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Research Article

# PHYTOCHEMICAL SCREENING AND FT-IR STUDIES ON WILD AND COMMON SOUTH INDIAN LEGUMES

**M.MARIMUTHU* AND P.GURUMOORTHI**

**Nutraceuticals Chemistry Lab, Department of Food Process Engineering, School of Bioengineering, SRM University, Kattankulathur-603203, Tamil Nadu, India, Email:** marimtu@gmail.com

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**ABSTRACT**

The present study deals with the preliminary phytochemical screening, functional group identification of seed extract of wild and common legumes were investigated. Preliminary phytochemical tests are helpful in finding and locating chemical constituents which are source of pharmacologically active principles. The results of the phytochemical screening carried out on the seed extract of legumes showed the presence of useful phytonutrients. The extracts were subjected for identification of functional groups using Fourier transform infrared spectrophotometer, presence of alcohol and hydroxyl groups, alkane groups, alkene groups, nitrogen-oxy groups, sulfur-oxy groups, aryl groups, aliphatic iodo groups. To sum up, the legumes of the present study reveals as one of the cheapest sources of phytonutrients.

**Keywords:** Legumes, Phytochemical screening, FTIR-Spectroscopy, Functional groups.

## INTRODUCTION

Phytochemical are the dependable sources for the treatment of different health problems. Phytochemical techniques played a significant role in searching raw materials and resources for pharmaceutical industry. Legumes are an inexpensive source of proteins with desirable characteristic such as abundance of carbohydrates, ability to lower the serum cholesterol, high fiber, low fat (except oilseeds), high concentration of polyunsaturated fatty acids and a long shelf life. In addition to B complex vitamins, minerals and fiber, legumes are also major sources of proteins and calories [1].They are known to contain certain bioactive compounds whose beneficial effects need to be explored for exploitation. Research has to be geared to exploiting the unconventional legume resources to meet the protein requirements of developing countries. Legumes produce primary and secondary metabolites and other phytochemicals such as pharmaceuticals, pesticides and industrial products[2]. They are also an excellent source of nutraceuticals constituents such as fibre, phytic acid and polyphenols such as flavonoids, isoflavones, lignans and tannins. The bean seed coat colour is attributed to the presence and quantity of polyphenols such as flavonol glycosides, condensed tannins and anthocyanins. These compounds have antioxidant, antimutagenic and anticarcinogenic activities and also free radical scavenging properties[3]

Fourier transform infrared spectrometry (FTIR) can be used to identify the structure of unknown composition or its chemical group, and the intensity of the absorption spectra associated with molecular composition or content of the chemical group[4, 5].Fourier transform infrared spectrometry is a physico-chemical analytical technique that does not resolve the concentrations of individual metabolites but provides a snapshot of the metabolic composition of a tissue at a given time[6].The FT-IR method measures the vibrations of bonds within chemical functional groups and generates a spectrum that can be regarded as a biochemical or metabolic "fingerprint" of the sample. By attaining IR spectra from plant samples, it might possible to detect the minor changes of primary and secondary metabolites [4, 5]. At present, particularly in phytochemistry, FTIR has been exercised to identify the concrete structure of certain plant secondary metabolites [7-9]. FT-IR is one of the most widely used methods to identify the chemical constituents and elucidate the compounds structures, and has been used as a requisite method to identify medicines in Pharmacopoeia of many countries [10]. Hence during the present investigations phytochemical screening of wild and common legumes is carried out to analyze the presence of chemical constituents that included primary & secondary metabolites, with a view to recommend their application in pharmaceutical industry.

## MATERIALS AND METHODS

### Samples collection

The *Mucuna pruriens* (velvet bean) were from natural stands of ecological region of Tamil Nadu, South India. *Macrotyloma uniflorum* (horse gram), *Phaseolus lunatus* (lima bean) and *Canavalia ensiformis* (jack bean) were purchased from local market in Madurai, Tamil Nadu, South India. After drying in the sun, the pods were thrashed to separate mature seeds. After thorough cleaning and removal of broken seeds and foreign materials, the seeds were stored in plastic containers at room temperature (25°C) until further use.

