

QUALITATIVE PHYTOCHEMICAL SCREENING TESTS OF ALPINIA GALANGA L.

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ABSTRACT

In this research work I have chosen the leaves and whole plant of *Alpinia galanga* (L.) are shade dried and pulverized and stored in an air tight container for future use. The crushed mass of leaves are defatted with petroleum ether for 12 hours at 60 to 80⁰C and carried out for the process of continuous hot extraction by soxhlet apparatus and decoction using the ethanol and water. To estimated the qualitative phytochemical constituents in crude mixture of aqueous extraction.

KEYWORDS: *Alpinia galanga* (L.), Hot soxhlet apparatus, Qualitative phytochemical tests.

INTRODUCTION

Medicinal plants and derived medicine are widely used in traditional cultures all over the world and they are becoming increasingly popular in modern society as natural alternatives to synthetic chemicals. In the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects.

The World Health Organization (WHO) estimated that approximately 80% of world population relies mainly on traditional medicines, mostly plant drugs in their health care. Today, Ayurveda coexists with modern system of medicine, and is still widely used and practiced. About 30% of the currently used therapeutics is of natural origin.

Alpinia galanga is also known as Greater galangal in English and Kulanjan in Hindi. Most of the South Indian physicians of traditional Ayurveda and Siddha medicine system use *Alpinia*

galanga to treat various kinds of disease including diabetes mellitus. The optimum time for harvesting *Alpinia galanga* was determined in Kerala, India during 1995-1999. Treatments consisted of harvesting at 3 month-intervals from 6 to 48 months after planting.

Harvesting the crop at 42 months after planting was the best for realizing maximum rhizome (45.4 t/ha) and oil (127.4 litres/ha) yields, and for obtaining oil of good quality (27.1% cineole [eucalyptol]). A substantial quantity of oil (127.4 litres/ha) was obtained from the roots (19.5 t/ha) 39 months after planting. The shoot yield (40.5 t/ha) and shoot oil yield (70.61 h/a) were highest at 18 months after planting. *A. galanga* reached a maximum height of 129.4 cm with more than 48 tillers per clump and 13 leaves per tiller in the experimental location.

MATERIALS AND METHODS

Collection, identification and authentication of plant material

An indigenous medicinal plant *Alpinia galanga* L. (Zingiberaceae) known by a local name called peddadumparashtram (Telugu). The fresh leaves of *Alpinia galanga* (L.) was collected in the month of June 2018 from village Pedur in Nellore district, Andhra Pradesh, India. The plant material was taxonomically identified and authenticated by expert botanist Dr. CVS Bhaskar, Principal / Lecturer in-charge, department of botany, V. R College, Nellore. The voucher specimen of the plant was deposited at the college for further reference.

Preparation of plant material

An indigenous medicinal plant *Alpinia galanga* L. (Zingiberaceae) the thoroughly washed leaves and whole plant of *Alpinia galanga* (L.) are shade dried and pulverized and stored in an air tight container for future use.

Preparation of Extract

Hot soxhlet extraction method

The authenticated leaves of *Alpinia galanga* (L.) are detached from the plant by hand picking washed with distilled water and cut in to bits. The leaves are carefully dried under the shade for 10 days to ensure complete dryness at room temperature. Leaves are kept in hot air oven at 45⁰C for 5 minutes and make crispy. The dried leaves were then subjected to size reduction, crush into coarse powder and further milled into a fine powder using an electric grinding machine.

The crushed mass of leaves are defatted with petroleum ether for 12 hours at 60 to 80°C and carried out for the process of continuous hot extraction by soxhlet apparatus and decoction using organic solvents such as ethanol and water.

A weighed quantity (60 gm) of the powder extracted with 500 ml of ethanol and water in ration of 70:30 for 16 to 18 hours at 40 to 60°C until it become colorless. The extracts subsequently concentrated by vacuum distillation until all the solvent has been removed to give an extract sample known as semisolid mass, kept in a petridish and stored in refrigerator until use.

