Section A-Research paper
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ISOLATION AND EVALUATION OF LEAVES OF SESBANIA SEBAIN AS A POTENTIAL ANTI-TB AGENT

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Abstract

The present study was carried out to investigate *Sesbania sesban (L) Merr* leaves pharmacognostic and phytochemical, in-vitro and in-vivo antidiabetic screening. *Sesbania sesban (L) merr* leaves was collected from local and surroundings areas in Karnataka and

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authenticated by renowned botanist. Authenticated plant material was subjected to morphological and microscopical analysis. Shade dried and powdered leaves was subjected to successive soxhlet extraction with organic solvents of increasing polarity like Pet. ether, chloroform, ethyl acetate and alcohol. All the extracts were evaluated by qualitative chemical examination for the presence of important phytoconstituents and confirmed by TLC studies.One phytoconstituents (COMP-I) were isolated from ethanolic extract by column chromatography and an attempt was made to characterize the isolated compounds by TLC, UV, FT-IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectroscopicstudies. Characterization revealed that COMP-I may be a flavonoid moiety. The in vitro anti-TB studies of *Sesbania sesban (L) merr* leaves were studied by invitro Micro plate Alamar Blue Assay (MABA) method and results revealed to be equipotent when compared to standard drugs like pyrazinamide, streptomycin and ciprofloxacin.In this study an attempt was made to provide scientific backup to the traditional claim. Since the results showed a significant anti-TB activity the traditional use of *Sesbania sesban (L) Merr* leaves for the anti-TB activity may be justified.

Keywords: *Sesbania sesban (L) Merr*, Micro plate almar assay, tuberculosis , anti-tubercular agent

Introduction

Herbal medicine, also called botanical medicine or phytomedicine, refers to the use of any plants or plant parts for medicinal purposes. Long practiced outside of conventional medicine, herbalist is becoming more mainstream as up-to-date analysis and research show their value in the treatment and prevention of disease. Plants had been used for medicinal purposes long before recorded history. For example, ancient Chinese and Egyptian papyrus writings describe medicinal plant uses. Indigenous cultures (e.g., African and Native American) used herbs in their healing rituals, while others developed traditional medicinal systems (e.g., Ayurveda and Traditional Chinese Medicine) in which herbal therapies were used systematically. Scientists found that people in different parts of the globe tended to use the same or similar plants for the same purposes[1-4].

Sesbania sesban(L)merr.(*Sesbania aegyptica pers)* Being so conspicuous and widely planted, this tree has names, number of common.

Tuberculosis :

Tuberculosis, commonly known as MTB, orTB, in the past also calledphthisis,pulmonalis, orconsumption, is a widespread, and in many cases fatal,bacteria infectious caused by various strains of mycobacteria, usually *Mycobacterium tuberculosis*.Tuberculosis typically attacks the lungs, but can also affect other parts of the body. It is spread through the air when people who have an active TB infection cough, sneeze, or otherwise transmit respiratory fluids through the air.Most infections do not have symptoms, known as latent tuberculosis. About one in ten latent infections eventually progresses to active disease which, if left untreated, kills more than 50% of those so infected[5].

Signs and symptoms:

The classic symptoms of active TB infection are achronic cough with blood tinged sputum, fever, night sweats, and weight loss(the latter giving rise to the formerly common term for the disease, "consumption"). Infection of other organs causes a wide range of symptoms.

General signs and symptoms includes Fever, Chills, Night sweats, Loss of appetite, Weight loss, Fatigue. Significant Nail clubbing may also occur[6].

Types of Tuberculosis :

Based on the site of infection, it is classified into two types .

a) pulmonary tuberculosis.

b) Extrapulmonarytuberculosis.

a) pulmonary tuberculosis :

Tuberculosis may infect any part of the body, but most commonly occurs in the lungs known as pulmonary tuberculosis.If a tuberculosis infection does become active, it most commonly involves the lungs (in about 90% of cases).Tuberculosis may become a chronic illness and cause extensive scarring in the upper lobes of the lungs. The upper lung lobes are more frequently affected by tuberculosis than the lower ones.The reason for this difference is not

entirely clear.It may be due either to better air flow within the upper lungs.Symptoms may includes chest pain and a prolonged cough producing sputum. About 25% of people may not have any symptoms (i.e. they remain "asymptomatic").

b) Extrapulmonary tuberculosis :

Extrapulmonary TB occurs when tuberculosis develops outside of the lungs, causing other kinds of TB.These are collectively denoted as "extrapulmonary tuberculosis".Extrapulmonary TB occurs more commonly in immuno suppressed persons and young children. In those with HIV, this occurs in more than 50% of cases.