### Preparation of raw seed samples

Dry mature seeds of different accessions (10 g each) were powdered in a Wiley Mill to 60-mesh size with suitable precaution to avoid contamination of samples. The powders were stored in plastic containers at room temperature (25°C) until further use.

### Solvent extraction

Solvent systems used for the extractions were Acetone, Ethanol, chloroform, Petroleum ether, hexane, methanol and aqueous. Soxhlet and flask extraction procedures were adapted for extraction. 10g grams of the powered samples were packed in muslin cloth and used for extraction by soxhlet apparatus at a temperature below the boiling temperature of each solvent. A portion of the powdered plant samples was soaked in the conical flask containing solvent, wrapped with aluminum foil and placed in shaker for 48 hours at 120-130 rpm. After 48 hours, the extracts were filtered using Whatman filter paper No: 1. the solvent was evaporated and the residue was dissolved in sterile dimethylsulfoxide (DMSO-9:1) in 50 mg/ml concentration. The extract was filtered using 0.22 micro filters (Type GV- Millipore) and stored at 4°C for further phytochemical screening study.

### FTIR Spectroscopic Analysis

Fourier transform infrared (FTIR) Spectrophotometer was used to identify the characteristic functional groups in the seed extract. A small quantity (5 mg) of the seed extract was dispersed in dry potassium bromide (KBr). The mixture was thoroughly mixed in a

mortar and pressed at pressure of 6 bars within 2 min to form a KBr thin disc. Then the disc was placed in a sample cup of a diffuse reflectance accessory. The IR spectrum was obtained using Perkin Elmer 2000 infrared spectrometer. The sample was scanned from 400 to 4000 $cm^{-1}$ for 16 times to increase the signal to noise ratio. Samples were run in triplicate and all of them were undertaken within a day period.

**Phytochemical screening of the seed extract**

A small portion of the seed powder extract was used for the phytochemical tests for compounds which include glycosides, tannins, phenols, flavonoids, alkaloids, saponins, steroids and terpenoids in accordance with the methods.

**Tests for Alkaloids**

Dragendorff's test: To the 1 mL of extract, add 1 mL of Dragendorff's reagent (potassium bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

Mayer's test: To the 1 mL of extract, add 1 mL of Mayer's reagent (Potassium mercuric iodide solution). Whitish yellow or cream coloured precipitate indicates the presence of alkaloids.

**Test for Glycosides**

Glycosides are compounds which upon hydrolysis give rise to one or more sugars (glycones) and a compound which is not a sugar (aglycone or genine). To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added, and observed for a reddish brown coloration at the junction of two layers and the bluish green color in the upper layer[11].

**Test for Terpenoids and steroids**

4mL of extract was treated with 0.5 ml of acetic anhydride and 0.5 mL of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet color was observed for terpenoid and green bluish color for steroids [11].

Salkowski test: 5 mL of each extract was mixed in 2 mL of chloroform, and concentrated sulfuric acid (3 mL) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids.

**Test for Flavonoids**

5mL of extract solution was treated with 1.5 mL of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids and orange color for flavones [11].

**Test for Tannins**

To 0.5 mL of extract solution 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue color was observed for Gallic tannins and green black for catecholic tannins [12].

**Test for saponins**

1mL of extract reacts with 2mL alcoholic vanillin solution and adds few drops conc. sulfuric acid. The formation of deep red colour indicates presence of saponins.

**Test for phenols**

2mL of extract was added to 2mL of ferric chloride solution (FeCl3), a deep bluish green solution is formed with presence of phenols. Lead tetra acetate test: 1mL of extract treats with 2 mL of lead tetra acetate. The result shows formation of precipitate the presence of tannins and phenols.

**RESULTS AND DISCUSSION**

The results of preliminary phytochemical analysis are tabulated (Table 1, 2). The phytochemical study revealed the presence of various phytocompounds in all the solvent extracts.

In the velvet bean seed extract, Alkaloids were present in all the extract. Steroids were found to be present in all the extracts except ethanol and water extracts. It was also observed that triterpenoids were present in acetone, petroleum ether and hexane extracts and the flavonoids were present only chloroform extract. The presence of phenols and tannin was observed in ethanol and water extract while glycosides were found only in hexane extract. The results show that saponins were present in all the extracts except hexane and methanol seed extracts.

