

# Preliminary Phytochemical Analysis of Leaf, Stem, Root and Seed Extracts of Arachis hypogaea L.

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#### ABSTRACT

The species *Arachis hypogaea* L. (peanut/groundnut) belongs to the Leguminous family possess rich in proteins and oils. However, it also contains some of the phytochemicals, which are useful in pharmaceutical industry. The phytochemicals present in peanut, have been screened from the extracts of different parts of the plant such as leaf, stem, root and seed by using various solvents. The phytochemical analysis showed the presence of alkaloids, lignins, fats and oils, whereas the tannins, flavonoids, sterols and quinones were found to be negative. The glycosides, phenols, saponins were feebly detected in all the types of extracts used. The present screening of phytochemicals in various parts of peanut plant suggest that these can also be used as food supplements to reduce the anti nutritional effects.

Keywords: Arachis hypogaea, Phytochemical analysis, Bioactive compounds, Fluorescence analysis.

#### **INTRODUCTION**

Foundnut/Peanut (*Arachis hypogaea L.*) is one of the major oil seed crops. It is cultivated worldwide and in India it is occupying about 7.0 million hectares area. Groundnuts are a good source of oil and fat ranging from 36 to 54%<sup>1</sup>. Among the fat, higher amounts of unsaturated fatty acids contain compared to saturated fatty acids<sup>2</sup>.

Groundnut contributes significantly to food security and alleviates poverty and the leaves and pods can be introduced into the soil as green manure or nitrogen source<sup>3</sup>. They can be rotated with other crops for soil improvement too. Hence, they are used to replenish soil that has been depleted of nitrogen<sup>4</sup>. Peanut is an enrich source containing fats, vitamins, minerals and dietary fibre with high calorific value and also rich in phytochemicals<sup>5</sup>, antinutrients, allergens and toxins which limit their frequent use as food supplements for long time<sup>6-7</sup>.

The literature survey revealed that the phytochemical analysis of seeds have been studied thoroughly<sup>8</sup>, whereas no work has been done on the other plant parts such as leaves, stem and root of peanut. Hence, the present investigation has been under taken to find out the various phytochemicals present in them for the purpose of using these bioactive substances in different food items as supplements to reduce the anti nutritional effects.

#### **MATERIALS AND METHODS**

#### **Plant Material**

The leaves, stem, roots and seeds of *A. hypogaea* were collected from the Research field, Department of Biotechnology, Kakatiya University, Warangal, Andhra Pradesh, India in August, 2012. The plant material was washed thoroughly with distilled water to remove surface

contaminants and was shade dried for 30 days. Each sample of the material was ground separately into fine powder using an electric blender and stored in air tight containers at ambient temperature.

## **Preparation of Crude Extracts**

50 ml of petroleum ether, methanol, benzene, chloroform and water (aqueous) were taken into sterilized conical flasks of 100 ml capacity then added 1 gm of each dried powdered sample. It was incubated for 72 hours. Later the extracts were filtered through Whatman No.1 filter paper. The supernatants were collected separately, labeled and used for the screening of various phytochemicals.

#### **Phytochemical Analysis**

The standard qualitative procedures are used for the identification of following bioactive compounds present in the leaves, stem, root and seed extracts of peanut<sup>9-14</sup>.

#### Tests performed for the presence of phytoconstituents

#### a) Tests for Alkaloids

**1) Dragendorff's test:** To 1 ml of each of the sample solution taken in a test tube few drops of Dragendorff's reagent (potassium bismuth iodide solution) was added. A reddish brown precipitate was observed indicating the presence of alkaloids.

**2)** Meyer's test: To 1ml of each of the sample solution few drops of Meyer's reagent (potassium mercuric chloride solution) was added. A creamish white precipitate was formed indicating the presence of alkaloids.

**3) Wagner's test:** To few ml of each of the sample solution, Wagner's reagent (lodine in potassium iodide) was added, which resulted in the formation of reddish brown precipitate indicating the presence of alkaloids.



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**4) Hager's test:** To 1 ml of each of the sample few drops of Hager's reagent (Picric acid) was added. Yellow precipitate was formed reacting positively for alkaloids.

**5) Tannic acid test:** When few ml of 10% Tannic acid was added to 1 ml of each sample, a buff colour precipitate was formed giving positive result for alkaloids.

**6)** FeCl<sub>3</sub> test: One drop of FeCl<sub>3</sub> solution was added to each of the test sample, formation of yellow precipitate was resulted reacting positively for alkaloids.

## b) Tests for Glycosides

**1. Raymond's test:** Test solution when treated with dinitrobenzene in hot methanolic alkali giving a violet colour.

**2. Legal's test:** When the test samples were treated with pyridine and sodium nitroprusside solution blood red colour appears.