Notable extrapulmonary infection sites includes the pleura(in tuberculous pleurisy), the central nervous system(in tuberculous meningitis), the lymphatic system (in scrofula of the neck),the genitourinary system(in urogenital tuberculosis)and the bones and joints (inPott disease),

When it spreads to the bones, it is also known as "osseous tuberculosis".a form of osteo myelitis.Sometimes, bursting of a tubercular abscess through skin results in tuberculous ulcer[8].

SYNONYMS:

Kan:Arinintajinamgi,

Hindi: Jainti, Jait, Rawasan,

English:Sesbsnia Sesban, Egyptian rattle pod, Frother, Iver bean, Sesban, Sesbania,

Bengali:Jainti, Jayant,

Guj:Jayanti, Rajashinganee,

Mal:Semp, Atti,

Punj: Jainta[9.]

SCIENTIFIC CLASSIFICATION:

Kingdom: plantae-plant

Subkingdom: Tracheobionta-vascular plants

Superdivision: spermatophyte-Seed plants

Division: magnoliophyta-Flowering plants

Class: magnoliophyta-Dicotyledons

Subclass: Rosidae

Order: Fabales

Family: Fabaceae or Leguminosae

Genus: Sesbania

Species: *Sesbania sesban (L) merr.* (Sesbania aegyptica pers)[10,11.]

Leaves Carbohydrates, Glycosides, Proteins, Amino acids, Saponins, Tannins,Alkaloid, Phenolic compounds, Flavonoids, Crude protein and Crude fibre, chikusetsusaponin-IV,3-o-L-rhamnopyranosyl-oleanolic acid, Ilexoside XL VIII, cholesterol, campesterol,beta-sitosterol. Fatty acids and amino acids[46] Various types of lignins composed of guaiacyl, syringyl and P-hydroxyphenylpropane building units and also antitumor principal kaempferol disaccharide[47.] Flowers contain cyanidin and delphinidinglucosides. Pollen and pollen tubes contain alpha-ketoglutaric, oxaloacetic and pyruvic acids[12.]

AYURVEDIC USES

The important formulations of *Sesbania sesban (L) merr* like Ratnagiri Rasa (used as antipyretic), The ayurvedic Pharmacopoeia of India recommends the use of the leaf indysuria[51.]

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Figure No: 1 Photographs of *Sesbania sesban (L) merr* leaves.

Various effects in cells, most of which can be described as being anti-diabetic and possibly slightly benefit other parameters associated with 'metabolic syndrome' (anti-inflammatory, anti-oxidant, etc.) Known for its nitrogen carrying and its antioxidant activities, it is a precursor of anabolic steroids and also used in cardiovascular diseases. The main adverse effects are decrease of lycopene levels and beta carotene, some rare cases of hemolytic anemia can also be observed High cholesterol. Taking beta-sitosterol significantly lowers total and bad (LDL) cholesterol levels, but it does not raise good (HDL) cholesterol levels[13.]

Experimental work

Extraction

PROCEDURE:

The leaves of *Sesbaniasesban (L) Merr*were shade dried pulverized and 100gm of coarse powder was further subjected to continuous hot percolation (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.

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.

QUALITATIVE PHYTOCHEMICAL INVESTIGATIONS.

The extracts of *Sesbania sesban (L) Merr* Leaves were subjected to qualitative chemical tests to detect the presence of various phytoconstituents as follows.

1) Tests for Carbohydrates

- **Molisch's test:**

  Treat the extract solution with few drops of alcoholic α-napthol. Add 0.2 ml of concentrated H_2SO_4 slowly through the sides of the test tube, purple to violet colored ring appears at the junction.

- **Benedict's test:**

  Treat the extract solution with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and upon boiling on water bath, reddish brown precipitate forms if reducing sugars are present.