From the jack bean seed extract, Alkaloids were found to be present in all the extract except acetone extract. Steroids were found to be present in all the extracts except hexane and petroleum ether extracts. It was also observed that triterpenoids were present in acetone and water extracts and the flavonoids were present only chloroform and petroleum ether seed extracts. The presence of phenols and tannin was observed in ethanol; hexane and petroleum ether extracts while glycosides were found only in acetone, chloroform, petroleum ether and water extract. The saponins were present in hexane and water seed extracts.

In all the solvent extracts of lima bean shows, alkaloids were absent in chloroform and water extract. Steroids were found to be present in ethanol, methanol, hexane and water extracts. It was also observed that triterpenoids were present in acetone, ethanol and hexane extracts and the flavonoids were absent in petroleum ether, hexane and acetone. The presence of phenols and tannin was observed in acetone, ethanol, methanol and petroleum ether while glycosides were found only in acetone, chloroform and petroleum ether extract. The results show that saponins were present in all the extracts except acetone.

In Horse gram seed extracts shows were alkaloids found to be present in all the extract except chloroform and acetone extract. Steroids were found to be present in acetone, chloroform, ethanol and methanol seed extracts. It was also observed that triterpenoids were present in acetone, methanol and water extracts and the flavonoids were present in only chloroform extract. The presence of tannin was observed in hexane, ethanol, petroleum ether and methanol and while glycosides were found all the extract except in ethanol and hexane extract. The saponins were present in hexane and water seed extracts. The preliminary phytochemical studies during the present investigations revealed that the wild and common legumes are mainly constituted of various primary & secondary metabolites which can be quantified for application in pharmaceutical industry.

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The Infra-red spectroscopic (IR) analysis of studied legumes, in a band width ranging from 400 to 4000 $cm^{-1}$, revealed the presence of different functional groups (Table 3). The peaks showed that the extract of legumes may have the compounds like alcohol, amides, amines, fluride, iodide, chloride, phosphine, bromide, sulfonates, aliphatic organo halogen, aliphatic and aromatic nitro compounds (Table 4). Further, it indicates the possible chemical bond and compound type as follows (Fig. 1 to 4). O-H, N-H stretching for alcohol (3398.97, 3399.83, 3399.44, 3401.66 $cm^{-1}$), N-H stretching and for amine salt (2926.54, 2926.26, 2925.45, 2925.48 $cm^{-1}$), C=O stretching for amide (1744.78, 1655.98, 1658.06, 1658.03, 1658.26 $cm^{-1}$), Asymmetric stretching for aliphatic and aromatic nitro compounds (1547.86, 1547.51, 1545.96 $cm^{-1}$), P-CH3 bending for phosphine (1408.19, 1408.06, 1407.00, 1402.32 $cm^{-1}$).C-F, C-Br stretching for fluride and aryl bromide (1249.82, 1078.47 $cm^{-1}$) and C-F, C-N stretching for aryl fluride and aliphatic amines (1161.05 $cm^{-1}$) only found in lima bean. C-Br, C-I stretching for bromide and iodide were present in only velvet bean. C-F, C-Br stretching for flurides and aryl flurides (1046.00 $cm^{-1}$) were only found in horse gram, P-H stretching for phosphine (985.09 $cm^{-1}$) was observed in only lima bean. S-O stretching for sulfonates (860.79, 859.79, 859.99, and 860.09 $cm^{-1}$) and C-Br, C-I stretching for bromides, iodides (572.33, 571.84, 574.61, and 573.89 $cm^{-1}$) were present in both wild and common legumes. C-I, C-Br, C-Cl stretching for iodides, bromides, chloride were found to be present in only

horse gram. From the above analysis we conclude that amines, aliphatic and aromatic nitro compounds, bromides, sulfonates, iodides and fluorides are the most commonly detected compounds in the legumes. The above result was analyzed with interpretation of infrared spectra, a practical approach by John Coates (2000). This analytical method is highly rapid, effective, visual and accurate for pharmaceutical research.

