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Qualitative phytochemical Screeing of Rhizomes On *Alpinia Calcarata* and *Alpinia Speciosa*

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Abstract
Rhizomes of *Alpinia calcarata* and *Alpinia speciosa* [Family: Zingiberaceae] commonly has been used in Ayurveda to treat different diseases such as antibacterial, anti-inflammatory, analgesic, diuretic and anticancer etc. This work was carried out to determine the potential applications of the rhizomes *Alpinia calcarata* and *Alpinia speciosa* by investigating its phytoconstituents. Hence this study, which attempt to both analyze the phytochemical composition and Percentage yield. Phytochemical analysis of the rhizomes extracted using soxhlet apparatus with different solvents revealed the presence of Polyphenols, Tannins, Flavonoids, Steroid, Glycosides and Alkaloids. Those results may be helpful for rationale use of this rhizomes in the modern system of health care.

Keywords: *Alpinia calcarata*, *Alpinia speciosa*, Phytochemicals, Percentage Yield.

1. Introduction
According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries used traditional medicine, which has compounds derived from medicinal plants [Aggarwal BB *et.al* 2007] [1]. Medicinal plants constitute the main source of new pharmaceuticals and healthcare products [Ivanova *et al.*, 2005] [2]. The use of medicinal plants in the industrialised societies has been traced to the extraction and development of several drugs from these plants as well as from traditionally used folk medicine [Shrikumar and Ravi, 2007] [3]. Extraction and characterization of several active Phytocompounds from these green factories have given birth to some high activity profile drugs. The use of herbal medicines has been on the rise in recent years due to their low prices. There is a common concept among people that herbal medicines have no side effects and that being natural in origin, herbs are safe.
Alpinia calcarata & *Alpinia speciosa* [Family: Zingiberaceae] is a rhizomatous perennial herb, which is commonly used in the traditional medicinal systems in Bangladesh, India, Indonesia, Thailand, Malaysia, SriLanka, Taiwan, Cambodia, and Vietnam . *Alpinia calcarata* is the mature rhizomes are branched and dense with a light to dark brown colour. The Leaf of the plant is simple, alternative 25 -35 cm long and 2.5 - 5 cm broad. Shell-flower is commonly called Shell ginger or Shell flower because its individual shell pink flowers, particularly when in bud, resemble sea shells. The fragrant flowers are waxy; light pink flower buds open to tubular flowers with yellow inside lips and red throats. The plant produces lance-shaped green leaves to 2' long and 5' wide.
Alpinia calcarata & *Alpinia speciosa* is widely used in Ayurvedic, Unani and Siddha Herbal System. *Alpinia calcarata* & *Alpinia speciosa* rhizomes are known to possess a broad spectrum of medicinal properties. Experimentally, rhizomes are shown to possess antibacterial, antifungal, anthelmintic, antinociceptive [Arambewela LSR, *et al*., 2004] [4], antioxidant [Arambewela LSR, *et al*., 2005] [5] aphrodisiac [Ratnasooriya WD, *et al*., 2006] [6], gastroprotective [Arambewella LSR, *et al*., 2009] [7], antidiabetic activities, rheumatism, fever, stomachache [The wealth of Indian raw materials, 2003] [8] and anticancer activity. It is also widely used to relieve colds and reducing swellings [Ahmed A D, *et al* 2005] [9] [Perveen R, *et al*., 2012] [10] etc. High blood pressure, Diuretic, stomach problems, analgesic [Leal-Cardoso, J.H *et al*., 2004] [11], anticandidal, antiplatelet, antispasmodic [Bezerra M.A, *et al*., 2000] [12] antiulcerous hypotensive [Lahlou S *et al*., 2003] [13] [de Moura R.S *et al*., 2005] [14] insecticidal, muscle relaxant and Uterine stimulant.
The main objective of our research work was to analyze the screening for Preliminary Phytochemical test for different solvents, Determination of Percentage Yield and Screening for

Preliminary Phytochemical test for ethanolic extract of *Alpinia calcarata* & *Alpinia speciosa* combined rhizomes.

2. Materials and Methods

2.1. Collection of the Sample

The present study included rhizomes of *Alpinia calcarata* & *Alpinia speciosa* were purchased from ABS Botanical Conservation Research & Training Centre Kaaripatti, Salem (Dt) T.N. India.

2.2. Preparation of plant extract

Fresh *Alpinia calcarata* & *Alpinia speciosa* rhizomes were washed in running water, cut into small pieces, and air dried for 12 - 15 days in the shade and coarsely powdered and used for extraction.

2.3. Extraction of plant material

The coarsely powdered rhizomes 100g was subjected to successive soxhlet extraction with solvents of increasing polarity Hexane, Petroleum ether, Ethyl acetate, Chloroform, Ethanol and aqueous respectively. Each extract were evaporated to dryness in a rotary evaporator. The extracts were preserved in an airtight container. Percentage yield was calculated for each extract. The condensed extracts were used for Preliminary Screening of Phytochemicals.

