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PHYSIOCHEMICAL ANALYSIS OF THREE LEGUME SPECIES DOLICHOS (*DOLICHOS LABLAB*), GLYCINE (*GLYCINE MAX*) AND ARACHIS (*ARACHIS HYPOGEA*)

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Abstract

To study in detail the Physiochemical analysis of the seeds such as *Dolichos lablab, Glycine max* and *Arachis hypogea*. The phytochemical screening and other recommended Parameters for standardizations were performed. Preliminary phytochemical Screening showed the Presence of Steroids, tannins, Proteins, flavonoids, terpenoids, carbohydrate, Protein with help of compound microscope the seeds are thoroughly identified, can be used as a rapid inexpensive binomial identification. And with FTIR study different kinds of Peaks values obtained. Each Peak value indicate the Presence of various functional groups. These functional groups and phytonutrients treat so many dangerous diseases and build the human of animal body healthy. So these three legume varieties are very important in our daily life.

***Key words**:* Physiochemical, Dolichos lablab, Glycine max, Arachis hypogea, Phytochemical, Carbohydrates.

Introduction

Legumes seeds are important sources of nutrients and can serve as high quality dietary protein sources to meet nutrient requirements (Perumal *et al*., 2001; Escudero *et al*., 2006). Legume seeds have an average of twice as much protein as cereals and the nutritive value of the proteins are usually high (Vijayakumari *et al*., 1997).

Hamana *et al*. (1992) have analysed the *Dolichos lablab* by HPLC and among unknown compounds *N*-(3-aminopropyl) aminoethanol [NH_2 $(CH_2)_3$ $NH(CH_2)_2$ OH] and *N*-(3-aminopropyl) amino-propanol [$NH_2(CH_2)_3$ $NH(CH_2)_3OH$] were identified by GC-MS.

Marrelli *et al*. (2013) have analysed non-genetically modified soybean and Round up Ready soybean. It showed a different polyphenolic content and lipophilic composition. Non-genetically modified soybean extract possessed twice the polyphenolic content of GM soybean and the highest number of sterols. Among them, γ-sitosterol was found to be the major constituent.

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 (Marka *et al*., 2013). Soybean meal or groundnut cake in the diets of broilers (Chauhan *et al*., 2004). Legumes are excellent source of medicinal property for the majority of the world population. It is a good challenge for scientists to provide efficient, safe and cheap medications. Black gram can be used as an antioxidant in treating several diseases infection (Anbuselvi and Rebecca, 2014).

The phytochemicals present in *Arachis hypogaea* 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 quinines 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 (Marka *et al*., 2013).

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Materials and method

Collection of plant samples for Biochemical studies

The seeds of the selected 13 legumes were collected from different agricultural fields.

Identification of legumes

Prequent field visits were made in different parts of the study area for identification and collection of legumes. The cultivator of legumes were consulted to know the common names of the legumes. The collected plants identified by referring the Flora of presidency of Madras (Gamble 1967).

Preparation of Plant Extracts

The dried seeds are powered and subjected to soxhlet extraction using Acetone, Chloroform, Ethanol and Distilled water (aqueous extract).

Phytochemical Screening

All the plant extracts were subjected to systematic phytochemical screening to determine the presence/ absence of secondary plant metabolites (Harborne 1999) as below.

Tests for Carbohydrates (Benedict's test)

About 2 ml of plant extracts were treated with 2ml of Benedict's reagent. The mixture was heated gently for 1-2 minutes. Formation of orange red precipitate indicates the presence of carbohydrates.

Tests for Protein (Biuret test)

3 ml test sample was taken into a test tube; to that 4% NaOH and few drops of 1% $CuSO_4$ was added. The tubes were observed for violet or pink colour formation.

Tests for Alkaloids (Wagner's test)

2-3 ml plant extract was taken into separate tubes. To that few drops of Wagner's reagent was added and observed reddish brown precipitate.

Detection of Flavonoids (Lead acetate Test)

The extracts were treated with few drops of 10% lead acetate solution. The formation of yellow precipitate confirmed the presence of flavonoids.