**TABLE 1: PHYTOCHEMICAL SCREENING OF WILD LEGUMES**

| Phytochemicals | Acetone | | Chloroform | | Ethanol | | Methanol | | Hexane | | Pet. Ether | | Water | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| Alkaloids | + | - | + | + | + | + | + | + | + | + | + | + | + | + |
| Steroids | + | + | + | + | - | + | + | + | + | - | + | - | - | + |
| Triterpenoids | + | + | - | - | - | - | - | - | + | - | + | - | - | + |
| Flavonoids | - | - | + | + | - | - | - | - | - | - | - | + | - | - |
| Tannins | - | - | - | - | + | + | - | - | - | + | - | + | + | - |
| Phenols | - | - | - | - | + | + | - | - | - | + | - | + | + | - |
| Glycosides | - | + | - | + | - | - | - | - | + | - | - | + | - | + |
| Saponins | + | - | + | - | + | - | - | - | - | + | + | - | + | + |

**(+)Present; (-) Absent, A –Velvet bean; B – Jack bean**

**TABLE 2: PHYTOCHEMICAL SCREENING OF COMMON LEGUMES**

| Phytochemicals | Acetone | | Chloroform | | Ethanol | | Methanol | | Hexane | | Pet. Ether | | Water | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | D | C | D | C | D | C | D | C | D | C | D | C | D |
| Alkaloids | + | - | - | - | + | + | + | + | + | + | + | + | - | + |
| Steroids | - | + | - | + | + | + | + | + | + | - | - | - | + | - |
| Triterpenoids | + | + | - | - | + | - | - | + | + | - | - | - | - | + |
| Flavonoids | - | - | + | + | + | - | + | - | - | - | - | - | + | - |
| Tannins | + | - | - | - | + | + | + | + | - | + | + | + | - | - |
| Phenols | + | - | - | - | + | + | + | - | - | + | + | - | - | - |
| Glycosides | + | + | + | + | - | - | - | + | - | - | + | + | - | + |
| Saponins | - | - | + | - | + | - | + | - | + | + | + | - | + | + |

**(+)Present; (-) Absent, C – Lima bean; D – Horse gram**

**TABLE 3: IR SPECTROSCOPIC ANALYSIS OF COMMON AND WILD LEGUMES, SHOWING FUNCTIONAL GROUPS**

| Wave number ($cm^{-1}$) | | | | |
|---|---|---|---|---|
| Wild legumes | | Common legumes | | |
| A | B | C | D | Functional group |
| 3399.44 | 3399.44 | 3398.97 | 3399.83 | Alcohol and hydroxyl compound |
| 2925.45 | 2925.45 | 2926.54 | 2926.26 | Saturated Aliphatic (alkane) |
| 1744.78 | 1744.78 | 1658.26 | 1658.03 | Oleifinic (alkene) |
| 1655.98 | 1655.98 | 1547.86 | 1547.51 | Nitrogen Oxy compound |
| 1545.96 | 1545.96 | 1408.19 | 1408.06 | Alcohol and hydroxyl compound |
| 1407.00 | 1407.00 | 1161.05 | - | Sulfur Oxy Compound |
| 1249.82 | 1249.82 | - | 1046.0 | Aliphatic organohalogen |
| 1050.16 | 1050.16 | 985.09 | - | Oleifinic (alkene) |
| 860.79 | 860.79 | 860.09 | 859.99 | Aromatic ring (Aryl ) |
| 572.33 | 572.33 | 547.61 | 573.89 | Aliphatic Iodo |

**(-) – Not observed, A – Velvet Bean; B – Jack bean; C –Lima bean; D – Horse gram**

**TABLE 4: IR SPECTROSCOPIC ANALYSIS OF COMMON AND WILD LEGUMES, WHICH SHOWING CHARACTERISTIC ABSORPTION PEAKS AT IR RANGE.**

