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A REVIEW PAPER ON PHARMACOGNOSTIC AND PHARMACOLOGICAL ACTIVITY OF AMORPHOPHALLUS PAEONIIFOLIUS

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ABSTRACT

The present study was aimed to investigate phytochemicals present in the tuber extract of *Amorphaphallus paeoniifolius* and development of new solvent system for thin layer chromatography of petroleum ether and ethanol extract of *Amorphaphallus paeoniifolius* tuber.Initially dried powder of *Amorphaphallus paeoniifolius* was extracted successively in Petroleum ether, Chloroform, Methanol and water and tested for the presence of different phytoconstituents. The tuber of *Amorphophallus paeoniifolius* contain phytoconstituents like alkaloids, steroids, fats & fixed oil, flavonoids, tannins, proteins and carbohydrates. Among the four extracts only the Petroleum ether and methanol extract were proceed to T.L.C. For TLC, new solvent system developed for the best separation of the phytoconstituents present in the extracts. The solvent system selected for the best results of TLC were Chloroform: Benzene: Diethyl ether of the ratio 2: 2: 0.5 for petroleum ether extract and Formic acid: Acetic acid: water of the ratio 100:11:11:27 for methanol extract. TLC resulted in identification of 3 spots for each solvent system.

KEYWORDS: *Amorphophallus paeoniifolius,* ethanol extract, Petroleum ether extract, Thin Layer Chromatography.

INTRODUCTION

Amorphophallus paeoniifolius known as Elephant foot yam is basically a crop of south East Asian origin. In India, it is commonly known as "*Suran*" or "*Jimmikand*". It grows in wild form in Philippines, Malaysia, Indonesia and other South East Asian countries. This tuber is consumed by many people as a food and widely used in many Ayurvedic preparations .In recent years the popularity of complementary medicine has increased. Over 50% of all modern drugs are natural product origin and they play an important role in drug development programs of the pharmaceutical industry. Epidemiological evidence suggests that dietary factors play an important role in human health and in the treatment of certain chronic diseases including cancer. The tubers of wild plants are highly acrid and cause irritation in throat and mouth due to excessive amount of calcium oxalate present in the tubers. The tubers are anodyne, anti-inflammatory, anti-haemorrhoidal, haemostatic, expectorant, carminative, digestive, appetizer, stomachic, anthelmintic, liver tonic, aphrodisiac, emmenagogue, rejuvenating and tonic. They are traditionally used in arthralgia, elephantiasis, tumors, inflammations, hemorrhoids, hemorrhages, vomiting, cough, bronchitis, asthma, anorexia, dyspepsia, flatulence, colic, constipation, helminthiasis hepatopathy, splenopathy, amenorrhea, dysmenorrhoea, seminal weakness, fatigue, anemia and general debility. The tuber is reported to have antiprotease activity, CNS depressant activity, analgesic activity, and cytotoxic activity.

MATERIALS AND METHODS

Plant material and its extract

Amorphophallus paeoniifolius (Araceae) tuber was boughrt from Asansol market, West Bengal, India in month of September 2007. The tuber was identified by the Botanical Survey of India, Botanical Garden, Howrah with ref no. CNH/I-I/ (272)/ 2008/ Tech. II/ 314. The tuber of the plant was dried under shade and made to a fine powder using a laboratory mill. The powdered drug (approx. 200 gm.) were then packed in the soxhlet apparatus and was extracted successively with petroleum ether (40-60) (Merck, India), chloroform (Merck, India), methanol (Merck, India) and distilled water. After completion of total, the extracted powder was discarded and the different extracts so obtained were further processed. The excess solvent in the extracts were removed by distillation and the concentrated extracts so obtained were further dried at a temperature not exceeding 40^0 C in water bath. The extracts were then collected kept in Petridish and stored in a dessicator at room temperature. The yield values and other physical properties were observed.

Preliminary photochemical screening

It involves testing of different extracts of *Amorphophallus paeoniifolius* for their contents of different classes of compounds. The methods used for detection of various phytochemicals were followed by qualitative chemical test to give general idea regarding the nature of constituents present in crude drug. The qualitative chemical tests for various phytoconstituents were carried out for all the extracts of *Amorphophallus paeoniifolius* as explained below.

A **Test For Alkaloids**

Mayer's Test

Alkaloids give cream color precipitate with Mayer's reagent [Potassium mercuric iodide solution].