Test for Terpenoids

1 ml of test sample was taken in a test tube and then 10 ml of methanol was added in it, shaken well and filtered to take 5 ml extract of plant sample. Then 2 ml of chloroform was mixed in the extract of selected plant sample and 3 ml of sulphuric acid was added in the selected sample extract. Formation of reddish brown color indicated the presence of terpenoids in the plants.

Tests for Tannins

With 2-3 ml test solution, 5% Fec Cl_3 solution was added and observed for deep blue-black colour reactions.

Tests for Steroids (Salkowski Reaction)

To 2 ml of sample, 2ml chloroform and 2 ml Concentrated H_2SO_4 were added and observed chloroform layer for red color and acid layer for fluorescence.

Test for Phenolic compounds (Ferric chloride test)

The extract was diluted to 5 ml with distilled water. To that few drops of neutral 5% ferric chloride solution was added. A dark green color indicated the resences of phenolic compounds.

Tests for Glycosides

To 2 ml of extract with dilute HCl and 2 ml Sodium nitropruside in yridine and sodium hydroxide solution were added. Formation of pink to blood red color indicates the presence of Cardiac glycosides.

Tests for Saponin (Foam test)

1 ml of plant extract was mixed with 4 ml of distilled water, the mixture was agitated in graduated cylinder for 15min, and formation of foam indicates saponin.

Fourier Transform Infrared Spectrioscopy study

The plant materials were analysis in ATR model FTIR Spectrophotometer (Bruker Co., Germany). About 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. Then the sample was loaded in FTIR spectroscope and the spectrum scan ranges from 500 to 4000 cm^{-1} with a resolution of 4 cm^{-1} was recorded using Attenuated Total Reflectance (ATR) technique beach measurement.

The reference spectra were acquired from the cleaned blank crystal prior to the presentation of each sample replicate. Samples were run in triplicate an all of them were undertaken within a day period. The obtained data were computerized analysed by IRPAL software and the functional groups were observed.

Result and Discussion

In the present study the three different species of legumes plants nutrients were analysed of the three species *Dolichos lablab*, *Glycine max* and *Arachis hypogea* Various nutrients are Positive in Acetone, chloroform, Ethanol and Aqueous extract. Protein, Carbohydrate, Alkaloids, are positive in all extracts. In *Dolichos lablab* Glycosides are negative in all extracts, *Glycine max* Terpenoids are totally negative in all

extracts. In *Arachis hypogea* flaronoids and Tannins are negative in all extracts. The different types of Phytochemicals present in the different species. These phytochemicals treat so many dangerous diseases like cholera, vomiting and diarrhea, astringent, night sweats, confusion, joint Pain, anti-diabetic and anticancer.

In *Dolichos lablab* seed, acetone extract showed positive for carbohydrates, flavonoids, tannins, steroids and phenol; aqueous extract showed positive for carbohydrates, alkaloids, flavonoids, tannins, phenol and saponin; chloroform extract showed positive for carbohydrate, alkaloids, flavonoids, steroidsand phenols; and ethanol extract showed positive for carbohydrate, alkaloids, flavonoids, terpenoids, steroids and phenols (Table 1).

In *Glycine max* seed, acetone extract showed positive for alkaloids, flavonoids, tannins, phenols and glycosides; aqueous extract showed positive for carbohydrates, protein, alkaloids, flavonoids, tannins, phenol and saponin; chloroform extract showed positive for alkaloids, steroids and glycosides; and ethanol extract showed positive for carbohydrate, protein, alkaloids, flavonoids, tannins, steroids, phenols and glycosides (Table 2).

In *Arachis hypogaea* seed, acetone extract showed positive for carbohydrate, protein, alkaloids and saponin; aqueous extract showed positive for carbohydrates, protein, alkaloids, terpenoids, steroids, glycosides and saponin; chloroform extract showed positive for carbohydrate, alkaloids and saponin; and ethanol extract showed positive for carbohydrate, protein, alkaloids, terpenoids, steroids, glycosides and saponin (Table 3).