Qualitative Phytochemical study

Qualitative screening of aqueous and ethanol extracts of leaves of *Alpinia galanga* (L.) (L.) are performed by using different standard methods for the detection of various classes of active phytochemical components.

Different test for each constituent

A.TEST FOR CARBOHYDRATES

The extract solution was made by suspending small amount of the extract in 10 ml of distilled water and filtered. The test solution was subjected to test for carbohydrates.

a) Molisch's test

To the extract solution, add few drops of alcoholic alpha naphthol (10g of alpha naphthol in 100 ml of 95% alcohol). A purple to violet color ring appears at the junction when 0.2 ml of concentrated sulfuric acid added slowly to the sides of the test tube.

b) Fehling's test

To the extract solution add equal volume of Fehling's A (Copper sulfate in distilled water) and Fehling's B (Potassium tartarate and Sodium hydroxide in distilled water) reagents and boiled, a brick red precipitate forms that indicates the presence of cuprous oxide, if reducing sugars are present.

c) Caramelisation

To the extract solution add strong sulfuric acid slowly down the side of the test tube, they undergo charring with the dehydration along with burning sugar smell, if carbohydrates are present.

d) Bromine water test

To the extract solution add 0.2 ml of bromine water gets decolorized by aldose but not by the ketose, if carbohydrates are present.

e) Borntrager's test

The layer was separated when chloroform was added to the extract solution. To this equal volume of dilute ammonia solution was added. Color change in the ammonia layer shows the presence of carbohydrates.

f) Benedict's test

To the extract solution add few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled on water bath for few minutes, a reddish brown precipitate forms if reducing sugars are present.

g) Selwinoff's test

To the extract solution add 2.5 ml of selwinoff's reagent and boiled for two minutes gives a red colored compound when linked with resorcinol. Fructose gives red color within half minute.

B. TEST FOR ALKALOIDS

To the extract solution add few drops of dilute hydrochloric acid, mix and filtered. The filtrate was subjected to different alkaloidal reagent tests.

a) Dragendorff's test

To the filtrate add few drops of dragendorff's reagent solution (potassium bismuth iodide); reddish brown precipitate indicates the presence of alkaloids.

b) Wagner's test

To the filtrate add few drops of wagner's reagent solution (iodine in potassium iodide), reddish brown precipitate indicates the presence of alkaloids.

c) Mayer's test

To the filtrate add few drops of mayer's reagent (potassium mercuric iodide solution), cream color precipitate indicates the presence of alkaloids.

d) Hager's test

To the filtrate add few drops of hager's reagent (saturated solution of picric acid), yellow color precipitate indicates the presence of alkaloids.

e) Tannic acid test

To the filtrate add 1 ml of 10% Tannic acid solution shows buff color indicates the presence of alkaloids.

C. TEST FOR FLAVONOIDS

a) To the extract solution add concentrated sulphuric acid yellowish orange colour indicates anthocyanins, orange to crimson indicates flavonones, yellow to orange colour indicates flavones.

b) To the extract solution add sodium hydroxide solution blue to violet colour indicates the presence of anthocyanins, yellow to orange colour indicates flavonones.

c) Millon's reagent test

To the extract solution add few drops of millon's reagent (Mercuric nitrate in nitric acid containing traces of nitrous acid). A white precipitate occurs, changes to red upon gentle heating.

d) Ferric chloride test

To the extract solution add few drops of neutral ferric chloride solution would result in the development of violet color or blackish red color indicates the presence of flavonoids.

e) Alkaline reagent test

To the extract solution add few drops of 20% sodium hydroxide solution, an intense yellow color appears, changes to colorless on addition of few drops of 20% hydrochloric acid that indicates the presence of flavonoids.

f) Lead acetate test

To the extract solution add few drops of 10% lead acetate solution. A formation of yellow precipitate indicates the presence of flavonoids.

g) Ammonia test

To the extract solution add few drops of 1% ammonia solution appearance of yellow color indicate the presence of flavonoids.

h) Shinoda test

To the extract solution add few turnings of magnesium and add concentrated hydrochloric acid in a drop wise manner. After few minutes a pink scarlet, rarely green to blue or crimson red color appears.

i) Zinc hydrochloride test

To the extract solution add a pinch of zinc dust and few drops of concentrated hydrochloric acid, after few minutes a red or magenta color indicates the presence of flavonoids.