2.4. Preparation of the hot ethanolic extract

Fresh *Alpinia calcarata* & *Alpinia speciosa* rhizomes powdered 500g were extracted with 1.5 L of ethanol using soxhlet extraction apparatus for 3 days. Each extracts were evaporated to dryness in a rotary evaporator. The extracts were preserved in an airtight container. It was used for the qualitative analysis of secondary metabolites.

3. Identification Test

The combined rhizomes of ethanolic extract *Alpinia calcarata* & *Alpinia speciosa* (1:1) was subjected to the qualitative phytochemical screening for the presence of some chemical constituents. Phytochemical test were carried out adopting standards procedure [Hegde *et al*., 2010] [15].

3.1. Carbohydrate:

a) Fehling's Test: To 2 ml of the plant extract 1ml of a mixture of equal parts of Fehling's solution A & B were boiled for a few minutes. Formation of red of brick red precipitate indicated the presence of reducing sugar.

b) Benedict's Test: To 0.5ml of the extract, 5ml of Benedict's reagent was added and boiled for 5 minutes. Appearance of red, yellow or green colour precipitate showed the presence of reducing sugar.

c) Molisch's Test: To 2ml of the extract, 2 drops of freshly prepared 20% alcoholic solution of alpha naphthol was added mixed and 2ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of the violet ring at the junction of the solution and its disappearance on the addition of excess alkali solution indicated the presence of carbohydrate.

3.2. Proteins

a) Biuret Test: Test solution was treated with 10% sodium hydroxide solution and two drops of 0.1% copper sulphate solution and observed for the formation of violet / pink color.

3.3. Amino Acids

a) Ninhydrin Test: Test solution when boiled with 0.2% solution of Ninhydrin, would result in the formation of purple colour suggesting the presence of aminoacids.

3.4. Steroids

a) Salkowki's Test: 1ml extract was dissolved in 10ml of chloroform and equal volume of concentrated sulphuric acid was added carefully along the sides of the test tube. Red colour formed in the chloroform layer, indicated the presence of steroids.

3.5. Thiols

To 0.5ml of the extract, enough ammonium sulphate was added to saturate the solution, 2 -4 drops of 5% sodium nitroprusside was then added followed by one or more drops of concentrated nitric acid. Transient magenta colour developed indicated the presence of thilos.

3.6. Alkaloids

A quantity 3ml of concentrated extract was taken into a test tube and 1ml HCI was added the mixture was heated gently for 20 min cooled and filter, the filtrate was used for following test.

a) Wagner test: 1ml of the extract was treated with Wagner's reagent; formation of brown reddish precipitate indicates presence of alkaloids.

b) Dragendorff's test: 2 drops of Dragendorff's reagent were added to 1ml of the extract. The development of a creamy precipitate was indicative of the presence of alkaloids.

c) Hager's test: 1ml of the extract was treated with Hager's reagent, presence of alkaloids confirmed by the yellow colored precipitate.

3.7. Flavonoids

a) Alkaline reagent test: Extract was treated with 10% NaOH solution, formation of intense yellow colour indicates presence of Flavonoid.

b) NH_4OH Test: 3ml of extract were 10% NH_4OH solution development of yellow fluorescence indicates positive test.

c) Mg turning test: Extract were treated with Mg turning and add conc. HCL to this solution add 5ml of 95% ethanol, formation of crimson red colour indicates Flavonoid.

d) Zn test: 2ml extract were treated with Zn dust and conc. HCL development of red colour indicates presence of Flavonoid.

3.8. Phenols

a) Ferric Chloride test: Test extract were treated with 4 drops of Alcoholic $Fecl_3$ solution. Formation of bluish black colour indicate the presence of Phenols.

3.9. Saponins

a) Foam test: 5ml extract was mixed with 20ml of distilled water then agitated in graduated cylinder. For 15 min formation of foam indicates Saponins.

3.10. Cardial Glycosides

a) Legal's test: To the extract 1ml of pyridine and few drops of freshly prepared sodium nitroprusside solution were added, appearance of pink to red colour indicates presence of glycosides.

b) Keller-Killani test: Plant extract treated with 2ml glacial acetic acid containing a drop of $Fecl_3$. A brown colour ring indicates the presence of positive test.

3.11. Tannins

4ml extract was treated with 4ml $Fecl_3$ formation of green colour indicates that presence of condensed tannins.