FTIR chromatogram were analysed by the 3 legume species. In *Dolichos lablab* 2 high peaks were found 996.98 and 1634.49. In this peaks mentioned various functional groups. In *Glycine max* the chromatogram showed 2 peaks, the peak value is 995.03 and 1633.20. In *Arachis hypogea* 4 highest Peaks were found 1154.98, 2853.74 and 2921.67. These Peaks also indicate the various functional groups.

11 peakes were found in the FTIR chromatogram of *Dolichos lablab*. Among these, 2 was found in high peaks 996.98 and 1634.49 (Fig. 1). The appropriate class of functional group and its structure were given in table 4.

8 peakes were found in the FTIR chromatogram of *Glycine max*.Among these, 2 was found in high peaks 995.03 and 1633.20 (Fig. 4). The appropriate class of functional group and its structure were given in table 5.

Table 1: Phytochemical tests of *Dolichos lablab* seed

Sl. No.	Phytochemical constituents	Presence (+) or Absence (-) in different extracts			
		Acetone	Aqueous	Chloroform	Ethanol
1	Carbohydrate	+	+	+	+
2	Protein	+	-	+	+
3	Alkaloids	-	+	+	+
4	Flavonoids	+	+	+	+
5	Terpenoids	-	-	-	+
6	Tannins	+	+	-	-
7	Steroids	+	-	+	+
8	Phenols	+	+	+	+
9	Glycosides	-	-	-	-
10	Saponin	-	+	-	-

Table 2: Phytochemical tests of *Glycine max* seed

Sl. No.	Phytochemical constituents	Presence (+) or Absence (-) in different extracts			
		Acetone	Aqueous	Chloroform	Ethanol
1	Carbohydrate	+	+	+	+
2	Protein	+	+	-	+
3	Alkaloids	+	+	+	+
4	Flavonoids	+	+	-	+
5	Terpenoids	-	-	-	-
6	Tannins	+	+	-	+
7	Steroids	-	-	+	+
8	Phenols	+	+	-	+
9	Glycosides	+	-	+	+
10	Saponin	-	+	-	-

Table 3: Phytochemical tests of *Arachis hypogaea* seed

Sl. No.	Phytochemical constituents	Presence (+) or Absence (-) in different extracts			
		Acetone	Aqueous	Chloroform	Ethanol
1	Carbohydrate	+	+	+	+
2	Protein	+	+	-	+
3	Alkaloids	+	+	+	+
4	Flavonoids	-	-	-	-
5	Terpenoids	-	+	-	+
6	Tannins	-	-	-	-
7	Steroids	-	+	-	+
8	Phenols	-	-	-	-
9	Glycosides	-	+	-	+
10	Saponin	+	+	+	+