**3. Bromine water test:** When treated with bromine water test solution gives yellow precipitate.

**4. Kellar Kiliani test:** 1ml of concentrated sulphuric acid was taken in a test tube then 5ml of extract and 2ml of glacial acetic acid with one drop of ferric chloride were added, reaction shows formation of a blue colour.

**5. Concentrated Sulphuric acid test:**  $Conc.H_2SO_4$  was added to test sample which resulted in appearance of reddish colour.

**6.** Molisch test: When alpha naphthol and concentrated  $H_2SO_4$  were added to test samples reddish violet ring at junction of two layers was resulted.

## c) Tests for Tannins and Phenolic Compounds

**1. Ferric chloride test:** When few drops of ferric chloride were added to sample solution a blackish precipitate appears.

**2. Gelatin test:** When gelatin and water were added to test samples formation of white precipitate was resulted.

**3. Lead acetate:** Few ml of test samples were taken in different test tubes followed by the addition of aqueous basic lead acetate, results in the formation of reddish brown bulky precipitate.

**4. Alkaline reagent:** When sodium hydroxide solution was added to the sample solution results in the formation of yellow to red precipitate.

**5. Mitchell's test:** Tannins give a water soluble irontannin complex with iron and ammonium citrate or iron and sodium tartarate.

**6. Ellagic acid test:** When 5% glacial acetic acid and 5% sodium nitrite were added to test samples a muddy niger brown colour appears, which is a positive result for phenols.

## d) Tests for Flavonoids

**1. Zinc Hydrochloride reduction test:** To test the sample solution for the flavonoids added a mixture of zinc dust and concentrated hydrochloric acid results in red colour.

**2. Lead acetate test:** When aqueous basic lead acetate was added to test sample produces reddish brown precipitate.

**3. Ferric chloride test:** To few ml of test samples taken separately, few drops of ferric chloride were added which resulted in the formation of blackish red precipitate.

**4.** Shinoda test (Magnesium hydrochloride reduction test): To the test solution few fragements of magnesium ribbon and concentrated hydrochloric acid were added drop wise and reddish to pink colour was resulted.

**5. Alkaline reagent test:** When sodium hydroxide solution was added to the test samples formation of intense yellow colour, which turns to colour less on addition of few drops of dilute acid indicates the presence of flavonoids.

## e) Tests for Sterols

**1. Libermann-Buchard test:** When samples were treated with few drops of acetic anhydride, boiled and few drops of concentrated sulphuric acid from the sides of the test tube were added, shows a brown ring at the junction of two layers and the upper layer turns green which shows the presence of steroids.

**2. Salkowski test:** Few drops of concentrated sulphuric acid were added to the test samples in chloroform, a red colour appears at the lower layer indicates the presence of sterols.

# f) Tests for Fats and Oils

**1. Stain test:** Press the small quantity of each extract between two filter papers, the stain on filter papers indicates the presence of the oils.

**2. Saponification test:** Added a few drops of 0.5N alcoholic potassium hydroxide to various extracts with a drop of phenolphthalein separately and heat on water bath for 1-2 hours, formation of soap or partial neutralization of alkali indicates the presence of oils and fats.

# g) Tests for Lignins

**1. Labat test:** When gallic acid is added to the test sample, it results in the formation of olive green colour.

**2. Furfuraldehyde test:** When furfuraldehyde is added to the test sample a red colour appears indicating the presence of lignin.

## h) Tests for Quinones

**Alcoholic KOH test:** When alcoholic KOH was added to the test samples red to blue colour appears reacting positively for quinines.



## i) Tests for Saponins

**1. Foam test:** 5ml of extract was shaken vigorously to obtain a stable persistent froth. The froth was then mixed with three drops of olive oil and observed for the formation of an emulsion, which indicated the presence of saponins.

## RESULTS

The preliminary phytochemical screening of leaf, stem, root and seed extracts of different solvents viz., aqueous, benzene, chloroform, methanol and petroleum ether of *A. hypogaea* have been carried out for the identification and analysis of biologically active phytochemicals by using standard procedures<sup>9-14</sup>. Fluorescence analysis of all the extracts under UV and normal light is presented in table 1. Various solvents have shown the different colors under UV and normal light fluorescence.