- **Barfoed's test:**

  General test for monosaccharides: Heat the test tube containing 1ml reagent and 1 ml of extract solution in a beaker of boiling water; if red cuprous oxide is formed within two minutes, a monosaccharide is present. Disaccharides on prolonged heating (about 10min) may also cause reduction, owing to partial hydrolysis to monosaccharides.

- **Selwinoff's test:**

  Hydrochloric acid reacts with ketose sugar to form derivative of furfuraldehyde, which gives red colored compound when linked with resorcinol. Add extract solution to about 5 ml of

reagent and boil. Fructose gives red color within half minute. The test is sensitive to 5.5mmol/lt. if glucose is absent. If glucose is present it is less sensitive and on addition of large amount of glucose it gives similar color.

- **Fehling's test:**

Equal volume of Fehling's A (Copper sulphate in distilled water) and Fehling's B (Potassium tartarate and Sodium hydroxide in distilled water) reagents are mixed along with few drops of extract solution, boiled, a brick red precipitate of cuprous oxide forms, if reducing sugars are present.

- **Caramelisation:**

Carbohydrates when treated with strong sulfuric acid, they undergo charring with the dehydration along with burning sugar smell.

- **Tollen's test:**

To 100mg of extract add 2ml of Tollen's reagent, a silver mirror is obtained inside the wall of the test tube, indicates the presence of aldose sugar.

- **Bromine water test:**

It gets decolorized by aldose but not by the ketose, because bromine water oxidizes selectively the aldehyde group to carboxylic group, giving raise to general class of compounds called aldonic acid.

2) Tests for Proteins &Aminoacids

- **Millon's Test:**

Extract solution + 2 ml of Millon's reagent (Mercuric nitrate in nitric acid containing traces of nitrous acid) white precipitate appears, which turns red upon gentle heating.

- **Ninhydrin Test:**

Amino acids and proteins when boiled with 0.2% solution of Ninhydrin (Indane 1, 2, 3-trione hydrate), produces violet color.

3) Tests for Sterols and Triterpenoids

- **Libermann-Burchard test:**

Extract treated with few drops of acetic anhydride, boil and cool, concentrated sulphuric acid is added from the side of the test tube, A brown ring at the junction of two layers and the upper layer turns green indicates the presence of sterols and formation of deep red color indicates the presence of triterpenoids.

- **Salkowski's test:**

Treat extract in chloroform with few drops of concentrated Sulfuric acid, shake well and allow to stand for some time, red color appears in the lower layer indicates the presence of sterols and formation of yellow colored lower layer indicating the presence of triterpenoids.

4) Tests for Glycosides

- **Test I:**

Extract 200 mg of the drug by warming in a test tube with 5 ml of dilute (10%) sulphuric acid on a water bath at 100^{o}C for two minutes, centrifuge or filter, pipette out supernatant or filtrate. Neutralize the acid extract with 5% solution of Sodium hydroxide (noting the volume of NaOH added). Add 0.1 ml of Fehling's solution A and B until alkaline (test with pH paper) and heat on a water bath for 2 minutes. Note the quantity of red precipitate formed and compare with that formed in Test II.

- **Test II:**

Extract 200 mg of the drug using 5 ml of and boil on water bath. After boiling add equal volume of water to the volume of NaOH used in the above test. Add 0.1 ml of Fehling's A and B until alkaline (red litmus changes to blue) and heat on water bath for two minutes. Note the quantity of the red precipitate formed.

Compare the precipitates of Test II with Test I. If the precipitate in Test-I is greater than in Test-II, then Glycoside may be present. Since Test II represent the amount of free reducing sugar already present in the crude drug, whereas Test-I represents the Glycoside after acid hydrolysis.

5) Tests for Alkaloids

- **Mayer's test: (Potassium mercuric iodide solution).**

To the extract/sample solution, add few drops of Mayer's reagent , creamy white precipitate is produced.

- **Dragendroff's test: (Potassium bismuth iodide solution).**

To the extract/sample solution, add few drops of Dragendroff's reagent, reddish brown precipitate is produced.

- **Wagner's test: (Solution of Iodine in Potassium Iodide).**

To the extract/sample solution, add few drops of Wagner's reagent, reddish brown precipitate is produced.

- **Hager's Test: (Saturated solution of Picric acid)**

To the extract/sample solution, add few drops of Hager's reagent, yellow precipitate is produced.

