



Original Research Article

DOI: <http://dx.doi.org/10.22192/ijcrms.2017.03.03.010>

Preliminary phytochemical analysis and antimicrobial evaluation of *Arachis hypogaea* L.

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Abstract

Arachis hypogaea L. is a very important nuts used for the treatments of various diseases such as loose cough, arthritis, constipation, recuperation after illness, etc. The present study aimed at an evaluation of the presence of different phytochemicals along with antimicrobial activity of aqueous, petroleum ether, chloroform and ethanol extracts of dry powder obtained from stem, leaf and seed of *Arachis hypogaea*. Phytochemical screening of Aqueous, petroleum ether, chloroform and Ethanol extracts revealed the presence of alkaloid glycoside, fixed oils and fats, tannin, saponins & phenols by positive reaction with the respective test reagent. Among the four solvents of extracts used, chloroform and ethanolic extracts of the plant were active against *E. coli*, *S. aureus*, *Fusarium sp* and *A. flavus*. The demonstration of antimicrobial activity against both bacteria and fungi is an indication that the plant is a potential source for production of drugs with a broad spectrum of activity.

Keywords: *Arachis hypogaea*, Phytochemical analysis, antibacterial activity, antifungal activity

Introduction

Now a days natural products derived from plants are being tested for presence of new drugs with new modes of pharmacological action. A unique attribute of higher plants is their capacity to produce a large number of bioactive compounds (Castello *et al.*, 2002). Recent studies are involved in the identification and isolation of new therapeutic compounds of medicinal importance from higher plants for specific diseases (Erturk *et al.*, 2006; Mohanta *et al.*, 2007).

Knowledge of the chemical constituents of plant is helpful in the discovery of therapeutic agent as well as new sources of economic materials like oil and gums. The most important bioactive constituents of these plants are alkaloids, tannins,

flavonoids and phenolic compounds (Kumar *et al.*, 2007). In India large number of plant species had been screened for their pharmacological properties but still a vast wealth of endangered species are unexplored.

The peanut or groundnut (*Arachis hypogaea*) is a species in the legume or "bean" family (Fabaceae). It has been considered not only as a highly nutritious food source for human beings but also a natural starting material for the production of soap and machine oil. In addition, *Arachis hypogaea* L. has been used for the treatments of various diseases such as loose cough, arthritis, constipation, recuperation after illness, etc. Peanut roots have been used as

poultry feed and fertilizer without sufficient scientific knowledge (Ho, 2000 and Fuhrman, 1995). Hence the present study aimed at an evaluation of the presence of different phytochemicals along with antimicrobial activity of aqueous, petroleum ether, chloroform and ethanol and dry powder obtained from stem, leaf and seed of *Arachis hypogaea*.

Materials and Methods

The plant parts of stem, leaf and seed were collected from mature plants and washed with water and then chopped into small fragments. The materials were then shade dried at ambient temperature (32°C) for 10 to 15 days and the drying operation was carried out under controlled conditions to avoid chemical changes. The dried samples were crushed into fine powder using an electronic blender. The powdered samples were stored in polythene containers at room temperature. The powdered samples were extracted by using soxhlet apparatus at 47°C petroleum ether, chloroform and ethanol were used as a solvent. After extraction, the extract was dried at 50°C in hot air oven. Extracted samples were stored at properly and can be used for phytochemical analysis and antimicrobial activity.

A qualitative phytochemical test to detect the presence of alkaloid, tannin, saponin, flavonoid, glycoside and phenol and quantitative estimation of alkaloids, saponin and glycosides were carried out using standard procedures as described by Sofowara, (1993), Trease and Evans (1989), Harborne (1973), Mohanta et al. (2007), Kumar et al. (2007) and Edeoga et al. (2005).

For Antimicrobial studies, the bacteria, *Escherchia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Vibrio cholerae*, *Salmonella typhi* and fungi, *Candida albicans*, *Aspergillus niger*, *A. flavus*, *Penicillium citrinum*, *Fusarium sp* were used for the experiment.

Antibacterial activity was carried out using well-diffusion method (Perez *et al.*, 1990). Petri plates were prepared with 20 ml of sterile Mueller Hinton Agar (MHA) (Hi-media, Mumbai) for

bacteria. The test cultures (10^5 dilution) were swabbed on the top of the solidified media and allowed to dry for 10 min. After solidification of media, wells were made in the seeded plates with the help of a sterile well borer (6 mm dia.). Well are filled with 25, 50, 75 and 100µl of the aqueous, petroleum ether, chloroform and ethanolic extracts of stem, leaves and seed extracts and the growth inhibition zones were measured after 24 h of incubation at 37°C. Zone of inhibition was recorded in millimeters and the experiment was repeated twice.

