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Phytochemical Screening and Thin Layer Chromatographic Analysis of *Arachis hypogaea* L. Seed Extracts

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Abstract: *Arachis hypogaea* L. belongs to the genus *Arachis*, family Leguminoseae is a legume plant. The seed of *Arachis hypogaea* contains various bioactive phytochemicals in addition to the identified oil constituents and other sources of nutrition. However, reports regarding the role of the seed part of *Arachis hypogaea* as source of phytochemicals and other pharmaceuticals were very minimal. The objective of this study was therefore to investigate the presence of various phytochemicals and analysis thin layer chromatographic profiles of crude seed extracts of *Arachis hypogaea*. The ground powder of dried seed of *Arachis hypogaea* was extracted using petroleum ether, methanol and aqueous, separately by putting on an incubated orbital shaker for 24 h. Each extracts were then filtered through Whatman No. 1 filter paper, concentrated using rotary vacuum evaporator and calculated the percentage yield. Standard qualitative procedures were employed to identify the major phytochemical constituents. The TLC profiles of each crude seed extracts were observed through UV-lamp followed by iodine vapour. Besides to this, fluorescence analysis of all the crude extracts under UV-lamp and ordinary light has been carried out. The phytochemical analysis of different solvent extracts of *Arachis hypogaea* seed studied herein revealed a strong, moderate and weak presence of alkaloids, glycosides, tannins, flavonoids, phenols, sterols, fats, oils, saponins and quinines. And from the TLC profile, two spots with R_f values 0.13 and 0.86 were observed in common in all the solvent extracts of *Arachis hypogaea* seed. The present study suggests that the seed of *Arachis hypogaea* L. can be used as food supplements in everyday diet andmay also useful in pharmaceutical industry being the phytochemicals it contains can be used as precursors for the isolation and characterization of pure bioactive compounds employed as ingredients in drug formulation.

INTRODUCTION

Groundnut/peanut (*Arachis hypogaea*) is a legume plant which is widely grown as a food crop. It is an herbaceous plant of which there are different varieties such as Boro light, Boro Red, Mokwa, Campala, Guta and Ela[1]. The genus *Arachis*, a member of the family *Leguminoseae* is widely distributed in the tropics and moderate regions. It has >70 wild species of which only *Arachis hypogaea* L. is domesticated and commonly cultivated. *Arachis hypogaea* L. is grown worldwide in the tropics and temperate zones merely as an oilseed crop[2, 3].

The chemical composition of *Arachis hypogaea* (L.) seed has been evaluated mainly in relation to protein level, amino acid composition and fatty acid compositions. *Arachis hypogaea* (L.) seed contains 44-56% oil and 22-30% protein on a dry seed basis and is a rich source of minerals (phosphorus, calcium, magnesium and potassium) and vitamins (E, K and B group)[1]. *Arachis hypogaea* (L) seed is also reported to contain 9.5-19.0% total carbohydrates as both soluble and insoluble carbohydrate[4].

In many studies, leaves, roots, flowers, whole plants and stems of various plants have been examined for useful phytochemicals, while the reports regarding the role of seeds as source of phytochemicals and other pharmaceutical sources were very few[5,6]. Same is true for *Arachis hypogaea* (L.) seed. The present study is designed to investigate the presence of various phytochemicals and to analysis the thin layer chromatographic profile in the seed of *Arachis hypogaea* (L.) which may evoke various therapeutic effects.

MATERIALS AND METHODS

Plant material: The healthy seed of *Arachis hypogaea* was collected from local market found in Haramaya town, Eastern Ethiopia on August, 2015. The seed was washed thoroughly with distilled water to remove some surface contaminants and dusts and shade dried. It was dehusked after oven dried at 70°C to separate the kernel from the pods. The completely dried seed was then ground to a fine powder using Waring commercial laboratory blender (Torrington, USA) and sieved by 1mm sieve. The powder was then stored in an air tight container at ambient temperature for further experimental works.

Chemicals and reagents: All the chemicals and reagents used in this study were purchased from high-tech. healthcare (India), BDH chemicals (England) and Merck (Germany).