#### Leaf extracts

Phytochemical analysis of various solvent extracts of leaf of *A. hypogaea* revealed the presence of alkaloids, glycosides, fats, oils, phenols, lignins, whereas tannins, flavonoids, sterols, quinones and saponins were completely absent (Table-2). The leaf extracts of benzene, chloroform and petroleum ether showed rich in alkaloids, whereas in aqueous and methanol extracts less amount was present. Glycosides, phenols, fats & oils and lignins were found to be present feebly. Lignin tests of benzene and chloroform solvent extracts of leaf have shown their presence, whereas in aqueous, methanol and petroleum ether extracts they were weakly present. Tannins, flavonoids, sterols, quinones and saponins tests of all solvent extracts of leaf were negative.

## Stem extracts

Phytochemical analysis of all the solvent extracts of stem revealed the presence of alkaloids, glycosides and lignins, whereas tannins, flavonoids, sterols, phenols, quinones and saponins were completely absent (Table-3). Analysis of alkaloids of benzene and petroleum ether extracts of stem were strongly positive, where as aqueous and chloroform extracts were weakly detected and methanol extracts showed complete absence of alkaloids. Tests performed for the presence of glycosides in all the solvent extracts of stem, benzene and methanol extracts were feebly detected and while in other solvent extracts of aqueous, chloroform and methanol have shown complete absence. Fats and oils were weakly detected in all the tests performed in all solvent extracts of stem. Tests performed for the presence of lignins were strongly positive in all solvent extracts whereas aqueous, methanol and petroleum ether extracts were weakly positive. Tannins, flavonoids, sterols, phenols, quinones and saponins tests of all solvent extracts of stem have shown their completely absence.

## **Root extracts**

Different solvent extracts of root *A. hypogaea* revealed the presence of high concentration of alkaloids and lignins. Whereas flavonoids, glycosides, tannins, sterols, fats & oils, quinones, saponins were completely absent (Table-4). Tests performed for alkaloid analysis of benzene, chloroform and petroleum ether extracts were strongly positive, where as aqueous and methanol extracts were weakly positive. Benzene and methanol extracts showed weakly presence of flavonoids while other solvent extracts were negative. All solvent extracts of root revealed to contain low amount of phenols. While lignins were strongly positive in all the tests performed by all the solvent extracts.

## Seed extracts

Phytochemical analysis of various solvent extracts of seed of *A. hypogaea* revealed the presence of alkaloids, fats & oils, lignins and saponins, whereas flavonoids, phenols and quinones were completely absent (Table-5). Alkaloids, lignins, saponins, fats and oils were strongly positive in all the tests performed of all the solvent extracts. Tests conducted for the presence of glycosides gave positive results in benzene and chloroform extracts while in other solvent extracts they were weakly detected. Tannins were weakly detected in all the five solvent extracts. Tests performed for the presence of sterols were screened positive in benzene, methanol and petroleum ether extracts whereas aqueous and chloroform extracts were found to be negative.

Table 1: Showing the fluorescence analy	vsis of various extracts of A	. hvpogaea under normal l	ight and UV light

Name of the	Colour of the extract under normal light				Colour of the extract under UV light				
extract	Leaf	Stem	Root	Seed	Leaf	Stem	Root	Seed	
Aqueous	Light Green	Light Green	Sea Green	Bluish White	Brown	Brown	Brown	White	
Benzene	Bluish Green	Light Green	Yellowish Green	White	Green	White	White	Blue	
Chloroform	Green	Fluorescent Pink	White	Bluish White	Yellowish Brown	Brown	Yellow	White	
Methanol	Pinkish Green	Pink	White	Blue	Dark Green	Green	Yellowish Green	White	
Petroleum ether	Muddy Green	Light Green	White	White	Green	Brown	White	Blue	



Aqueous Benzene Chloroform Methanol Petroleur						
Phytochemical Test		Extract	Extract	Extract	Extract	Extract
	Dragendorff's test	+	+	+	+	+
Alkaloids	Mayer's test	_	+	+	_	+
	Wagner's test	+	+	+	+	+
	Hager's test	_	+	+	+	+
	Tanicacid test	+	+	+	_	+
	Raymond's test	_	_	_	_	_
	Legal's test	+	+	_	_	+
Chronoidea	Bromine water test	+	+	+	+	+
Glycosides	Kellar Kiliani test	_	_	_	_	_
	Conc. H <sub>2</sub> SO <sub>4</sub> test	_	_	_	_	_
	Molisch test	+	+	+	+	+
	FeCl <sub>3</sub> test	_	_	_	_	_
	Gelatin test	_	_	_	_	_
Tannins	Lead acetate test	_	_	_	_	_
	Alkaline reagent test	_	_	_	_	_
	Mitchell's test	_	_	_	_	_
	Zn-HCl reduction test	_	_	_	_	_
	Lead acetate test	_	_	_	_	_
Flavonoids	FeCl <sub>3</sub> test	_	_	_	_	_
	Shinoda's test	_	_	_	_	_
	Alkaline reagent test	_			_	
64	Libermann Burchard test	_	_	_	_	_
Sterols	Salkowski test	_	_	_	_	_
	Stain test	+	+	+	+	+
Fats & oils	Saponification test	_	_	_	_	_
Dhanala	FeCI <sub>3</sub> test	_	_	_	_	_
Phenols	Elagic acid test	+	+	+	+	+
	Labat test	_	+	+	_	+
Lignins	Lignin(furfuraldehyde) test	+	+	+	+	+
Quinones	Alcoholic KOH test	_	_	_	_	_
Saponins	Foam test	_	_	_	_	_