D. TEST FOR GLYCOSIDES**General test**

The extract solution was hydrolyzed by dilute hydrochloric acid solution and neutralized by dilute sodium hydroxide solution. To this add 0.5 ml of Fehling's A and B solution, a red precipitate indicates the presence of glycosides.

Test 1

To 200mg of dried extract add 5 ml of 10% dilute sulphuric acid boil for two minutes and filter. The filtrate was neutralized with equal volume of 5% sodium hydroxide solution. Add 0.1 ml of Fehling's A and B solution until becomes alkaline and boil for two minutes. The quantity of red precipitate appeared was noted and compared with that formed in Test 2.

Test 2

To 200 mg of dried extract add 5 ml of distilled water boil for two minutes and filter. Add equal volume of distilled water and 0.1 ml of Fehling's A and B solution to the filtrate until becomes alkaline and boil for two minutes. The quantity of red precipitate appeared was noted and compared with that formed in Test 2. The quantity of red precipitate appeared was noted.

Compare the Test 2 precipitate with Test 1. If the Test 2 precipitate is more than that of Test 1, indicates the presence of glycosides. Because Test 1 signifies the amount of free reducing sugar previously present in the crude drug, whereas Test 2 signifies the glycoside after acid hydrolysis.

Test for different glycosides**a) Test for cardiac glycosides****i) Keller Killani test**

To the extract solution add few drops of glacial acetic acid and ferric chloride solution, mix and add concentrated sulfuric acid to the sides of the test tube. Formation of two layers was observed. Upper acidic layer changes to bluish green and lower reddish brown layer.

ii) Legal's test:

To the extract solution add pyridine (made alkaline by adding sodium nitroprusside solution) forms pink to red color.

iii) Baljet's test

To the extract solution add sodium picrate develops yellow to orange color.

iv) Bromine water test

To the extract solution add bromine water yellow precipitate develops.

v) Raymond's test

To the extract solution add dinitrobenzene in hot methanolic alkali forms violet color.

b) Test for anthraquinones glycosides**i) Borntrager's test**

To the extract solution add 5 ml of 10% sulphuric acid heat on water bath for 5 minutes, filter, cool the filtrate add equal volume of benzene shake gently for 2 minutes and a benzene layer was separated. To this add 10% ammonia solution, ammonical layer is separated shows rose to pink color indicates the presence of anthraquinones.

ii) Modified Borntrager's test

The extract solution is hydrolyzed by adding 5 ml of dilute hydrochloric acid and 5 ml of 5% ferric chloride solution. The hydrolyzed extract solution was carried out same procedure as described under Borntrager's test.

E. TEST FOR SAPONINS**a) Foam test**

i. About 0.3 g of the extract was vigorously shaken with 6 ml of distilled water in a test tube, if foam or frothing (appearance of creamy mass of small bubbles) stable for about 15 minutes indicates the presence of saponins.

ii. About 0.3 g of the extract was vigorously shaken with 6 ml of distilled water in a test tube stable persistent froth was formed. To this froth add 2 to 3 drops of olive oil and observe for the formation of emulsion indicates the presence of saponins.

b) Haemolysis test

To the test solution add 1ml of 1.8% sodium chloride solution and 2 to 3 drops of blood. Mix the contents gently and observe under the microscope for haemolysis that indicates the presence of saponins.

F. TEST FOR STEROLS & TRITERPENOIDS

About 0.5 g of extract is treated with chloroform, filtered and the filtrate was subjected to tests for sterols and triterpenoids.

a) Libermann Buchard test

To the chloroform extract solution add few drops of acetic anhydride boil and cool. Add concentrated Sulfuric acid from the sides of the test tube, a brown ring appears at the junction of two layers green color in the upper layer indicates the presence of sterols and formation of deep red color in the lower layer indicates the presence of triterpenoids.

b) Salkowski test

To the chloroform extract solution add concentrated sulphuric acid to the sides of the test tube shakes well and allow standing for few minutes. Two layers are formed at the junction, red color in the lower layer indicates the presence of sterols and yellow color in the upper layer indicates the presence of triterpenoids.

