixed with 1.25 mL of distilled water and 75 µL of 5 % sodium nitrite solution. The solutions were incubated for 5 min and then 150 µL of 10 % aluminium chloride solution was added. After 6 min 500 µL of 1 M sodium hydroxide and 275 µL of distilled water was added, after proper mixing of the solution the intensity of pink colour was obtained at 510 nm. The standard curve was established using various concentrations of catechin. The flavonoid content was expressed as catechin equivalents (CNE) in mg/g of dry material [14].

Estimation of *ortho*-dihydric phenols: 1 mL of the extract solution was taken and mixed with equal volume of 0.5 N HCl and 1 mL of Arnow's reagent, 2 mL of 1 N NaOH and 4.5 mL of distilled water was added. The solution were mixed thoroughly (pink colour was appeared) and the absorbance at 515nm was measured. The standard curve was established using various concentrations of catechol. The *ortho*-dihydric phenols content content was expressed as catechol equivalents (CLEs) in mg/g of dry material [15].

RESULTS AND DISCUSSION

Yield of different extracts of *A. allughas:* Extraction of botanical compounds from the plant materials is entirely dependent on the type of solvent and the extraction procedure. Table-1 represents the plant extracts with their % yield of dried rhizomes and leaves in different solvents (non-polar to polar sequentially). The % yield was found high in petroleum ether extract for leaves (0.78 %) and rhizome (0.83 %). Among other solvents used for study, methanolic extract showed minimum extraction value of 0.66 % for leaves and 0.67 % for rhizome. The other extracts that is hexane and dichloromethane extract showed moderate yield for both rhizome and leaves.

Phytochemical screening of rhizome and leaves of *A. allughas*: Preliminary phytochemical screening of the various extracts of rhizomes and leaves of *A. allughas* revealed the presence of various bioactive components which include glycosides, alkaloids, tannin, saponin, phytosterol, phenol and flavonoid, *etc*. The present investigation showed that methanolic extract of rhizomes and the leaves of *A. allughas* were certainly much better than the other extracts. This might be possibly due to the better solubility of the active compound in organic solvents of highest polarity [16]. Saponin, phytosterol, phenol and flavonoid was found in all the extracts (polar and non-polar) of both rhizomes and leaves of *A. allughas*. AARDE and AALDE showed the presence of the glycoside in the rhizome and leaves extracts of *A. allughas*. AARDE, AARME, AALDE and AALME were indicative for the presence of the carbohydrates. The presence of the tannin was indicated by the methanolic extract (AARME and AALME) of both the leaves and rhizome. However, the presence of the diterpene was indicated by the dichloromethane extract (AARDE and AALME) of both the rhizome and the leaves. The presence of the terpenoids, glycoside, flavonoids indicates the presence of the antioxidant and antimicrobial activity in this plant and can be used in the pharmaceuticals for the treatment of various diseases [17]. All the extracts of rhizomes and leaves showed the complete absence of the fat and oil as well as the resin. The results of comparative phytochemical screening of rhizomes and leaves are given in Table-2. The phytochemicals are reported to possess antioxidant, antimicrobial and pharmacological action properties. Some of the alkaloid are reported to possess the cardiovascular activity [18].

TABLE-1
YIELD OF DIFFERENT EXTRACTS OBTAINED FROM RHIZOME AND LEAVES OF *A. allughas*

Plant's name	Yield of extract (%)
A. allughas, rhizome petroleum ether extract (AARPE)	0.83
A. allughas, rhizome hexane extract (AARHE)	0.77
A. allughas, rhizome dichloromethane extract (AARDE)	0.73
A. allughas, rhizome methanolic extract (AMRME)	0.67
A. allughas, leaves petroleum ether extract (AALPE)	0.78
A. allughas, leaves hexane extract (AALHE)	0.72
A. allughas, leaves dichloromethane extract (AALDE)	0.68
A. allughas, leaves methanolic extract (AALME)	0.66

Total phenols, flavonoids and *ortho*-dihydric phenol: The total phenolic content in the rhizome and leaves extract of *A. allughas* was obtained in the range of 105.15 ± 5.006 to 306.66 ± 2.777 mg/g (GAE) (gallic acid equivalent) and 123.33 ± 1.892 to 411.81 ± 2.405 mg/g GAE, respectively. The highest phenolic content was observed in the AALME. The different

TABLE-2
PHYTOCHEMICAL SPECIFICATIONS OF DIFFERENT EXTRACTS OF RHIZOMES AND LEAVES OF *A. allughas*

Phytoconstituent	Test	AARPE	AARHE	AARDE	AARME	AALPE	AALHE	AALDE	AALME
Alkaloids	Hager's test	–	–	+	+	+	+	+	+
Carbohydrates	Molisch's test	–	–	+	+	–	–	+	+
Glycosides	Modified Borntrager's test	–	–	+	–	–	–	+	–
Saponins	Froth test	+	+	+	+	+	+	+	+
Phytosterols	Salkowski's test	+	+	+	+	+	+	+	+
Fats & Oils	Stain test	–	–	–	–	–	–	–	–
Resins	Acetone-water test	–	–	–	–	–	–	–	–
Phenols	Ferric Chloride test	+	+	+	+	+	+	+	+
Tannins	Gelatin test	–	–	–	+	–	–	–	+
Flavonoids	Alkaline Reagent test	+	+	+	+	+	+	+	+
Proteins & Aminoacids	Biuret test	–	–	–	–	–	–	–	–
Diterpenes	Copper acetate test	–	–	+	–	–	–	+	–