For antifungal activity, the same method as for bacteria was adopted. Instead of nutrient agar, potato dextrose agar was used. The inoculated medium was incubated at 25°C for two days for the *Candida albicans* and three days for other fungi tested. Zone of inhibition was recorded in millimeters and the experiment was repeated thrice.

Results and Discussion

Table 1 and 2 shows the results of the preliminary phytochemical analyses of the different stem, leave and seed extracts of *Arachis hypogaea*. Phytochemical screening of Aqueous, petroleum ether, chloroform and Ethanol extracts revealed the presence of alkaloid glycoside, fixed oils and fats, tannin, saponins & phenols by positive reaction with the respective test reagent. Phytochemical screening showed that maximum presence of phytoconstituents in petroleum ether extracts. According to Sofowora, 1986, the presence of secondary metabolites in plants, produce some biological activity in man and animals and it is responsible for their use as herbs. The presence of these phytochemicals in the investigated plant parts of *Arachis hypogaea* would be responsible for the demonstrated antimicrobial activity of the extracts. In this regard, the higher concentration of these phytochemicals in the root extract may have been responsible for a relatively higher antimicrobial activity demonstrated by the root extract on the tested important human pathogens.

Table 1: Colour of the various extract of *A. hypogaea*

Name of the extract	Colour of the extract		
	stem	leaf	seed
Aqueous	Pale Green	Green	Bluish White
Petroleum ether	Pale Green	Green	White
Chloroform	Green	Pinkish Green	white
Ethanol	Muddy green	Pinkish Green	Bluish white

Table 2: Qualitative Phytochemical analysis of *A. hypogaea*

TEST	STEM				LEAF				SEED			
	AE	PE	CE	EE	AE	PE	CE	EE	AE	PE	CE	EE
Alkoloids	+	+	+	+	+	+	+	+	+	+	+	+
Cardiac glycosides	+	-	-	+	+	+	+	+	-	+	+	+
Flavonoids	-	-	-	-	-	-	+	-	-	+	-	-
Steroids	+	-	-	-	-	+	-	-	-	-	-	-
Terpenoids	-	-	-	-	-	-	-	-	-	+	-	-
Tannins	-	-	+	-	-	+	-	-	-	+	+	-
Saponins	-	-	-	-	+	-	+	-	+	+	+	+
Fixed oils & fats	-	+	-	+	+	+	+	+	+	+	+	+
Phenols	-	-	-	-	+	-	+	+	+	-	-	+

AE- Aqueous extract, PE – Petroleum ether extract, CE- Chloroform extract, EE- Ethanol extract

Antibacterial activity of aqueous, petroleum ether, chloroform and ethanol extracts of leaf stem, and seed extracts were tested against bacteria like *Escherchia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Vibrio cholerae*, *Salmonella typhi* and fungi like *Candida albicans*, *Aspergillus niger*, *A. flavus*, *Penicillium citrinum*, *Fusarium sp*. Four different concentrations (25, 50, 75 and 100 µl/l) of aqueous, petroleum ether, chloroform and ethanol showed varying degree of inhibitory effect.

The aqueous extract of the leaf stem, and seed extracts were active against bacteria and fungi with inhibition (Table 3). Out four concentrations, 100 µl/l of leaf extracts gave the maximum zone of inhibition (10.38 mm) against *E.coli*. The lowest zone was found in all extracts against *Salmonella typhi* compared other organisms tested.

The petroleum ether extract of leaf stem, and seed extracts showed different antimicrobial activity towards the test organisms of both bacteria and fungi. The petroleum ether extract of all three showed low activity against *S. typhi* and *A. flavus*, high activity (6.98 mm and 5.50mm) against *Fusarium sp* and *S. aureus* respectively (Table 4).

Table 5 shows results of the antimicrobial susceptibility test of the extracts against the test organisms. From the results, seed extracts were the most effective and the highest activity was demonstrated against *S. aureus* (9.56 mm zone of inhibition), *E.coli* (8.15 mm zone of inhibition) and *Fusarium sp* (6.52 mm zone of inhibition), followed by the stem extracts with the highest activity against *S. aureus* (8.28 mm) and *E.coli* (5.56 mm zone of inhibition each). In all three extracts tested, the lowest activity registered against *S. typhi*.

