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QUALITATIVE ANALYSIS OF PHYTOCHEMICALS IN TWO LEGUME SPECIES

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Abstract

Legume seeds are important sources of nutrients and can serve a high quality dietary protein sources to meet nutrient requirements. The important phytochemicals present in the two legumes species *Phaseolus vulgaris* L. and *Vigna unguiculata* (L) Walp. For Qualitative analysis four extracts were used, Maximum of the nutrients are Positive in Aqueous and Ethanol extract. All the Phytochemicals were medicinally very active that are used treat cough, asthma hypertensive and cardiac depressant properties.

Key words: Legume, Phytochemical, Ethanol, asthma.

Introduction

Legumes which are mainly the edible dry seeds are one of the major classes of seeds that play an important role in human nutrition (Iqbal *et al*., 2006). Legumes are grown agriculturally, primarily for their grain seed called pulse, for livestock forage and silage, and as soil-enhancing green manure. A legume fruit is a simple dry fruit that develops from a single carpel and usually dehisces (opens along a seam) on two sides (Messina, 1999).

Beans and legumes are an inexpensive food, commonly found in diets all over the world. High in fiber, calcium, complex carbohydrates, healthy fats, folate, potassium, magnesium and iron, beans and legumes are also a great source of protein.Protein content differs between types of legumes, but each kind offers its own unique nutritional profile, so make an effort to eat a variety.

Beans are such good sources of protein that the USDA includes them in both the vegetable and protein food groups. Proteins consist of chains of amino acids and there are nine amino acids. Men should get 56 grams and women need 46 grams of protein daily. There is evidence that a portion of pulses (roughly one cup daily) in a diet may help lower blood pressure and reduce LDL cholesterol levels, though there is a concern about the quality of the supporting data.

Legumes are excellent sources of protein, carbohydrates, essential micronutrients, and fiber. Substituting legumes for food that are high in saturated fats or refined carbohydrates is likely to lower the risk of cardiovascular disease. Although legumes are rich in a number of compounds that could potentially reduce the risk of certain cancers, the results of epidemiological studies are too inconsistent to draw any firm conclusions regarding legume intake and cancer risk in general.

The seed color of beans is determined by the presence and concentration of flavonol glycosides, anthocyanins, and tannins (Beninger and Hosfield, 2003; Tsuda *et al*., 2000). Recently, *Phaseolus vulgaris* L. is gaining increasing attention as a functional or nutraceutical food, due to its rich variety of phytochemicals with potential health benefits such as proteins, amino acids, complex carbohydrates, dietary fibers, oligosaccharides, phenols, saponins, flavonoids, alkaloids, tannins, among others (Amole *et al*., 2013; Mishra *et al*., 2010).

The young leaves, green pods and green seeds are used as vegetables whilst the dry seeds are used in the preparation of various food dishes (Anyia and Herzog,

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2004). The haulms are also used as a quality feed for livestock (Akande *et al*., 2010). The plant is well adapted to hot and dry climates, has the ability to fix atmospheric nitrogen, curbs soil erosion through food ground cover and contributes to soil fertility through decayed residues (Anyia and Herzog, 2004). Furthermore, the trade of the fresh produce, seeds and foods processed from cowpea provides many farmers with a source of income (Anyia and Herzog, 2004). Previous studies have shown that cowpea leaves contain flavonoids (Lattanzio *et al*., 1997) of which some are known to exhibit antimicrobial properties (Aziz *et al*., 1998).

Materials and methods

Collection of Plant Samples For Biochemical Studies

The seeds of the selected legumes were collected from agricultural fields in Kanyakumari District. The seeds were shade dried and finely powdered using electric blender and then used for further studies.

Identification 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 were subjected to soxhlet extraction using Acetone, Chloroform, Ethanol and Distilled water (aqueous extract). Each 5grams of plant material was filled separately in the thimble and extracted successively with 60 ml of solvents using a soxhlet extractor for three hours. After solvent evaporation, each of these solvent extract was weighed and preserved in room temperature until further use.

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 yellos 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% $FecCl_3$ 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.

Results and discussion

Phaseolus vulgaris L. is a sub-erect or twining annual herb, its Vernacular name is Tingalavari, *Vigna unguiculata* (L) Walp. is a twining bushy glabrous annual Plant. Both contain active Phytochemicals and healthy nutrients such as carbohydrates, Protein, Alkaloids, Flavonoids, Terpenoids, Tannins, Steroids, phenols, Glycosides and Saponin in different solvent extracts such

Table 1: Phytochemical tests of *Phaseolus vulgaris*L.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 *Vigna unguiculata* (L) Walp.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	-	+	-	-

as acetone, chloroform, Ethanol and Aqueous. Presence of these nutrient contents are Tabulated.

Screening of Phytochemical Constituents was qualitatively tested for the two samples with different solvent extracts. In *Phaseolus vulgaris* L. and *Vigna unguiculata* (L) Walp. seed, acetone extract showed positive for carbohydrate, Protein, Alkaloids, flavonoids, Terpenoids, Tannins Steoids and Phenols, aqueous extract showed positive for all phytochemicals except Tepenoids, chloroform extract showed positive for carbohydrate, Alkaloids, Flavonoids, Terpenoids and Phenol and Ethanol extract showed positive for all the phytochemicals except Alkaloids and saponin.

The seeds have astringent, laxative, diuretic, anthelmintic, antibacterial and galactogogue properties. The seeds are help in relieving the condition like anorexia, jaundice and general debility.

Preliminary phytochemicals investigations focus on the importance of separation of the natural compounds from their mixture as they may be used for various clinical practices (Ganatra *et al*., 2013). In the present study, the qualitative phytochemical screenings of the two bean seeds or nuts were determined by performing standard phytochemical test protocols followed by Harborne (1999) and Sofowora (1993).

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 *viz*. *Phaseolus vulgaris* L., *Vigna unguiculata* (L) Walp.

The phytochemical tests of *Phaseolus vulgaris* L. seed extracts revealed that the presence of carbohydrate, protein, alkaloids, flavonoids, terpenoids, tannins, steroids, phenols, glycosides and saponins. The bioactive components of *Phaseolusvulgaris* L. seed extract was analysed by Atchibri *et al*. (2010), the result of the study indicated that presence of alkaloids, flavonoids, glycosides, polyphenols, steroids polyphenols, saponins, steroids, tannins and terpenoids. (Pradeepkumar *et al*., 2015). Saponins are glycosides of both triterpenes and sterols having hypertensive and cardiac depressant properties (Trease and Evans, 1985), hence the presence of these metabolites in *Phaseolus vulgaris* L. seeds tend to support its medical uses. This could be responsible for their antibacterial properties as reported by Sofowora (1993). The presence of tannins also, showed that the seed could be used as purgative, cough, asthma and hay fever according Gills (1992).The present study concluded that the two legume species are rich in phytochemicals and treat so many diseases.

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