Preparation of crude extracts: About 5.00 g powder of the sample was soaked in conical flasks containing 50 mL of petroleum ether, methanol and water (aqueous), separately and put on an incubated orbital shaker for 24 h. Each solvent extracts were filtered through Whatman No. 1 filter paper. The supernatants were subjected to fluorescence analysis both under UV-lamp (365 nm) and ordinary light. All the extracts were then concentrated using rotary vacuum evaporator (JAICO, India) and the percentage yield was calculated. Each dried extract was subjected to phytochemical and Thin Layer Chromatographic (TLC) analysis.

Phytochemical analysis: The standard qualitative procedures were used for the identification of following bioactive compounds present in the different extracts of *Arachis hypogaea* L. seed[7-9].

Tests performed for the presence of phytoconstituents
Tests for alkaloids
Meyer's test: To 1 mL 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.

Wagner's test: To few mL of each of the sample solution, Wagner's reagent (Iodine in Potassium Iodide) was added which resulted in the formation of reddish brown precipitate indicating the presence of alkaloids.

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.

Ferric chloride test: One drop of $FeCl_3$ solution was added to each of the test sample, formation of yellow precipitate was resulted reacting positively for alkaloids.

Tests for glycosides
Kellar Kiliani test: 1 mL of concentrated sulphuric acid was taken in a test tube then 2 mL of extract and 2 mL of glacial acetic acid with one drop of ferric chloride were added, reaction shows formation of a blue color.

Molisch test: When α-naphthol and concentrated sulphuric acid were added to test samples, reddish violet ring at junction of two layers was resulted.

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

Concentrated sulphuric acid test: Concentrated sulphuric acid was added to test sample which resulted in appearance of reddish color.

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

Tests for tannins and phenolic compounds

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

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

Lead acetate test: 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.

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

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

Tests for flavonoids

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

Ferric chloride test: To 2 mL of test samples taken separately, few drops of ferric chloride were added which resulted in the formation of blackish red precipitate.

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

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 color.

Tests for sterols

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

Tests for fats and oils

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

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

Tests for quinones

Alcoholic potassium hydroxide test: When alcoholic potassium hydroxide was added to the test samples, red to blue color appears reacting positively for quinines.

Tests for saponins

Foam test: 5 mL 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.

Thin Layer Chromatographic (TLC) analysis: Each solvent extract of *Arachis hypogaea* L. seed *was* subjected to TLC (silica gel 60 F254, 20×20 cm, Merck) analysis. TLC plates were cut with ordinary household scissors. Plate markings were carried out with soft pencil. Glass capillary tubes were employed to spot each extract on the TLC plate. Three different ratios of solvent systems were developed in three separate chambers. These are: solvent system 1 (petroleum ether: methanol, 2:1), solvent system 2 (petroleum ether: methanol, 1:1) and solvent system 3 (petroleum ether: methanol, 1:2). After pre-saturation with the mobile phase for 20 min, a development process of a spotted TLC was made. In each case, the developed TLC plate was then dried and visualized by exposure of the plate first to Uv-lamp (254 nm) followed by iodine vapour to observe different bands on the plate. The corresponding retention factor (R_f) value of each band was calculated as: R_f = Distance travelled by the solute/Distance travelled by the solvent.

RESULTS AND DISCUSSION

Fluorescence analysis of all the extracts under Uv-lamp (365 nm) and ordinary light has been shown (Table 1).

Percentage yield of different extracts: The amounts (g) obtained from petroleum ether, methanol and aqueous extracts of *Arachis hypogaea* L. seed were 1.69, 0.43 and 0.39, respectively. The percentage yield of each extracts was calculated (Table 2).