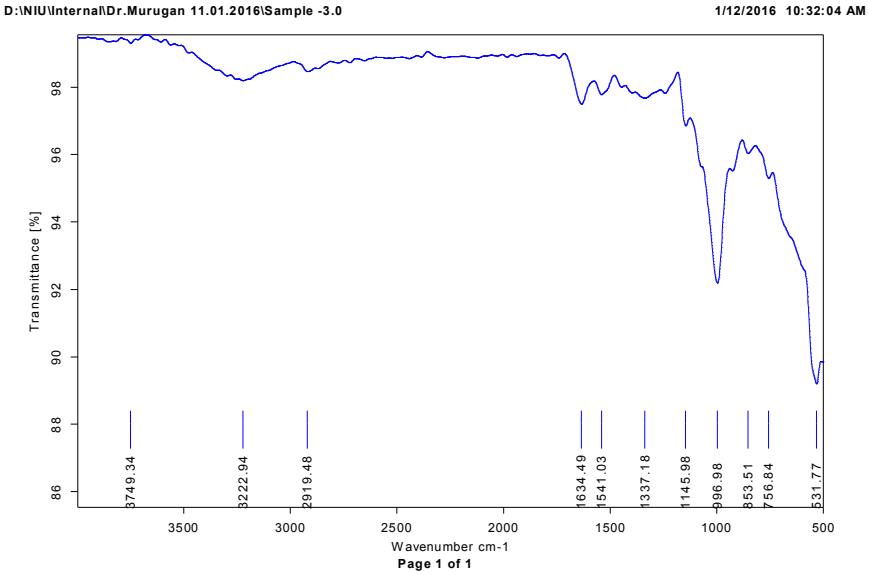

Fig. 1: FTIR chromatogram of *Dolichos lablab* seed

Table 4: FTIR-functional group of *Dolichos lablab* seed

Sl. No.	Peak value	Class of functional group	Structure	Assignment
1	996.98	Misc.	P-H phosphine	P-H bending
		Misc.	P-OR ester	P-OR ester
2	1634.49	Amides	RCONH2	NH out of plane
		Amines	RNH2	NH2 in plane bend
		Misc.	C=N	C=N

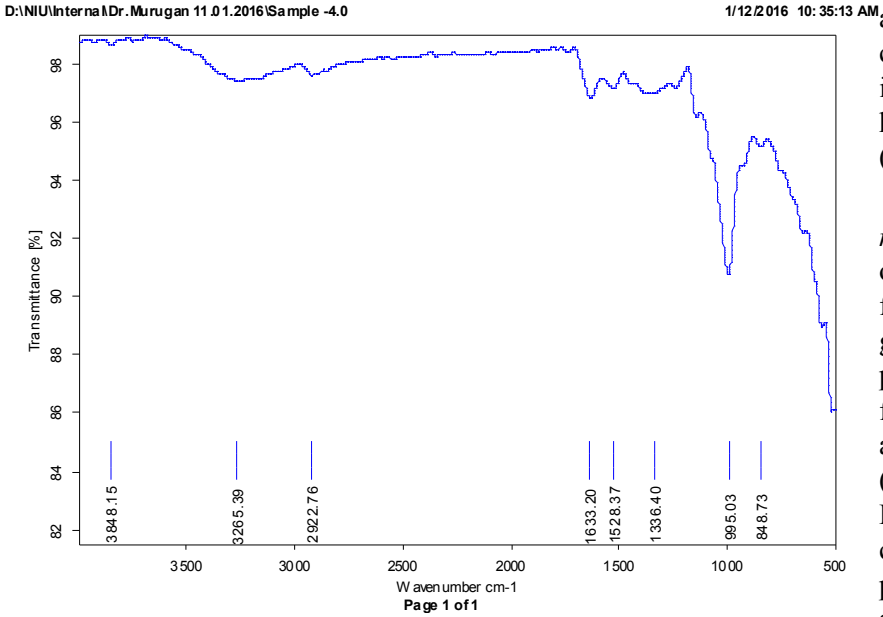

Fig. 2: FTIR chromatogram of *Glycine max* seed

15 peakes were found in the FTIR chromatogram of *Arachis hypogaea*. Among these, 4 was found in high peaks 1154.69, 1742.98, 2853.74 and 2921.67 (Fig. 3). The appropriate class of functional group and its structure were given in table 6.

In this present study, some major phytochemical tests includes carbohydrate, protein, alkaloids, flavonoids, terpenoids, tannins, steroids, phenols, glycosides and saponins were determined in four solvent extract of such as acetone, aqueous, chloroform and ethanol for the entire selected seeds samples such as *Dolichos lablab, Glycine max* and *Arachis hypogaea*.

The phytochemical tests of *Dolichos lablab* seed extracts showed the presence of carbohydrate, alkaloids, flavonoids, terpenoids, tannins, steroids, phenols, glycosides and saponins. In the study of Deoda *et al*., (2012), phytochemical investigation of *Dolichos lablab* confirms the presence of Carbohydrates, Glycosides, Saponins, Flavanoids, Polyphenols, Tannins and Lipids. Al-Snafi (2017) confirms the presence of sugar, alcohols, phenols, alkaloids, steroids, essential oils, tannins, flavonoids, coumarins, saponins, terpenoids, glycosides, anthnanoids. Also, the preliminary pharmacological studies revealed that *Dolichos lablab* possessed antidiabetic, anti-inflammatory, analgesic, cytotoxic, antioxidant, hypolipidemic, insecticidal, antimicrobial, antilithiatic, hepatoprotective, antispasmodic effects (Al-Snafi, 2017).