6) Tests for Phenolic Compounds

- **Ferric chloride test:**

Extract solution gives blue-green color with few drops of $Fecl_3$.

- **Shinoda Test (Magnesium Hydrochloride reduction test)**

To the extract solution, add few fragments of magnesium ribbon and concentrated Hydrochloric acid drop wise, yellowish; yellow- orange occasionally orange color appears after few minutes.

- **Zinc-Hydrochloride reduction test:**

To the extract solution, add a mixture of Zinc dust and concentrated Hydrochloric acid. It gives yellowish, yellow- orange occasionally orange color appears after few minutes.

7) Tests for Flavonoids

- **Shinoda Test (Magnesium Hydrochloride reduction test)**

To the extract solution add few fragments of magnesium ribbon and concentrated Hydrochloric acid drop wise, pink scarlet, crimson red or occasionally green to blue color appears after few minutes.

- **Zinc-Hydrochloride reduction test:**

To the extract solution, add a mixture of Zinc dust and conc. Hydrochloric acid. It gives red color after few minutes.

- **Alkaline reagent test:**

To the extract solution, add few drops of Sodium hydroxide solution, formation of an intense yellow color that turns to colorless on addition of few drops of dilute acetic acid indicates the presence of flavonoids.

8) Tests for Tannins

- **Gelatin test:**

Extract solution with 1% gelatin solution containing 10% sodium chloride gives white precipitate.

- **Ferric chloride test:**

Extract solution gives blue-green color precipitate with $FeCl_3$.

- **Vanillin Hydrochloride test:**

Extract solution when treated with few drops of Vanillin Hydrochloride reagent gives purple red color.

- **Alkaline reagent test:**

Extract solution with sodium hydroxide solution gives yellow to red precipitate within short time.

9) Test for Anthraquinone glycosides

- **Modified Borntrager's Test:**

To the 5 ml of extract add 5 ml of 5% Ferric chloride & 5 ml dil. HCL. Heat for 5 min in boiling water bath, cool, add benzene or any organic solvent. Shake well. Separate organic layer, add equal volume of dil. Ammonia. Ammonical layer shows pinkish red colour.

10) Test for Steroidal glycosides

- **Kedde's test:**

Extract with chloroform, evaporate to dryness, and add one drop of 90% of alcohol and 2 drops of 2% 3,5-dinitro benzoic acid (3,5, dinitrobenzene carboxylic acid - Kedde's reagent) in 90% alcohol. Make alkaline with 20% sodium hydroxide solution. A purple color is produced. The color reaction with dinitrobenzoic acid depends upon the presence of anα,β unsaturated – γ lactone in theaglycone[62, 63, 64.]

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 mobile phase moves through stationary phase by capillary action, sometimes assisted by gravity or pressure. TLC separation takes place in the open layers with each component having the same total migration time but different migration distances. Mobile phase consists of a single solvent or mixture of solvent. Numerous fixed sorbent have been used including Silica gel, Cellulose, Polyamide, Alumina, Ion exchanger & Chemically bonded Silica gel.

The stationary phase of theTLC is prepared using various techniques such as pouring, dipping and spraying. However readymade prepared stationary phase (TLC plates) is also available in the market. 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 - 120^0C 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 100-120^0 C so that adsorbent activity is retained. The R_f values are calculated using following formula[14.]

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

INVITRO ANTI-TB ACTIVITY

MICROPLATE ALAMAR BLUE ASSAY:

The anti mycobacterial activity of compounds were assessed against M. tuberculosis using microplate Alamar Blue assay (MABA).This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with propotional and BACTEC radiometric method. Briefly, 200µl of sterile deionzed water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate.The final drug concentrations tested were 100 to 0.2 µg/ml. Plates were covered and sealed with parafilm and incubated at 37ºC for five days.After this time, 25µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. Finally the readings were noted based on the visual colour change. Pink colour in the well indicates growth of the bacteria and blue colour indicates no bacterial growth.

The MIC was defined as lowest drug concentration which prevented the color change from blue to pink[15].

Results

PHARMACOGNOSTIC INVESTIGATION

Table No: 01 Morphological evaluation of *Sesbania sesban (L) Merr* leaves.