Phytochemical analysis: Phytochemical analysis of different solvent extracts of *Arachis hypogaea* seed revealed a strong, moderate and weak presence of the various classes of phytochemicals (Table 3, ++ = strong presence, + = moderate presence, - = absence). Alkaloids were detected positively in methanol and aqueous extracts whereas petroleum ether extract was found to be negative for all tests. Tests performed for the presence of glycosides showed a strong positive result in all solvent extracts. Tannins were screened positively in methanol and aqueous extracts while they were absent in petroleum

Table 1: Fluorescence analysis of different extracts of *Arachis hypogaea* seed

Name of extracts	Color of the seed extract under ordinary light	Color of the seed extract under Uv-lamp (365 nm) light
Petroleum ether	White	White
Methanol	White	White
Aqueous	Bluish white	Blue

Table 2: The percentage yield of different extracts of *Arachis hypogaea* seed

Extract name	Yield of extract (g)	Percentage yield (% w/w)
Petroleum ether	1.69	33.8
Methanol	0.43	8.6
Aqueous	0.39	7.8

Table 3: Phytochemical profile of different extracts of *Arachis hypogaea* seed

Phytochemicals	Petroleum ether	Methanol	Aqueous
Alkaloids			
Meyer's test	-	++	+
Wagner's test	-	+	+
$FeCl_3$ test	-	+	+
Hager's test	-	+	+
Glycosides			
Kellar kiliani test	+	+	+
Molisch test	-	-	+
Bromine water test	+	-	+
Legal test	++	+	+
Conc. Sulphuric acid test	+	++	+
Tannins			
$FeCl_3$ test	-	-	-
Gelatin test	-	+	+
Lead acetate test	-	+	+
Alkaline reagent test	-	-	+
Phenols			
$FeCl_3$ test	-	-	-
Ellagic acid test	-	-	+
Flavonoids			
Lead acetate test	-	+	+
$FeCl_3$ test	-	-	-
Alkaline reagent test	-	-	+
Zinc hydrochloride reduction test	-	-	-
Sterols			
Salkowski test	++	+	-
Fats and oils			
Stain test	+	+	+
Saponification test	+	+	+
Quinones			
Alcoholic KOH test	-	+	+
Saponins			
Foam test	+	+	+

ether extract for all tests. Tests conducted for the presence of phenols showed a very weak positive result only in aqueous extract. Flavonoids were detected weakly in methanol and aqueous. Tests done for the presence of sterols gave a strong positive result in petroleum ether extract while in methanol extract they were weakly detected and absent in aqueous extract. The screening tests performed for the presence of fats and oils revealed positive results in all solvent extracts. Quinones were slightly detected in methanol and aqueous extracts. Saponins were positively detected in all solvent extracts.

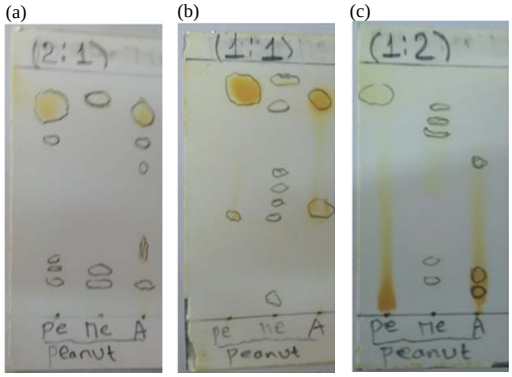

Fig. 1(a-c): TLC profiles with solvent system 1 (petroleum ether: methanol, 2:1), solvent system 2 (petroleum ether: methanol, 1:1) and solvent system 3 (petroleum ether: methanol, 1:2) of different *Arachis hypogaea* seed extracts (Pe: petroleum ether, Me: methanol, A: aqueous)

Thin Layer Chromatographic (TLC) analysis: As shown in Fig. 1, in solvent system 1 (petroleum ether: methanol, 2:1), petroleum ether extract shows five spots with R_f = 0.13, 0.19, 0.23, 0.71 and 0.86, methanol extract shows three spots with R_f = 0.13, 0.19 and 0.86, aqueous extract shows five spots with R_f = 0.13, 0.32, 0.63, 0.71 and 0.86. In solvent system 2 (petroleum ether: methanol, 1:1), two spots were observed in petroleum ether extract with R_f = 0.40 and 0.90. Methanol extract shows seven spots with R_f = 0.09, 0.36, 0.43, 0.49, 0.56, 0.80 and 0.90, aqueous extract shows two spots with R_f values 0.42 and 0.85. In solvent system 3 (petroleum ether: methanol, 1:2), petroleum ether extract shows one spot with R_f = 0.86, methanol extract shows five spots with R_f = 0.13, 0.21, 0.73, 0.76 and 0.82, aqueous extract shows three spots with R_f = 0.09, 0.17 and 0.61 (Table 4).