Table 3: Preliminary antimicrobial activity of aqueous extracts of *A. hypogaea* (zone of inhibition in mm)

TEST	STEM ($\mu\text{g/l}$)				LEAF ($\mu\text{g/l}$)				SEED ($\mu\text{g/l}$)			
	25	50	75	100	25	50	75	100	25	50	75	100
Bacteria												
<i>Escherchia coli</i>	0.00	2.54	3.2	4.54	1.86	5.30	8.24	10.38	0.00	3.66	4.99	6.64
<i>Klebsiella pneumoniae</i>	0.00	0.00	3.15	4.30	2.93	4.69	5.72	6.48	0.00	0.00	2.78	4.59
<i>Staphylococcus aureus</i>	2.66	3.23	4.00	5.40	0.00	0.00	3.54	5.30	1.86	3.78	3.96	6.60
<i>Vibrio cholerae</i>	0.00	0.00	1.52	2.18	0.00	0.00	0.00	1.45	0.00	0.00	0.00	0.00
<i>Salmonella typhi</i>	0.00	1.33	2.46	3.63	0.00	0.00	0.00	1.58	0.00	0.00	0.00	0.00
Fungi												
<i>Aspergillus niger</i>	0.00	1.06	1.50	2.16	1.00	1.93	3.53	3.10	1.03	1.90	2.03	2.10
<i>A. flavus</i>	0.00	0.00	1.10	2.45	0.00	0.00	2.22	1.78	0.00	0.00	0.00	0.00
<i>Penicillium citrinum</i>	0.00	0.00	0.00	1.23	0.00	0.00	1.06	1.06	1.26	1.50	1.36	1.23
<i>Fusarium sp</i>	1.23	1.40	2.00	1.70	0.00	0.00	1.30	1.33	0.00	1.30	2.34	3.50
<i>Candida albicans</i>	1.10	1.23	1.70	3.20	0.00	1.23	2.26	3.03	1.09	1.73	3.33	4.20

Table 6 shows results of the antimicrobial susceptibility test of the extracts against the test organisms. From the results, seed extracts were the most effective and the highest activity was demonstrated against *S. aureus* (9.88 mm zone of

inhibition), *Fusarium sp* (6.43 mm zone of inhibition) and *E. coli* (6.12 mm zone of inhibition), followed by the leaf extracts with the highest activity against *E.coli* (6.31 mm zone of inhibition each).

Table 4: Preliminary antibacterial activity of Petroleum ether extracts of *A. hypogaea* (zone of inhibition in mm)

TEST	STEM ($\mu\text{g/l}$)				LEAF ($\mu\text{g/l}$)				SEED ($\mu\text{g/l}$)			
	25	50	75	100	25	50	75	100	25	50	75	100
Bacteria												
<i>Escherchia coli</i>	0.00	0.00	1.80	2.90	1.46	2.60	3.76	3.93	1.40	1.80	4.96	3.23
<i>Klebsiella pneumoniae</i>	0.00	1.30	2.36	2.76	0.00	0.00	0.00	2.76	1.76	1.74	2.80	3.29
<i>Staphylococcus aureus</i>	1.56	2.40	2.63	3.53	1.52	2.74	4.52	3.66	0.00	2.33	4.30	5.50
<i>Vibrio cholerae</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Salmonella typhi</i>	0.00	1.00	1.55	2.46	0.00	0.00	0.00	1.52	0.00	0.00	0.00	1.00
Fungi												
<i>Aspergillus niger</i>	0.00	1.36	2.84	3.66	1.24	1.78	1.56	1.00	1.22	2.86	3.46	5.12
<i>A. flavus</i>	0.00	0.00	1.52	2.84	0.00	0.00	2.56	3.48	0.00	0.00	0.00	0.00
<i>Penicillium citrinum</i>	0.00	1.60	3.48	5.76	0.00	2.28	3.54	3.96	0.00	0.00	3.54	4.94
<i>Fusarium sp</i>	1.82	2.51	2.96	4.00	1.74	2.33	3.54	4.28	2.42	2.76	5.90	6.98
<i>Candida albicans</i>	1.55	1.54	2.42	2.68	0.00	1.81	2.18	2.67	0.00	0.00	2.81	3.19

Table 5: Preliminary antibacterial activity of Chloroform extracts of *A. hypogaea* (zone of inhibition in mm)