Dragendorff's test

Alkaloids give reddish brown precipitate with Dragendorff's reagent [Potassium bismuth iodide solution].

Wagner's test

Alkaloids give a reddish brown precipitate with Wagner's reagent [Solution of iodine in potassium iodide].

Hager's test

Alkaloids give yellow color precipitate with Hager's reagent [saturated solution of Picric acid].

Tannic acid test

Alkaloids give buff color precipitate with 10% Tannic acid solution.

B. Test for Glycosides

The extracts were tested for free sugars

The extract is hydrolyzed with mineral acid and then tested for the glycone and aglycone moieties.

Raymond's test

Test solution when treated with dinitro- benzene in hot methanolic alkali, gives violet color.

Legal's test

Treat the extract with pyridine and add alkaline sodium nitroprusside solution, blood red color appears.

Bromine water test

Test solution when treated with bromine water gives yellow precipitate.

Chemical Tests For Specific Glycosides

Test For Saponin Glycosides

Froth Test

Place 1ml solution of drug in water in a semi-micro tube and shaken well and noted for a stable froth.

Hemolysis test

Add 0.2ml solution of saponin (prepared in 1% normal saline) to 0.2ml of v/v blood in normal saline and mix well, centrifuge and note the red supernatant compare with control tube containing 0.2ml of 10% blood in normal saline diluted with 0.2ml of normal saline.

Test For Anthrauinone

Glycosides Borntrager's Test

Boil the test material with 1ml of dilute sulphuric acid in a test tube for 5min (anthracene glycosides are hydrolyzed to aglycone and sugars by boiling with acids) centrifuge or filter while hot (if centrifuged hot, the plant material can be removed while anthracene aglycones are still sufficiently soluble in hot water, they are however insoluble in cold water), pipette out the supernatant or filtrate, cool and shake with an equal volume of dichloromethane (the aglycones will dissolve preferably in dichloromethane) separate the lower dichloromethane layer and shake with half its volume with dilute ammonia. A rose pink to red color is produced in the ammonical layer (aglycones based on anthroquinones give red color in the presence of alkali).

Modified Borntrager's test

Boil 200mg of the test material with 2ml of dilute sulphuric acid, 2ml of 5% aqueous ferric chloride solution for 5min and continue the test as above. As some plant contains anthracene aglycone in a reduced form, if ferric chloride is used during the extraction, oxidation to anthroquinones takes place, which shows response to the Borntrager's test.

Test For Cardic Glycosides

Kedde's Test

Extract the drug with chloroform, evaporate to dryness, add one drop of 90% alcohol and 2 drops of 2% 3,5-dinitro benzoic acid(3,5-dinitro benzene carboxylic acid-Kedde's reagent) in 90% alcohol. Make alkaline with 20% sodium hydroxide solution. A purple color is produced. The color reaction with 3, 5-diinitrobenzoic acids depends upon the presence of an β- unsaturated-o lactones in the aglycone.

Keller killiani test [test for Deoxy sugars]

Extract the drug with chloroform and evaporate it to dryness. Add 0.4ml of glacial acetic acid containing a trace amount of ferric chloride. Transfer to a small test tube; add carefully 0.5ml of concentrated sulphuric acid by the side of the test tube, blue color appears in the acetic acid layer.

Test for Cyanogenetic Glycosides

Place 200mg of drug in a conical flask and moisten with few drops of water, there should be no free liquid at the bottom of the flask (the test will not work if there is any liquid in the flask as the hydrogen cyanide produced will dissolve in the water rather than, come off as a gas to react with the paper). Moisten a piece of picric acid paper with sodium carbonate solution (5% aqueous) and suspended by means of cork in the neck of the flask, warm gently at about 37°C. Observe the change in color. Hydrogen cyanide is liberated from cyanogenetic glycoside by the enzyme activity and reacts with sodium picrate to form the reddish purple sodium isopicrate.

C.Test For Tannins And Phenolic

Compounds Gelatin Test

Test solution with 1 % gelatin solution containing 10% sodium Chloride gives white precipitate.

Ferric chloride test

Test solution gives blue green color with ferric chloride.

Vanillin Hydrochloride test

Test solution when treated with few drops of vanillin hydrochloride reagent gives purplish red color. Tannins get precipitated in the solution when treated with heavy metals.

Tannins yield bulky precipitate with phenazone especially in the presence of Sodium and phosphate.

Alkaline reagent test

Test solution with sodium hydroxide solution gives yellow to red precipitate within short time.