+ = Presence; – = Absence

TABLE-4
CORRELATION COEFFICIENTS (R) FOR RELATIONSHIPS BETWEEN DIFFERENT PHYTOCHEMICALS OF EXTRACTS OF RHIZOMES AND LEAVES OF *A. allughas*

Correlation coefficient (R)							
Alpinia allughas rhizome extracts				*Alpinia allughas* leaves extracts			
Total phenols	Total Phenols	Flavonoids	*o*-Dihydroxy phenol	Total phenols	Total Phenol	Flavonoids	*o*-Dihydroxy phenol
	1	0.972**	0.935**		1	0.982**	0.978**

**Significant at $\alpha = 0.01$

flavonoid content found in the various extracts of rhizome and leaves of *A. allughas* was determined spectrophotometrically based on the formation of flavonoid aluminium complexes which possess the maximum absorptivity at 510 nm. The total flavonoid content of the extracts varied from 20.12 ± 0.222 to 41.53 ± 0.769 mg/g CNE (catechin equivalents) and 27.17 ± 0.222 to 52.82 ± 0.587 mg/g CNE, respectively. AALME had the highest content of the flavonoids followed by AALDE > AARME > AALHE > AARDE > AALPE > AARHE > AARPE in that decreasing order. The *ortho*-dihydric phenol content found in the various extracts ranged from 32.00 ± 1.802 to 54.00 ± 0.5 mg/g CLE) (catechol equivalents) and 34.00 ± 0.500 to 53.83 ± 0.288 mg/g CLE, respectively in which AARME had the highest *ortho*-dihydric phenolic content followed by the other extracts (Table-3). In our earlier report we have already reported the total phenolic content, flavonoid content, *ortho*-dihydric phenolic content as well as the preliminary phytochemical screening (by TLC) of the rhizomes of *A. allughas* and phenolic content of leaves of *A. allughas* [19-21]. In the present investigation an attempt has been made in order to compare the efficacy of rhizome part as well as leaves part of *A. allughas*. Thus a comparison has been made in terms of the presence of different types of phytochemicals, difference in their quantification as well as difference in their correlations in between such parameters. While comparing the phenolic content, flavonoid content and *ortho*-dihydric phenolic content of the rhizome and leaves of *Alpina allughas* observed that the leaves were more rich in phytochemicals. The phytochemicals such as phenolics, flavonoid are reported to possess the antioxidant activity and radical scavenging activity [18,22,23]. We have already reported the efficacy of *A. allughas* in terms of their essential oil composition, essential elements, antifungal and antioxidant potential [20,21,24,25]. It has been reported by many workers that there exist a direct correlation among phenolic contents and antioxidant activity [20,21,26]. Some authors have also reported strong antioxidant potential in this plant [27,28]. In present communication it was observed that the leaves of *A. allughas* was found to be a rich source of phytochemicals and a better antioxidant source as compared to its rhizomes.

Correlation between phytochemicals of extracts of rhizomes and leaves of *A. allughas*: In present study the total phenols were correlated with flavonoid and *ortho*-dihydroxy flavonoid for both rhizome and leaves extracts of *A. allughas* separately. The results recorded in Table-4 showed good correlation of total phenols with flavonoids and *ortho*-dihydroxy phenol for both the rhizome and leaves of *A. allughas* at α = 0.01 (level of significance). The observed data again distinguishes the greater potency and efficacy of *A. allughas* leaves as compared to its rhizomes with a higher correlation coefficient (0.982** and 0.978**) among these parameters.

TABLE-3
TOTAL PHENOLIC, FLAVONOID CONTENT AND *o*-DIHYDRIC CONTENT OF VARIOUS EXTRACTS FROM RHIZOMES AND LEAVES OF *A. allughas*

Sample name	Total phenol (mg/g GAE)	Flavonoid (mg/g CNE)	*o*-Dihydric phenol (mg/g, CLE)
AARPE	105.15 ± 5.006^{a}	20.12 ± 0.222^{a}	32.00 ± 1.802^{a}
AARHE	128.18 ± 2.405^{b}	25.76 ± 1.017^{b}	41.50 ± 0.866^{c}
AARDE	206.36 ± 6.363^{c}	35.76 ± 0.384^{c}	48.33 ± 0.763^{d}
AARME	296.06 ± 1.049^{e}	41.53 ± 0.769^{d}	54.00 ± 0.5^{e}
AALPE	123.33 ± 1.892^{b}	27.17 ± 0.222^{b}	34.00 ± 0.500^{a}
AALHE	218.48 ± 1.388^{d}	37.30 ± 0.384^{c}	38.83 ± 0.577^{b}
AALDE	320.30 ± 1.388^{f}	45.00 ± 0.769^{e}	48.83 ± 0.763^{d}
AALME	348.18 ± 1.818^{g}	52.82 ± 0.587^{f}	53.83 ± 0.288^{e}

Values are means of three replicates ± SD. Within a column, mean values followed by the same letter are not significantly different according to Tukey's test ($p < 0.05$).

Conclusion

The presence of different phytochemicals that is alkaloid, carbohydrates, glycoside, saponin, phytosterol, phenol, tannin present in different extracts (polar and nonpolar) of rhizomes and leaves of *A. allughas* indicates the presence of good antioxidant potential and pharmacological properties in this plant. Due to the presence of higher phytochemicals and a good correlation in between phytochemicals present in leaves in comparison to rhizomes of *A. allughas* it can be concluded that leaves can be a good sources of natural antioxidants, food preservative to reduce the oxidative stress and for the development of new drug molecules.

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