TEST	STEM ($\mu\text{g/l}$)				LEAF ($\mu\text{g/l}$)				SEED ($\mu\text{g/l}$)			
	25	50	75	100	25	50	75	100	25	50	75	100
Bacteria												
<i>Escherchia coli</i>	0.00	2.56	4.58	5.56	0.00	3.64	4.18	5.86	2.86	4.58	6.84	8.15
<i>Klebsiella pneumoniae</i>	0.00	3.48	4.72	5.46	1.24	1.26	3.83	3.42	0.00	0.00	2.34	2.82
<i>Staphylococcus aureus</i>	2.36	5.84	7.45	8.28	2.12	5.26	6.06	9.00	3.00	4.89	6.84	9.56
<i>Vibrio cholerae</i>	0.00	0.00	0.00	1.39	0.00	0.00	2.43	2.55	0.00	0.00	2.14	2.16
<i>Salmonella typhi</i>	0.00	1.29	1.62	2.03	0.00	0.00	0.00	0.00	0.00	0.00	1.26	1.74
Fungi												
<i>Aspergillus niger</i>	0.00	0.00	2.12	2.83	0.00	0.00	2.42	2.69	0.00	1.86	2.78	2.66
<i>A. flavus</i>	0.00	0.00	0.00	1.33	0.00	0.00	0.00	1.24	0.00	0.00	1.00	1.28
<i>Penicillium citrinum</i>	2.33	3.62	4.86	3.16	0.00	1.53	1.62	1.35	0.00	1.86	2.53	1.68
<i>Fusarium sp</i>	2.54	2.91	3.94	4.03	2.33	3.33	3.59	4.09	3.94	4.63	5.42	6.52
<i>Candida albicans</i>	0.00	0.00	1.86	2.18	0.00	1.23	2.16	2.51	1.14	1.18	2.08	2.18

Table 6: Preliminary antibacterial activity of ethanol extracts of *A. hypogaea* (zone of inhibition in mm)

TEST	STEM ($\mu\text{g/l}$)				LEAF ($\mu\text{g/l}$)				SEED ($\mu\text{g/l}$)			
	25	50	75	100	25	50	75	100	25	50	75	100
Bacteria												
<i>Escherchia coli</i>	2.33	3.62	4.82	5.3	2.51	4.26	5.13	6.31	3.54	4.87	5.53	6.12
<i>Klebsiella pneumoniae</i>	1.58	1.54	2.38	4.01	1.76	1.74	1.80	2.23	0.00	1.63	1.93	1.86
<i>Staphylococcus aureus</i>	2.15	3.34	4.75	5.65	2.83	3.52	3.98	4.58	5.88	6.53	7.24	9.88
<i>Vibrio cholerae</i>	1.23	1.40	2.46	3.95	0.00	1.50	1.80	1.30	1.63	1.06	1.36	2.30
<i>Salmonella typhi</i>	2.40	3.30	3.50	4.10	0.00	1.53	1.73	2.70	2.76	3.74	2.80	2.23
Fungi												
<i>Aspergillus niger</i>	0.00	0.00	1.03	1.10	0.00	1.30	1.93	2.06	0.00	1.53	2.16	3.13
<i>A. flavus</i>	0.00	0.00	0.46	1.10	0.00	0.00	0.00	0.46	0.00	0.00	0.00	0.00
<i>Penicillium citrinum</i>	1.50	1.60	2.16	2.20	1.23	1.53	2.30	2.50	0.00	0.00	1.33	1.46
<i>Fusarium sp</i>	1.50	1.06	1.36	3.10	1.9	1.50	2.20	5.66	1.09	2.73	4.96	6.43
<i>Candida albicans</i>	0.00	2.09	1.94	3.30	2.62	2.08	1.26	1.10	1.78	2.78	3.9	5.40

Several plants which are rich in alkaloids have been shown to possess antimalarial activity against a number of microorganisms. The inhibitory activity exhibited by the secondary metabolites tends to agree with the reports of Leven et al. (1979) and Scherbonvaski (1971) both of which linked the antibacterial properties of plants to the presence of secondary metabolites. For example, Adebayo et al. (1983) investigated the antimicrobial activity of leaf extract of *Eugenia uniflora* and reported that alkaloids, tannins and glycosides were detected and that the ethyl acetate and methanolic leaf extracts of the plant were active against *E. coli*, *P. vulgaris*, *K. pneumoniae* and *A. niger*.

The results obtained in this study showed that the aqueous, petroleum ether, chloroform and ethanol extracts of stem leaf and seeds of *T. avicennioides* has antimicrobial activities on test organisms used in this study. It may therefore be suggested that the constituents of this plant can be used in chemotherapy. This study represents the preliminary report on *Arachis hypogaea* and reported that alkaloid glycoside, fixed oils and fats, tannin, saponins & phenols were detected and that the chloroform and ethanolic extracts of the plant were active against *E. coli*, *S. aureus*, *Fusarium sp* and *A. flavus*.

The demonstration of antimicrobial activity against both bacteria and fungi is an indication that the plant is a potential source for production of drugs with a broad spectrum of activity. The results of the study also supports the traditional application of the plant and suggests that the plant extracts possess compounds with antimicrobial properties that can be used as antimicrobial agents in novel drugs for the treatment of several bacterial and fungal infections.

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Mythily K. & Revathi. K. (2017). Preliminary phytochemical analysis and antimicrobial evaluation of *Arachis hypogea* L. Int. J. Curr. Res. Med. Sci. 3(3): 76-82.

DOI: <http://dx.doi.org/10.22192/ijcrms.2017.03.03.010>