Mitchell's test

With iron and ammonium citrate or iron and sodium tartarate. Tannins give a water-soluble iron-tannin complex,which is insoluble in solution of Ammonium acetate.

D.Test for Flavonoids

Shinoda test (Magnesium Hydrochloride reduction test)

To the test Solution, add few fragments of Magnesium ribbon and add concentrated Hydrochloric acid drop wise, pink scarlet, crimson red or occasionally green to blue color appears after few minutes.

Zinc Hydrochloride reduction test

To the test solution add a mixture of Zinc dust and conc. Hydrochloric acid. It gives red color after few minutes.

Alkaline reagent test

To the test solution add few drops of sodium hydroxide solution; formation of an intense yellow color, which turns to Colorless on addition of few drops of dil. acid, indicates presence of Flavonoids.

E.Test for Proteins & Amino Acids

Millons test

Test solution with 2ml of Millons reagent (Mercuric nitrate in nitric acid containing traces of nitrous acid), whiteprecipitate appears, which turns red upon gentle heating.

Ninhydrin test

Amino acids and Proteins when boiled with 0.2% solution of Ninhydrin (Indane 1, 2, 3 trione hydrate), Violet colorappears.

F.Test for Sterols & TriterpenoidsLibermann-

Buchard test

Extract is treated with few drops of acetic anhydride, boil and cool, con. Sulfuric acid is added from the sides of the test tube, shows a brown ring at the junction of two layers and the upper layer turns green which shows the presence of Steroids and formation of deep red color indicates the presence of triterpenoids.

Salkowski test

Treat extract in Chloroform with few drops of cone. Sulfuric acid, shake well and allow standing for some time, red color appears at the lower layer indicates the presence of Steroids and formation of yellow colored lower layer indicates the presence of Triterpenoids.

G. Test for Carbohydrates

Molisch's test

Treat the test solution with few drops of alcoholic alpha napthol. Add 0.2ml of con. Sulfuric acid slowly through the sides of the test tube, a purple to violet color ring appears at the junction.

Benedict's test

Treat the test solution with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and upon boiling on water bath, reddish brown precipitate forms if reducing sugars are present.

Camnelisation

Carbohydrates when treated with strong sulfuric acid, they undergo charring with the dehydration along with burning sugar smell.

Selwinoff’s test

Hydrochloric acid reacts with ketose sugar to form derivatives of furfuraldehyde, which gives red colored compound when linked with resorcinol. Add compound solution to about 5ml of reagent and boil. Fructose gives red color within half minute. The test is sensitive to 5.5mmol / liter if glucose is absent, but if glucose is present it is less sensitive and in addition of large amount of glucose can give similar color.

Fehling's test

Equal volume of Fehling's A (Copper sulfate in distilled water) and Fehling's B (Potassium

tartarate and Sodium hydroxide in distilled water) reagents are mixed and few drops of sample is added and Boiled, a brick red precipitate of cuprous oxide forms, if reducing sugars are present.

H. Test for Fats & Fixed Oils Stain test

Press the small quantity of extract between two filter papers, the stain on I filter paper indicates the presence of fixed oils.

Saponification test

Add a few drops of 0.5N of alcoholic potassium hydroxide to small quantities of various extracts along with a drop of Phenolphthalein separately and heat on a water bath for 1-2 hrs. The formation of soap or partial neutralization of alkali indicates the presence of Fixed oils and Fats.

Thin Layer Chromatography

Thin layer chromatography (TLC) is a chromatography technique used to separate mixtures. Thin layer chromatography is performed on a sheet of glass, plastic, or aluminum foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide, or cellulose. This layer of adsorbent is known as the stationary phase. After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobilephase) is drawn up the plate via capillary action. Because different analytes ascend the TLC plate at different rates, separation is achieved. The petroleum ether and methanol extracts of the *Amorphophallus paeoniifolius* were subjected to thin layer chromatographic analysis, to find the presence of number of chemical constituents to support the chemical test.

The details of procedure are as following

Analytical TLC plates were prepared by pouring the silica gel G slurry on the glass plates. Prepared chromo plates were then divided of the liquid associated with thin layer by drying the thin layer plates, for 30 minutes in air and then in an oven at 110^0C for another 30 minutes. The petroleum ether extract in petroleum ether solvent and methanol extract in methanol solvent were applied as a single spot in a row along one side of chromo plate, about 2 cm from the edge, by using capillary tubes [13]. To make a choice of suitable solvent system, firstly elutropic series of different solvents was tried by running on the TLC plate8. TLC plate containing the sample spot was placed at 45 ^{0} angle in the development chamber covering the bottom of the plate by the solvent up to nearly 1 cm. The solvent front was

marked and the plate was finally allowed to dry. The colored substances were visual on the chromatogram. Colourless components were detected by using visualizing agent, iodine vapours. The qualitative evaluation of the plate was done by determining the migrating behavior of the separated substances given in theform of R_f value.