The phytochemical tests of *Glycine max* seed extracts showed the presence of carbohydrate, protein, alkaloids, flavonoids, tannins, steroids, phenols, glycosides and saponins. The phytochemicals such as alkaloids, flavonoids, saponin, tannins and phenols are present seed of *Glycine max* (Mbagwu *et al*., 2011). Presence of Phytosterols, flavonoids, phenolic compounds, tannins, carbohydrates, proteins, amino acids, fixed oils and fats are confirmed by Arora *et al*. (2013b).

The phytochemical studies revealed the presence of Saponins, Tannins, Alkaloids, Steroids and Numerous other chemicals. These compounds have significant therapeutic application against human and aquaculture pathogens including bacteria, fungi or virus. The methanol extract of *Glycine max* was exhibited high activity against *E.*

Table 5: FTIR-functional group of *Glycine max* seed

Sl. No.	Peak value	Class of functional group	Structure	Assignment
1	995.03	Alkenes	RCH=CH2	=CH out of plane
		Misc.	P-H phosphine	P-H bending
		Misc.	P-OR ester	P-OR ester
2	1633.20	Amides	RCONH2	NH out of plane
		Amines	RNH2	NH2 in plane bend
		Misc.	C=N	C=N

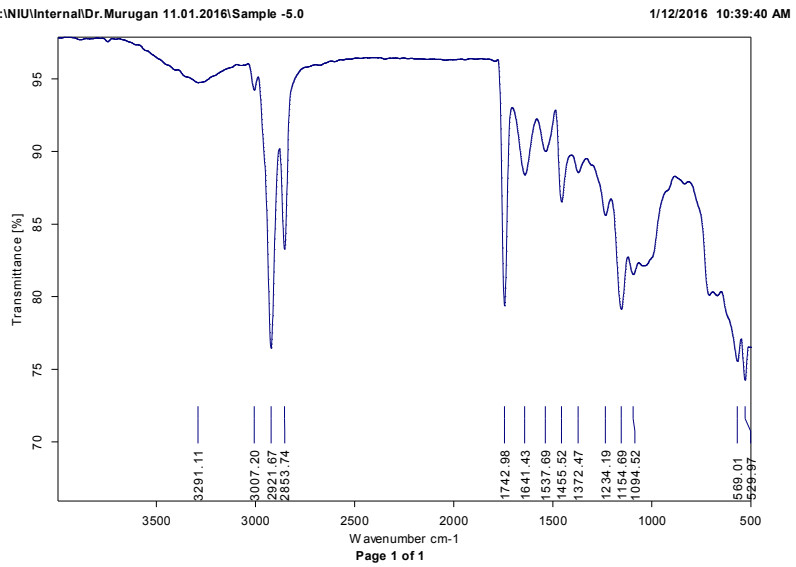

Fig. 3: FTIR chromatogram of *Arachis hypogaea* seed

Table 6: FTIR-functional group of *Arachis hypogaea* seed

Sl. No.	Peak value	Class of functional group	Structure	Assignment
1	1154.69	Alkyl halides	R-F	C-F stretch
		Amines	RNH2	C-N stretch
		Amines	R2NH	C-N stretch
		Misc.	C=S thiocarbonyl	C=S thiocarbonyl
		Misc.	S=O sulfone	S=O sulfone 2
		Misc.	P-H phosphine	P-H bending
		Misc.	P-O phosphine oxide	P-O phosphine oxide
		Misc.	P-O phosphate	P-O phosphate
		Carboxylic acid	RCO-OH	C-O stretch
		Esters	RCOOR	C-O stretch
		Alkyl halides	CH2X	C-H wag (-CH2X)
2	1742.98	Ketones	R2CO 5-ring	C=O stretch doublet
		Amides	RCONHR 4-ring	C=O stretch
3	2853.74	Alkanes	RCH2CH3	CH stretch
		Carboxylic acid	RCO-OH	Dimer OH
		Alkanes	-CH2-	-CH2
4	2921.67	Alkanes	RCH2CH3	CH stretch
		Carboxylic acid	RCO-OH	Dimer OH
		Alkanes	-CH2-	-CH2

coli and *V.harveyi* (Kumaran and Citarasu, 2015).