## Table 2: Analysis of phytochemicals from leaf extracts of A. hypogaea

#### Table 3: Analysis of phytochemicals from stem extracts of A. hypogaea

Phytochemical Test		Aqueous Extract	Benzene Extract	Chloroform Extract	Methanol Extract	Petroleum ether Extract
Alkaloids	Dragendorff's test	+	+	+	_	+
	Mayer's test	_	+	_	_	+
	Wagner's test	+	+	+	_	+
	Hager's test	_	+	+	_	+
	Tanicacid test	+	+	_	_	+
	Raymond's test	_	_	_	_	_
	Legal's test	_	+	_	+	_
Chronoidee	Bromine water test	_	_	_	_	_
Glycosides	Kellar Kiliani test	_	+	_	+	_
	Conc. H <sub>2</sub> SO <sub>4</sub> test	_	+	_	+	_
	Molisch test	+	+	+	+	+
	FeCl <sub>3</sub> test	_	_	_	_	_
	Gelatin test	_	_	_	_	_
Tannins	Lead acetate test	_	_	_	_	_
	Alkaline reagent test	_	_	_	_	_
	Mitchell's test	_	_	_	_	_
	Zn-HCI reduction test	_	_	_	_	_
	Lead acetate test	_	_	_	_	_
Flavonoids	FeCl <sub>3</sub> test	_	_	_	_	_
	Shinoda's test	_	_	_	_	_
	Alkaline reagent test	_	_	_	_	_
Chanala	Libermann Burchard test	_	_	_	_	_
Sterols	Salkowski test	_	_	_	_	_
	Stain test	+	+	+	+	+
Fats & oils	Saponification test	_	_	_	_	_
Phenols	FeCl <sub>3</sub> test	_	_	_	_	_
	Elagic acid test	_	_	_	_	_
Lignins	Labat test	+	+	+	+	+
	Lignin(furfuraldehyde) test	+	+	+	+	+
Quinones	Alcoholic KOH test	_	_	_	_	_
Saponins	Foam test	_	_	_	_	_



Phytochemical Test		Aqueous Extract	Benzene Extract	Chloroform Extract	Methanol Extract	Petroleum ether Extract
	Dragendorff's test	+	+	+	+	+
	Mayer's test	_	+	+	_	+
Alkaloids	Wagner's test	+	+	+	+	+
	Hager's test	_	+	+	-	+
	Tanicacid test	+	+	+	+	+
	Raymond's test	_	-	_	-	_
	Legal's test	_	_	_	_	_
Glycosides	Bromine water test	_	-	_	-	_
Glycosides	Kellar Kiliani test	_	-	_	-	_
	Conc. H <sub>2</sub> SO <sub>4</sub> test	_	_	_	_	_
	Molisch test	_	_	_	_	_
	FeCI <sub>3</sub> test	_	_	_	_	_
	Gelatin test	_	_	_	_	_
Tannins	Lead acetate test	_	_	_	_	_
	Alkaline reagent test	_	_	_	_	_
	Mitchell's test	_	_	_	_	_
	Zn-HCI reduction test	_	+	_	+	_
	Lead acetate test	_	+	_	+	_
Flavonoids	FeCl <sub>3</sub> test	_	_	_	_	_
Flavonolds	Shinoda's test	_	+	_	+	_
	Alkaline reagent test	_	_	_	_	_
Chanala	Libermann Burchard test	_	_	_	_	_
Sterols	Salkowski test	_	_	_	_	_
Fats & oils	Stain test	_	_	_	_	_
Fats & olis	Saponification test	_	_	_	_	_
Phenols	FeCl <sub>3</sub> test	_	_	_	_	_
	Elagic acid test	+	+	+	+	+
Lignins	Labat test	+	+	+	+	+
	Lignin(furfuraldehyde) test	+	+	+	+	+
Quinones	Alcoholic KOH test	_	_	_	_	_
Saponins	Foam test	_	_	_	_	_