4. Observations and Results

Table 1: Preliminary phytochemical test of different solvents in *Alpinia calcarata* rhizome

S. No	Test	Hexane	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Water
1.	Carbohydrates	-	-	-	-	+	+
2.	Proteins	-	-	-	+	+	+
3.	Steroids	+	+	+	+	+	+
4.	Thiols	-	-	-	-	-	-
5.	Alkaloids	-	-	-	+	+	-
6.	Flavanoids	-	+	+	+	+	+
7.	Phenols	-	-	-	-	+	+
8.	Saponins	-	-	-	-	-	-
9.	Glycosides	-	-	-	-	-	-
10.	Tanins	-	-	-	+	+	+

(+) = Present, (-) = Negative

Table 2: Preliminary phytochemical test of different solvents in *Alpinia speciosa* rhizome

S.no	Test	Hexane	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Water
1.	Carbohydrates	-	+	-	+	+	+
2.	Proteins	-	-	-	+	+	+
3.	Steroids	-	+	-	-	+	-
4.	Thiols	-	-	-	-	+	+
5.	Alkaloids	-	-	-	+	+	-
6.	Flavanoids	-	+	+	+	+	+
7.	Phenols	-	+	-	-	+	+
8.	Saponins	-	-	-	-	-	-
9.	Glycosides	-	-	-	-	-	-
10.	Tanins	-	+	-	_+	+	+

(+) = Present, (-) = Negative

Table 3: Percentage Yield in various solvents of *Alpinia calcarata* & *Alpinia speciosa* rhizomes.

S.no	Solvents	*Alpinia calcarata (%)*	*Alpinia speciosa (%)*
1.	Hexane	2.34	2.70
2.	Petroleum ether	0.06	1.50
3.	Chloroform	0.52	0.08
4.	Ethyl acetate	0.36	1.60
5.	Ethanol	2.64	5.28
6.	Water	1.24	1.96

Table 4: Phytochemical test for Ethanolic extract in *Alpinia calcarata* & *Alpinia speciosa* combined rhizomes.

S.no	Test	Ethanolic extract
1.	Carbohydrates	+
2.	Proteins	+
3.	Steroids	+
4.	Thiols	-
5.	Alkaloids	+
6.	Flavanoids	+
7.	Phenols	+
8.	Saponins	-
9.	Glycosides	+
10.	Tannins	-

(+) = Present, (-) =Negative

5. Discussion

The preliminary qualitative phytochemical screening of the crude powder of two rhizomes was done to assess the presence bioactive components. The presence of alkaloids, Flavonoids, Steroids, Tannins, Polyphenolic compounds and reducing sugars was determined. The results are shown in table 1, 2.

Since the rhizomes was found to more constituents. It would thus mean that in this study, the rhizomes ethanolic extract had the highest number of bioactive compounds. Since the yield of bioactive metabolites in a rhizomes extract also various considerably with the solvent of extraction it is plausible that the ethanolic extracts were generally more potent. This is in agreement with many literatures reporting of differences in the activities of rhizome extracts obtained from the same morphological part of a plant using different solvents.

According to the percentage yield in various solvents of *Alpinia calcarata* and *Alpinia speciosa* rhizomes were calculated. The extractive yield was considerably more in ethanol whereas Hexane, Petroleum ether, Chloroform, Ethyl acetate and Water extract gives less percentage. The results are shown in table 3.

The preliminary phytochemical reported that the ethanolic extract of *Alpinia calcarata* and *Alpinia speciosa* individually and combination of ethanolic extract *Alpinia calcarata*, *Alpinia speciosa* (1:1) the presence of alkaloids, flavonoids, phenols, steroids, tannind, proteins and carbohydrates. It was investigated that flavanoids were found to be present in *Alpinia calcarata* and *Alpinia speciosa* rhizomes have

excellent antioxidant activities and are important bioactive components in rhizomes which can cause inhibition of the oxidative modification of the human lipoprotein [Swapana N *et al.*, 2012] [16] while our studies also showed the same results that is flavonoids were detected in it. It also reveals that the various biological activities and therapeutic uses exhibited by the drug plant possess strong antioxidant activity. The results are shown in table 4.

6. Conclusion

Phytochemical screening of ethanolic extracts *Alpinia calcarata* and *Alpinia speciosa* rhizomes traditional Chinese medicinal plants [Cai Y *et al.* 2004] [17] had revealed the presence of flavonoids, alkaloids, phenols, steroids, tannins, proteins and carbohydrates by positive reaction with the respective test reagent. Results obtained in this investigation indicate that *Alpinia calcarata* and *Alpinia speciosa* rhizomes extract, rich in flavonoids exhibited highest antioxidant and reducing activities. Medicinal plants are used for discovering and screening of the phytochemical constituents which are very helpful for manufacturing of new drugs. The phytochemical analysis and present studied show nearly the similar results due to the presence of the constituents. The phytochemical analysis of the medicinal plants are also important and have commercial interest in both research institutes and pharmaceuticals companies for the manufacturing of the new drugs for treatment of various diseases.

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