G. TEST FOR TANNINS**a) Ferric chloride test**

To the extract solution add 1 ml of 1% neutral ferric chloride solution shows green or violet color indicates the presence of tannins.

b) Gelatin test

To the extract solution add 1% gelatin solution containing 10% sodium chloride shows white precipitate indicates the presence of tannins.

c) Lead acetate test

To the extract solution add 10% lead acetate solution formation of white color indicates the presence of tannins.

d) Match stick test

Match stick is dipped in extract solution and air dried. Add a drop of concentrated hydrochloric acid on match stick and hold near the flame. It turns pink to purple and red color indicates the presence of tannins.

e) Alkaline reagent test

To the extract solution add 10% sodium hydroxide solution forms yellow to red precipitate indicates the presence of tannins.

f) Vanillin hydrochloride test

To the extract solution add 1 ml of vanillin hydrochloride reagent shows pink to red color indicates the presence of tannins.

H. TEST FOR PHENOLS**a) Ferric chloride test**

To the extract solution add 5% of dilute ferric chloride solution violet colour indicates the presence of phenols.

b) Zinc hydrochloride test

To the test solution add a pinch of zinc dust and few drops of concentrated hydrochloric acid, after few minutes a yellow or orange color indicates the presence of phenols.

c) Shinoda test

Add few fragments of magnesium ribbon into the test solution and also add concentrated hydrochloric acid in a drop wise set aside for few minutes, yellow or orange color indicates the presence of phenols.

I. TEST FOR PROTEINS AND AMINO ACIDS**a) Millon's test:**

To the extract solution add 2 to 3 ml of millon's reagent (mercuric nitrate in nitric acid containing traces of nitrous acid) forms white precipitate and changes to red upon gentle heating indicates the presence of proteins and free amino acids.

b) Ninhydrin test

To the extract solution add 0.2% ninhydrin (indane 1, 2, 3-trione hydrate) solution shows purple color indicates the presence of proteins and free amino acids.

c) Biuret test

To the extract solution add 1% copper sulphate solution forms pink or purple color indicates the presence of proteins and free amino acids.

J. TEST FOR QUINONES

About 0.2 g of the extract was treated with concentrated hydrochloric acid formation of yellow precipitate indicates the presence of quinones.

RESULTS**Qualitative phytochemical screening:**

The qualitative phytochemical screening was taken on to know the presence of different phytochemical constituents in hydroalcoholic extract of the leaves of *Alpinia galanga* (L.) by using standard method. The preliminary phytochemical screening of hydroalcoholic extract was revealed the highest amount of various medicinal active constituents like carbohydrates, alkaloids, flavonoids, glycosides, saponins, sterols and triterpenoids, tannins, phenols, proteins and amino acids and quinones respectively. Table No. 2.

PERCENTAGE YIELD:

The percentage yield of hydroalcoholic extracts of leaves of *Alpinia galanga* (L.) is 18.764 gm w/w. Table No. 1

Table No. 1: Percentage yield of leaf extracts of *Rumex vesicarius* (L.)

| S. No | Solvent | Weight of extracts (gm) | | Percentage yield (% w/w) |
|-------|-----------------|-------------------------|-------------------|--------------------------|
| | | Practical yield | Theoretical yield | |
| 1. | Hydro alcoholic | 20.64 | 110 | 18.764 |

Table No. 2: Phytochemical Investigation of Different Extracts of *Alpinia galanga* (L.) Leaf.