## Table 4: Analysis of phytochemicals from root extracts of A. hypogaea

#### Table 5: Analysis of phytochemicals from seed extracts of A. hypogaea

Phytochemical Test	Table 5. Analysis of phy	Aqueous Extract	Benzene Extract	Chloroform Extract	Methanol Extract	Petroleum ether Extract
	Dragendorff's test	+	+	+	+	+
Alkaloids	Mayer's test	+	+	+	+	+
	Wagner's test	+	+	+	+	+
	Hager's test	+	+	+	+	+
	Tanicacid test	+	+	+	+	+
	Raymond's test	_	+	+	_	+
	Legal's test	+	+	+	_	+
Glycosides	Bromine water test	_	+	+	_	+
Glycosides	Kellar Kiliani test	_	_	_	-	_
	Conc. H <sub>2</sub> SO <sub>4</sub> test	+	_	_	+	_
	Molisch test	_	+	+	+	+
	FeCl <sub>3</sub> test	_	_	_	-	_
	Gelatin test	+	+	+	+	+
Tannins	Lead acetate test	_	_	_	_	_
	Alkaline reagent test	_	_	_	_	_
	Mitchell's test	_	+	+	_	+
	Zn-HCI reduction test	_	_	_	_	_
	Lead acetate test	_	_	_	_	_
Flavonoids	FeCl <sub>3</sub> test	_	_	_	_	_
	Shinoda's test	_	_	_	_	_
	Alkaline reagent test	_	_	_	_	_
Sterols	Libermann Burchard test	_	+	_	+	+
Sterois	Salkowski test	_	+	_	+	+
Fats & oils	Stain test	+	+	+	+	+
rals & Ulls	Saponification test	+	+	+	+	+
Phenols	FeCl <sub>3</sub> test	_	_	_	_	_
	Elagic acid test	_	_	_	_	_
	Labat test	+	+	+	+	+
Lignins	Lignin(furfuraldehyde)test	+	+	+	+	+
Quinones	Alcoholic KOH test	_	_	_	_	_
Saponins	Foam test	+	+	+	+	+



Tests of flavonoids, phenols and quinones were screened negative in all the solvent extracts. Glycosides, tannins and sterols were weakly detected in all the solvent extracts in tests performed for their presence. All the tests for lignins showed the presence in all the solvent extracts used. Saponins tests were also showed the strongly positive for all the extracts.

# DISCUSSION

The leaf, stem, root and seed extracts of the A. hypogaea analyzed are rich in phytochemicals. The results of the preliminary phytochemical screening of the legumes studied clearly showed that the legumes are nutritious and contained some phytochemicals such as alkaloids, glycosides, tannins, flavonoids, sterols, fats, oils, phenols, lignins, guinones and saponins. All these phytochemicals present in these legumes compared favorably with those reported from some medicinal plants found in Nigeria<sup>15</sup>. Strongly presences of alkaloids in various parts of leaf, stem, root and seed extracts of A. hypogaea are useful in the prolonging of the action of several hormones and acting as stimulants. Flavonoids are present in roots enable food to be tasty which is in line with the work of Dakora<sup>16</sup> that flavonoids promote peculiar taste in prepared foods. Flavonoids are capable of treating certain physiological disorder and diseases. They are potent water soluble, super anti-oxidant and free radical scavengers which prevents oxidative cell damage and have strong anti-cancer activity which adds protection against all stages of carcinogenesis<sup>17</sup>. Presence of glycosides in agricultural plants of A. hypogaea as potential precursors of defensive metabolites could lead to a new appreciation of their roles in crop resistance to pests. The highest value of fat and oils were present in groundnut seeds and low in leaf and stem is an important part of the diet of living organisms and also are useful in many industries. Saponins are present in all the legumes especially seeds of A. hypogaea studied and are contained in appreciable quantities, which mean it has cholesterol binding properties, and help in hemolytic activities<sup>17</sup>. The highest value of tannins was in A. hypogaea seeds which serve as astringent properties for healing of wounds and inflaming mucous membrane<sup>17</sup>. Phenols are found in different percentages of leaf and root extracts. The presence of phenol indicated that these legumes have the ability to block specific enzymes that causes inflammation. They also modify the prostaglandin pathways thereby protecting platelets from clumping.

## CONCLUSION

In conclusion the present screening of phytochemicals in various parts of peanut plant possess alkaloids, lignins, fats, oils, glycosides, phenols and saponins. This suggests that these various parts of the peanut can be used as food supplements in every day diet for normal metabolic activities of living organisms to reduce the anti nutritional effects and which are also useful in pharmaceutical industry.

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