The phytochemical analysis of the plants is very important because of its commercial value in the Pharmaceutical companies for the development of newer drugs (Beena *et al*., 2016).

The phytochemical tests of *Arachis hypogaea* seed extracts showed the presence of carbohydrate, protein, alkaloids, terpenoids, steroids, glycosides and saponins. Marka *et al*. (2013). 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, quinones and saponins (Marka *et al*., 2013).

Flavonoids are capable of treating certain physiological disorder and diseases, super anti-oxidant and free radical scavengers which prevents oxidative cell damage and have strong anti-cancer activity. The Presence of glycosides in *Arachis hypogaea* uses potential precursors of defensive metabolites. Saponins present in the seeds of *Arachis hypogaea* have cholesterol binding properties, and help in hemolytic activities. The tannins in seeds serve as astringent properties for healing of wounds and inflaming mucous membrane. Phenols are found in different percentages of leaf and root extracts, they have the ability to block specific enzymes that causes inflammation (Okwu, 2004). The ethanolic extract of *Arachis hypogaea* shows the presence of phytochemical such as tannin, saponin, phylobatanin, flavonoid, terpenoid and cardiac glycoside (Prabasheela, 2015).

Among the selected seeds analysed *Dolichos lablab* seed extracts showed positive result for protein; *Glycine max* showed the negative for terpenoids. *Arachis hypogaea* seed extracts showed negative for flavonoids, phenols and tannins. Medicinal values of the plants have assumed an important dimension in the past few decades. Plants produce a diverse group of secondary metabolites with antimicrobial, anti-inflammatory, antioxidant potential *etc*. Antioxidant compounds block the action of free radicals present

in our body which have been implicated in the pathogenes is of many diseases and in the aging process (Aruoma, 2003).

Alkaloids, comprising a large group of nitrogenous compounds are widely used as cancer chemotherapeutic agents. Alkaloids also interfere with cell division; hence the presence of alkaloids in the plant makes it a possible remedy in the treatment of cancer. Glycosides have been found to be effective in congestive heart failure, regardless of the cardiac rhythm and that the beneficial effect is brought about by its direct action to increase the force of myocardial contraction. It also acts directly on the smooth muscles of the vascular system.

Plants producing phenolic compounds are exhibit antioxidant activity by scavenging the free radicals generated during metabolic process in the body. Flavonoids possess substantial anti-mutagenic and anti carcinogenic activities due to their antioxidant and anti-inflammation properties (Li-Weber, 2009).

Dolichus lablab shows the presence of Misc. with P-H phosphine, P-OR ester, C=N; Amides with RCONH2, Amines with RNH2 structures. *Glycine max* shows the presence of Alkenes with RCH=CH2, Misc. with P-H phosphine, P-OR ester, C=N; Amides with RCONH2 and Amines with RNH2 structures. *Arachis hypogaea* shows the presence of Alkyl halides with R-F, CH2X; Amines with RNH2, R2NH; Misc. with C=S thiocarbonyl, S=O sulfone, P-H phosphine, P-O phosphine oxide, P-O phosphate; Carboxylic acid with RCO-OH; Esters with RCOOR; Ketones with R2CO 5-ring; Amides with RCONHR 4-ring; Alkanes with RCH2CH3, CH2, RCH2CH3 structures.

Conclusion

The current study discussed the chemical constituents and Pharmacological effects of Dolichos lablab, Glycine max and Arachis hypogea as promising medicinal plants with wide range of Pharmocological activities which could be utilized in several medicinal uses.

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