| Wave number ($cm^{-1}$) | | | | Type of Bond | Compound type |
|---|---|---|---|---|---|
| Wild legumes | | Common legumes | | | |
| A | B | C | D | | |
| 3399.44 | 3401.66 | 3398.97 | 3399.83 | O-H, N-H(s) | Alcohol, Amines |
| 2925.45 | 2925.28 | 2926.54 | 2926.26 | N-H(s) | Amine salts |
| 1744.78 | - | - | - | C=O (s), N-H(b) | Amides |
| 1655.98 | 1658.06 | 1658.26 | 1658.03 | C=O(s), N-H(b) | Amides |
| 1545.96 | - | 1547.86 | 1547.51 | Asym.(s), Asym.(s) strong | Aliphatic, Aromatic nitro compounds |
| 1407.00 | 1402.32 | 1408.19 | 1408.06 | P-CH3(b) | Phosphine |
| 1249.82 | 1078.47 | - | - | C-F(s), C-Br(s) | Flurides, Aryl bromides |
| - | - | 1161.05 | - | C-F(s), C-N(s) | Aryl fluride, Aliphatic amines |
| 1050.16 | - | - | - | C-Br(s), C-I(s) | Bromides, Iodides |
| - | - | - | 1046.0 | C-F(s), C-Br(s) | Flurides, Aryl bromides |
| - | - | 985.09 | - | P-H(b) | Phosphine |
| 860.79 | 859.79 | 860.09 | 859.99 | S-O(s) | Sulfonates |
| 572.33 | 571.84 | 573.89 | 574.61 | C-Br(s), C-I(s) | Bromides, Iodides |
| - | 547.54, 529.71 | - | - | C-I(s), C-Br(s) | Iodides, Bromides |

**(-) – Not observed, A – Velvet Bean; B – Jack bean; C –Lima bean; D – Horse gram**

**(s)-Stretching; (b)-Bending**

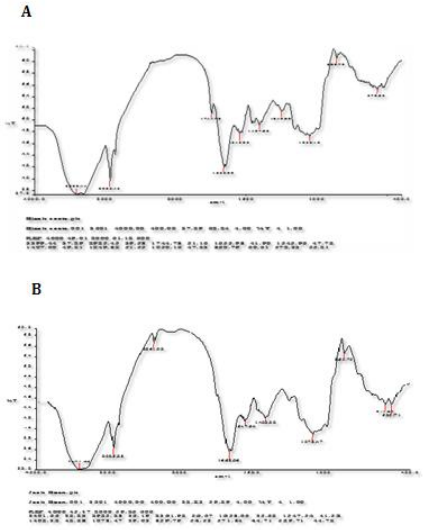


**FIGURE 1:FTIR SPECTROSCOPIC ANALYSIS OF WILD LEGUMES**

**A- FTIR Spectrum of velvet bean; B- FTIR Spectrum of Jack bean**

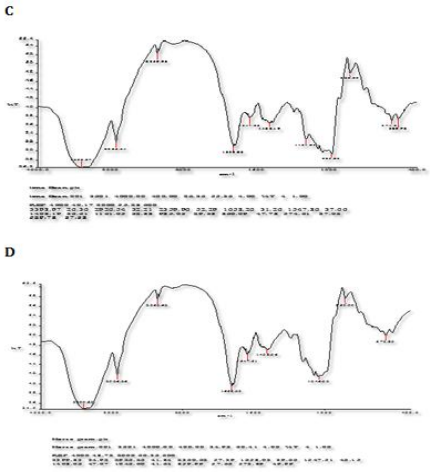


**FIGURE 2: FTIR SPECTROSCOPIC ANALYSIS OF COMMON LEGUMES**

**C-FTIR Spectrum of Lima bean; D-FTIR Spectrum of Horse gram**

## CONCLUSION

The present study was concluded that legumes as rich source of phytochemicals with the presence of glycosides, tannins, phenols, flavonoids, alkaloids, saponins, steroids and terpenoids. Thus this legume can be utilized as an alternative source of useful drugs. The presence of characteristic functional groups of alcohol and hydroxyl groups, alkane groups, alkenes groups, nitrogen-oxy groups, sulfur-oxy groups, aryl groups, aliphatic iodo groups are responsible for various medicinal properties of legumes. In future these legumes could be used as good pharmaceutical and therapeutic agents. Further studies are needed with this legume to isolate, characterize and elucidate the structure of the bioactive compounds of this legume for drug formulation.

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