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Phytochemical Analysis of *Sesbania sesban (L) merr* Leaves

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ABSTRACT

In order to give scientific background to the traditional claim of the plant, the leaves of *Sesbania sesban (L) merr* were subjected for phytochemical analysis. The present study deals with various pharmacognostical examinations like organoleptic, microscopical studies, physical evaluation and preliminary Phytochemical screening of various extracts were also carried out Qualitative chemical examination of various extracts revealed the presence of flavonoids, steroids, and phenolic compounds. An attempt has been made to isolate a phytoconstituents from successive ethanolic extract using column chromatography and further characterized by spectroscopic methods and upon analysis by UV, FT-IR, NMR, and GC-MS the isolated compound was characterized as a flavonoid.

INTRODUCTION

Medicinal plants are useful in curing and healing of various diseases in human being as well as in animals because of the presence of various medicinal constituents or phytochemical constituent. These phytoconstituents are the preliminary and secondary metabolites of the plants play a very important role in their growth development and survival. *Sesbania sesban (L) merr belongs* to family Fabaceae or Leguminosae (Sesbania aegypticapers) claim to posess various activities like Antidiabetic[1.] Hemoglobin glycosylation[2.]Antioxidant and Antimicrobial Activity[3.]Purgative, demulcent, Maturant, Anthelmintic and for all pains and inflammation, literature studies revals the presence several chemical compounds[4] like Saponins, Tannins, Alkaloid, Phenolic compounds, in barks[5,] root[6] and flowers[7.] The present study was aimed to carry out phytochemical analysis of *sesbania sesban (L) merr* leaves which are responsible for various activities including diabetes.

MATERIALS AND METHODS:

Plant collection: The leaves of *Sesbania sesban (L) Merr* were collected from the local & surroundings areas of Hubli in Karnataka, and authenticated by prof. k Prabhu Dept. of pharmacognosy S.C.S. College of pharmacy Harapanahalli.

Extraction with different solvents:

Procedure: The leaves of *Sesbania sesban (L) Merr* were shade dried pulverized and 100gm of coarse powder was further subjected to continuous hot percolation[8] (soxhlation) successively using solvents with increasing polarity such as Petroleum ether (40-60°C), Chloroform, Ethylacetate and Ethyl alcohol. After the exhaustive extraction, the solvent was removed under reduced pressure using rotary flash evaporator then finally dried in desiccator over sodium sulfite. This procedure was repeated for several times to get sufficient amount of extracts for further processing, after drying, the respective extracts were weighed and percentage yields were calculated.

Qualitative phytochemical investigations [9, 10, 11]

The extracts of *Sesbania sesban (L) Merr* Leaves were subjected to various qualitative chemical tests and detected the presence of various phytoconstituents as reported in table no 2.

Chromatographic studies: Thin layer chromatographic (TLC) studies were carried out for different extracts to substantiate the presence of Phytoconstituents detected in qualitative chemical tests & to detect numbers of phytoconstituents present in each extract. TLC is mode of chromatography in which sample is applied as a small spot on to the origin of a thin sorbent layer supported on a glass, plastic or metal plate. The prepared plates are allowed for setting (air drying). This is done to avoid cracks on the surface of adsorbent. After setting the plates are activated by keeping in an oven at 100 - 120°C for 1hr. Activation of TLC plates is nothing but removing water/moisture and other adsorbed substances from the surface of any adsorbent, by heating at 100-120°C so that adsorbent activity is retained. The R_f values are calculated using following formula[12].

$$\text{Resolution Factor } (R_f) = \frac{\text{Distance traveled by the solute from the origin}}{\text{Distance traveled by the solvent front from the origin}}$$

Isolation of phytoconstituents: Isolation of phytoconstituents from successive Alcoholic extract was carried out, as this particular extract showed eight spots respectively. From this one compound were isolated by column chromatography.

Column chromatography: Column chromatography is one of the most useful methods for the separation and purification of both solids and liquids. Column chromatography is another solid-liquid technique in which the two phases are a solid (stationary phase) and a liquid (moving phase). The most common adsorbents used are silica gel G and alumina. The sample is dissolved in a small quantity of solvent (the eluent) and applied to the top of the column.