RESULT AND DISCUSSION

The physical properties of the extracts of the tuber. All the extracts were almost pasty in nature with characteristic smell. Percentage of yield of the petroleum ether, chloroform, methanol and aqueous extracts of *Amorphophallus paeoniifolius* were 1.25, 2.01, 3.2, 5.29 respectively. Table 2 represents the various phytochemicals present in different extracts. The petroleum ether extract contain alkaloids, steroids, fats & fixed oil. The chloroform extract contain alkaloids. The methanol extract contain alkaloids, steroids, flavonoids and carbohydrates. The aqueous extract contains flavonoids, tannins, proteins and carbohydrates. The solvent system selected for the TLC of petroleum ether extract (Fig.1) was Chloroform: Benzene: Diethyl ether of the ratio 2: 2: 0.5. TLC resulted in identification of 3 spots with the R_f Value 0.96, 0.704, 0.3. The solvent system selected for the TLC of methanol extract (Fig. 2) was Ethyl acetate: Formic acid: Acetic acid: water of the ratio 100:11:11:27. TLC also resulted in identification of 3 spots with the R_f Value 0.148, 0.531, 0.851.

CONCLUSION

The tubers of *Amorphophallus paeoniifolius* contain phytoconstituets like alkaloids, steroids, fats & fixed oil, flavonoids, tannins, proteins and carbohydrates. The TLC results of the petroleum ether extract and methanol extract show that at least three different phytoconstituents were present in each extract of *Amorphophallus paeoniifolius* tuber. More detailed study must be done for farther isolation leading to the pure compounds.

The physical properties of the extracts of the tuber.

Physical characteristics	**Petroleum ether extract**	**Chloroform extract**	**Methanolextract**	**Aqueous extract**
Percentage yield	1.25	2.01	3.2	5.29
Color	Pale yellow	Yellowish brown	Reddish	Reddish brown
Odour	Characteristic	Characteristic	Characteristic	Characteristic
Consistency	Viscous	Viscous	Viscous pasty mass	Highly Viscous

Phytochemical Tests for various extracts.

Phytochemicals		Petroleum extract	Chloroform extract	Methanol Extract	Aqueous Extract
Alkaloids	Mayer's Test	+	+	++	-
	Dragendorff's Test	+	+	+	-
	Wagner's Test	-	+	+	-
	Hager's Test	+	+	+	-
	Tannic Acid Test	+	+	+	-
Glycosides	Raymond's Test	-	-	-	-
	Legal's Test	-	-	-	-
Tannins	Gelatin Test	-	-	-	+
	Ferric Chloride Test	-	-	-	+++
	Alkaline Reagent Test	-	-	-	++
Flavonoids	Shinoda Test	-	-	++	+
	Zinc Hydrochloride Reduction Test	-	-	+++	++
	Alkaline Reagent Test	-	-	++	++
Proteins and Amino Acids	Millon's Test	-	-	-	++
	Ninhydrin Test	-	-	-	+++
Sterol and terpenoid	Libermann-Buchard test	++	-	+	-
	Salkowski test	++	-	+	-
Carbohydrates	Molisch's test	-	-	-	+
	Benedict's test	-	-	-	+
	Camnelisation	-	-	-	+
	Selwinoff's test	-	-	-	+
	Fehling's test	-	-	-	++
Fats & Fixed Oils	Stain test	+	-	-	-
	Saponification test	++	-	-	-

+ = Present, - = absent.

TLC Plate of the Petroleum ether extract.

The solvent system for the TLC of petroleum ether extract was Chloroform: Benzene: Diethyl ether of the ratio 2: 2:

0.5. The R_f values of the 3 distinct spots were 0.96, 0.704, 0.3.

TLC Plate of the Methanol extract.

The solvent system selected for the TLC of methanol extract was Ethyl acetate: Formic acid: Acetic acid: water of the ratio 100:11:11:27. The R_f values of the 3 distinct spots were 0.148, 0.531, 0.851.

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