The different extracts of *Arachis hypogaea* seed analyzed herein are rich in phytochemicals. From the result of the preliminary phytochemical screening studied in this work, it is clear that the plant seed is nutritious and contains some phytochemicals that is alkaloids, glycosides, tannins, flavonoids, sterols, fats, oils, phenols, quinines and saponins in different degree of presence. The TLC results indicated that three spots with similar R_f values (0.13, 0.19 and 0.86) were shown both in petroleum ether and methanol extracts and two spots with R_f = 0.13 and 0.86 were observed in all extracts in solvent system 1. In solvent system 2, one spot with R_f value 0.90 was observed in petroleum ether and methanol extracts in common. From all the solvent systems, solvent system 1 (petroleum ether: methanol, 2:1) is the best and

Table 4: R_f values of TLC solvent systems for different extracts of *Arachis hypogaea* seed

	Solvent system 1		Solvent system II		Solvent system 3	
Extract name	No. of spots detected	R_f values	No. of spots detected	R_f values	No. of spots detected	R_f values
Petroleum ether	5	0.132	2	0.40	1	0.86
	-	0.19	-	0.90	-	-
	-	0.23	-	-	-	-
	-	0.71	-	-	-	-
	-	0.86	-	-	-	-
Methanol	3	0.13	7	0.09	5	0.13
		0.19	-	0.36	-	0.21
		0.86	-	0.43	-	0.73
	-	-	-	0.49	-	0.76
	-	-	-	0.56	-	0.82
	-	-	-	0.80	-	-
	-	-	-	0.90	-	-
Aqueous	5	0.13	2	0.42	3	0.09
		0.32	-	0.85	-	0.17
		0.63	-	-	-	0.61
		0.71				
		0.86				

suitable one to separate the compounds with good resolution which are present in those extracts by column chromatography techniques.

Regarding health benefits, the strong presences of alkaloids in seed extracts of *Arachis hypogaea* are helpful in the prolonging of the action of several hormones and acting as stimulants. Flavonoids are present to some extent in seed extracts of *Arachis hypogaea* which enable food content to be tasty as flavonoids promote peculiar taste in prepared foods. Besides to this, flavonoids are capable of treating certain physiological disorder and diseases being they are potent water soluble, super antioxidant and free radical scavengers[7, 10, 11]. The presence of glycosides in *Arachis hypogaea* seed extracts as potential precursors of defensive metabolites could lead to a new appreciation of their roles in crop resistance to pests. Strong presence of fats and oils in seed extracts of *Arachis hypogaea* are an important parts of the diet of living organisms and also are useful in many industries. The presence of saponins in *Arachis hypogaea* seed extracts are useful as cholesterol binding compounds and help in hemolytic activities[7, 11]. Tannins which were screened positively in the plant seed serve as astringent properties for healing of wounds and inflaming mucous membrane[7, 11]. The presence of phenols in different degree indicated that the legume plant has the ability to block specific enzymes that causes inflammation[7, 11].

CONCLUSION

The preliminary phytochemical screening of different solvent extracts of *Arachis hypogaea* seed presented herein shows the presence of alkaloids, glycosides, tannins, phenols, sterols, flavonoids, fats, oils, quinines and saponins in different degree. This suggests that this plant seed can be used as food supplement in every day diet for normal metabolic activities of living organisms to reduce the anti nutritional effects. Moreover, it may also useful in pharmaceutical industry being the phytochemicals it contains can be used as precursors for the isolation and characterization of pure bioactive compounds employed as ingredients in drug formulation.

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