| S. No | | Chemical tests | Aqueous Extract |
|-------|---|--|--|
| 1. | | Tests for carbohydrates a) Molisch's test (general test) b) Caramelisation c) Bromine water test d) Borntrager's test | Positive Positive Positive Negative |
| | A | Tests for reducing sugars a) Fehling's test b) Benedicts test | Positive Positive |
| | B | Test for hexose sugars a) Selwinoff's test | Negative |
| 2. | | Tests for alkaloids a) Dragendorff's test b) Wagner's test c) Mayer's test d) Hager's test e) Tannic acid test | Positive Positive Positive Positive Negative |
| | A | Tests for flavonoids | |
| | B | Tests for anthocyanins a) Sulphuric acid test b) Sodium hydroxide test | Negative Positive |
| | C | Tests for flavonones a) Sulphuric acid test b) Sodium hydroxide test | Negative Positive |
| | D | Millon's reagent test | Negative |
| 3. | E | Ferric chloride test | Positive |
| | F | Alkaline reagent test | Positive |
| | G | Lead acetate test | Negative |
| | H | Ammonia test | Positive |
| | I | Shinoda test | Positive |
| | | Zinc hydrochloride test | Positive |
| | | | |
| 4. | A | Tests for glycosides Tests for cardiac glycosides a) Keller Killani test b) Legal's test c) Baljet's test d) Bromine water test | Negative Positive Positive Positive |
| | B | Tests for anthraquinones glycosides a) Borntrager's test b) Modified Borntrager's test | Positive Positive |
| | | Tests for saponins a) Foam test b) Haemolysis test | Positive Positive |
| | | Tests for sterols and triterpenoids a) Libermann buchard test b) Salkowski test | Positive |

| | | | |
|-----|--|---|--|
| | | | Positive |
| 7. | | Tests for tannins a) Ferric chloride test b) Gelatin test c) Lead acetate test d) Match stick test e) Alkaline reagent test | Positive Positive Negative Positive Negative |
| 8. | | Tests for phenols a) Ferric chloride test b) Zinc hydrochloride test c) Shinoda test | Positive Positive Positive |
| 9. | | Tests for proteins and amino acids a) Millons test b) Ninhydrin test c) Biuret test | Positive Positive Positive |
| 10. | | Test for quinones a) Hydrochloric acid test | Positive |

Note: ('+') Positive indicates presence; while ('-') Negative indicates for absence.

DISCUSSION

The present research work takes account of the phytochemical investigation and pharmacological evaluation of leaves of *Alpinia galanga* (L.) for nephroprotective activity.

Phytochemical screening

The therapeutic value of plants lies in several chemical substances has a distinct physiological action on the human body. Different phytochemicals have been found to acquire an extensive range of activities to protect against chronic diseases. For instance, Alkaloids have medicinal uses for centuries as it has familiar biological activity of cytotoxicity and also protect against chronic diseases. Flavonoids obtain a wide range of therapeutic properties such as antioxidants, antimicrobial, antirheumatism, antihypertensive and diuretic. Flavonoids are rich in vegetables that aid in reducing the frequency of cancer of different organ systems and facilitates in health promoting disease preventing dietary compound. Glycosides are naturally cardioprotective drugs used in the treatment of congestive heart failure, cardiac arrhythmia and have been useful in the treatment of asthma. Saponins are accountable for its anti-inflammatory, antidote, antiyeast, antimicrobial, antifungal and also have a role to protect against hypercholesterolemia and antibiotic activities. They also have the function of precipitating and coagulating red blood cells. Other features of saponins include bitterness, hemolytic activity, cholesterol binding activity and foam formation in aqueous solutions. Steroids and triterpenoids are known to produce antibacterial effect and antianalgesic effect on central nervous system. These are imperative compounds

particularly with compounds such as sex hormones. The phenolic compounds like flavonoids and tannins are a major group of compounds which are known for primary antioxidants or free radical scavengers. Since these compounds were found in the leaves of *Alpinia galanga* (L.). Hence it might be answerable for the effective nephroprotective activity.

CONCLUSION

The present study revealed the *Alpinia galanga* (L.) has more active substances like, Alkaloids, Glycosides, Flavonoids, Carbohydrates, Phenols, Tannins, Saponins, Proteins and Amino acids, Quinones, sterols, anthraquinones, Triterpenoids. These phytoconstituents seemed to have a potent drug for various diseases.

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