Scheme of isolation of flavonoid

- Ethyl alcohol extract.
- Column chromatography.
- Elutes are tested for phytoconstituents by TLC.
- Isolated compounds are subjected to Spectral analysis.

Details of Column chromatography:

Adsorbent **:** Silica gel 'G' for column chromatography.

Activation **:** 110°C for 1 hour.

Length of the column **:** 55cm.

Diameter **:** Outer- 3.4cm, Inner- 3 cm

Length of the adsorbent **:** 30cm.

Rate of elution **:** 30-40 drops / min.

Volume of elute collected **:** 10 ml each.

Eluent **:** Chloroform: Ethyl acetate: Methanol: Glacial acetic acid.

(4.5:4:2.5:1)

Preparation of the sample: 2gm of ethyl alcohol extract was dissolved in 10 ml of methanol and mixed with 2 gm of silica gel (60-120 mesh size) and dried in vacuum oven at 45°C. The absorbed material obtained was transferred to column[13, 14].

Column packing: 100gms of silica gel 'G' for column chromatography [laboratory grade] was activated in hot air oven at 110°C for 1hr. above said solvent system was used to pack silica gel in the glass column. The glass wool was fixed at bottom. The slurry of activated silica with chloroform was charged in to the column in small portion, with gentle tapping after each addition in order to ensure uniform packing. The small quantity of eluent was allowed to remain at the top of the column (about 4cms) in order to prevent the drying and possible cracking of the packed column. Tapping is necessary to avoid the air bubble if any present in the column during packing.

Column packed is kept for stabilization overnight. Then the sample was charged to the column, a small cotton pad was kept over the sample to prevent mixing of dust particles with the sample and column was eluted with the mobile phase. About 220 fractions of 10 ml each were collected in the test tubes and each fraction was subjected to TLC to analyze the eluting number of compounds by single spotting. These fractions were grouped together according to

their Rf values of phytoconstituents judged from the TLC analysis. Eluents of same R_f values were pooled together[15].

Purification by recrystallization: Isolated & separated compounds A was dissolved in pure methanol and evaporated on the water bath.

Characterization of isolated compound 1: The physical characteristics like appearance, color, texture, odour, and solubility of compounds A were determined and further characterized by spectroscopy as follows. UV, FT-IR, ^{1}H-NMR, ^{13}C-NMR and MS studies.

RESULT:

Successive extraction

Table No. 01: percentage yield and physical characteristic of various extract of *Sesbania sesban (L) merr* leaves

Extract	% Dry wt gms	Colour	Odour	Consistency
Petroleum ether(40-60^0)	4.34	Yellowish brown	characteristic	powder
chloroform	6.69	Dark green	characteristic	Sticky mass
Ethyl acetate	1.70	Dark green	characteristic	Sticky mass
Alcoholic	3.02	Greenish black	characteristic	Sticky mass

Table No. 02: Preliminary phytochemical of various extracts of *sesbania sesban (l) merr* leaves

Nature	Successive fractions			
	P. Ether	Chloroform	Ethyl acetate	Alcoholic
Alkaloids	-ve	-ve	-ve	+ve
Steroids	+ve	+ve	-ve	+ve
Carbohydrates	-ve	-ve	-ve	+ve
Phenolic	-ve	-ve	-ve	+ve
Flavonoids	-ve	-ve	+ve	+ve
Glycosides	-ve	-ve	-ve	-ve
Triterpenoids	+ve	+ve	-ve	-ve
Tannins	-ve	-ve	+ve	+ve

Keywords:

P.E = Petroleum ether. +ve = Present and –ve = Absent

EVALUATION OF ALCOHOLIC EXTRACT BY TLC

Stationary phase : silica gel G

Mobile phase : chloroform: ethyl acetate: methanol: GAA

Proportion : 4.5: 4: 2.5: 1.5 (v/v/v)

Detection : UV 254

Preparation of sample: Extract was dissolved in methanol.

Solvent front : 8cm

Number of spots : 8

Rf values: 0.27, 0.36, 0.43, 0.5, 0.52, 0.6, 0.72 and 0.81.

TLC of alcoholic extract

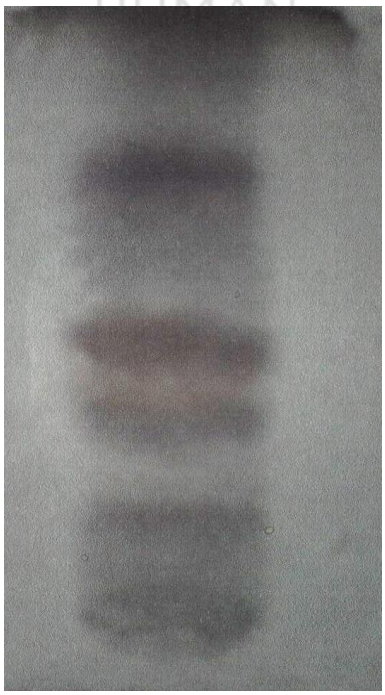

Figure No. 01: TLC of alcoholic extract

ISOLATED COMP-1

Mobile : chloroform: ethyl acetate: methanol: GAA

Proportion : 4.5: 4: 2.5: 1.5

Detection : UV-254

Table No. 03: TLC evaluation of Comp-1

Isolated COMP-1	Evaluation of chromatogram		
	Under visible range	Under UV range	Rf value
COMP-1	Yellow	Light green spot	0.67

TLC of Isolated COMP-1

Figure No. 02: TLC of isolated COMP-1

Table No. 04: characterization of isolated comp-1

Spectra	Characters
UV	Open peak with λ_{max} at 267nm
FT-IR	Peaks at following wave number are observed. Wave number 3394.22 -OH Stretching 2927.26 -C-H Stretching 2362.02 -C=C Stretching 1734.93 -C=O Stretching 1690.77 -CO-CH3
^{1}H-NMR	Peaks at following δ values are observed. δ value 0.88 -m(4H) 2.10 -s(1H) 3.4 -d(2H) 4.7 -m(4H) 7.1 -s(1H), Ar-H 8.2- s(1H), Ar-H
^{13}C-NMR	Peaks at following δ values are observed. δ value 23.01-s(1C) 29.04-s(1C) 39.02-s(1C) 59.06-s(1C) 72.07-m(4C) 78.04-(1C) 83.08-(1C)
MS	Base peak at 263 Molecular ion peak 290

DISCUSSION:

Preliminary phytochemical analysis of various extracts carried out and results revealed that presence of flavonoids, phenolic compounds, steroids, Carbohydrates, Alkaloids, Glycosides, triterpinoids and tannins in alcoholic extract so Further chromatographic studies were done for confirmation of above phytoconstituents. The alcoholic extract was found to contain eight spots in TLC then an attempt was made to isolate these phytoconstituents by column chromatography with isocratic elution. The isolated compounds 1 were further characterized by chromatography and spectral analysis such as, UV, FT-IR, NMR and Mass spectroscopy, UV spectra have shown one peak with λ_{max} at 267 nm. IR spectra has shown wave numbers at 3394.22 – OH Stretching, 2927.26 – C-H, Stretching, 2362.02– C=C Stretching,1734.93– C=O Stretching.

^{1}H-NMR Peaks at following δ values are observed. δ value, 0.88-m(4H), 2.10-s(1H), 3.4 -d(2H), 4.7-m(4H), 7.1-s(1H), Ar-H, 8.2-s(1H), Ar-H ^{13}C-NMR has shown δ Values at 23.01--s(1C), 29.04 --s(1C), 39.02--s(1C),59..06--s(1C), 72.07--m(4C), 78.04--(1C),83--(1C). MS spectra showed, Base peak at 263 and molecular ion peak at 290. With these findings the isolated comp 1 was identified as flavonoid.

CONCLUSION:

From the present studies it was concluded that comp-1 was isolated and characterized as Flavonoid may be responsible for various traditional claims but further it has to be subjected for various in-vitro and in-vivo studies for further confirmation.

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