Sl . No	Features	Observation
1	Colour	Dark green
2	Odour	Odorless
3	Taste	Bitter

4	Shape	Lanceolate, oblong and ovate
5	Size	Length:0.5-1.2cm,breath:0.3-0.6cm

PHOTOGRAPH OF AERIAL PARTS OF *SESBANIA SESBAN (L) MERR*

Figure No: 02 photograph of aerial parts of *Sesbania sesban (L) merr*

PHOTOGRAPH OF *SESBANIA SESBAN (L) MERR* LEAVES

Figure No: 03 photograph of *sesbania sesban (l) merr* leaves

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TRANSVERSE SECTION OF *SESBANIA SESBAN (L) MERR* LEAVES

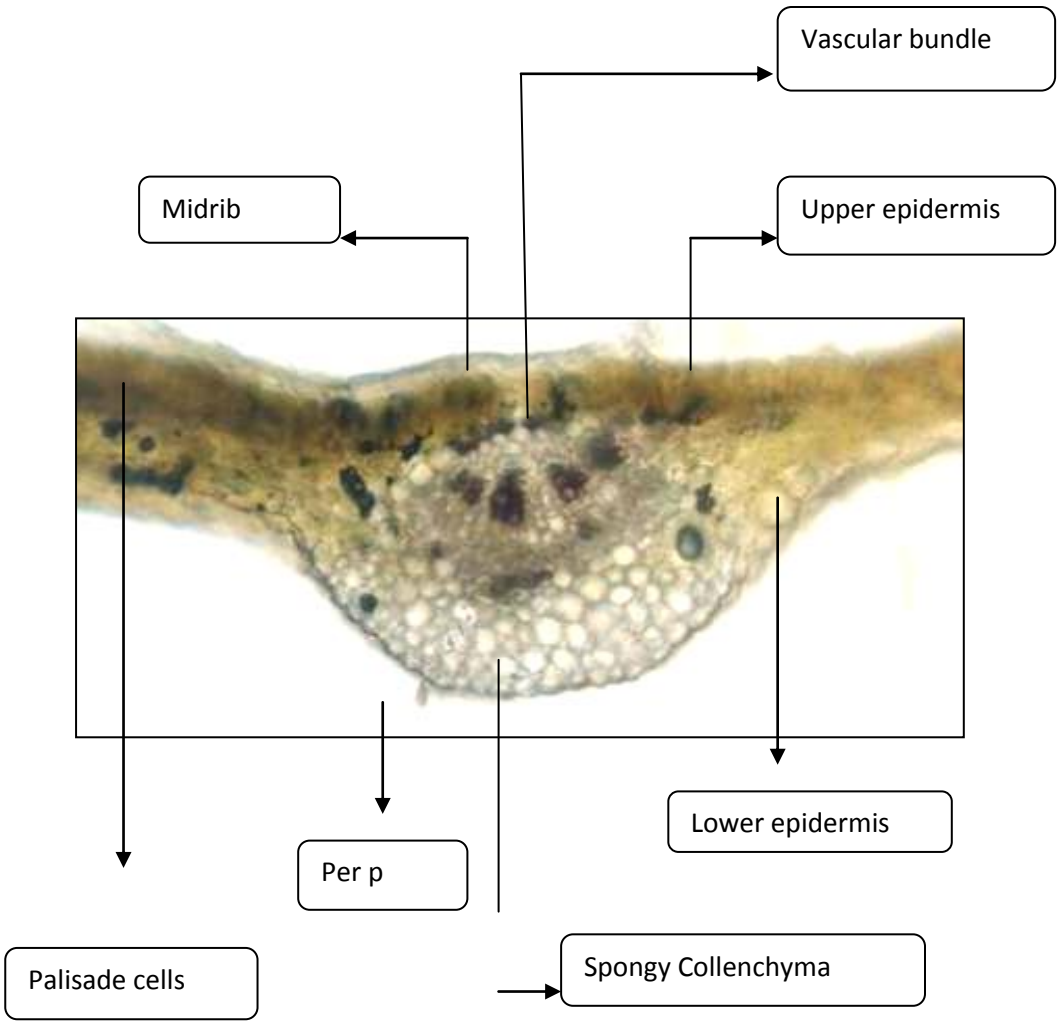


Figure No: 4 Transverse section of *Sesbania sesban (L) Merr* leaves

TRANSVERSE SECTION OF *SESBANIA SESBAN (L) MERR* LEAVES WITH STAIN

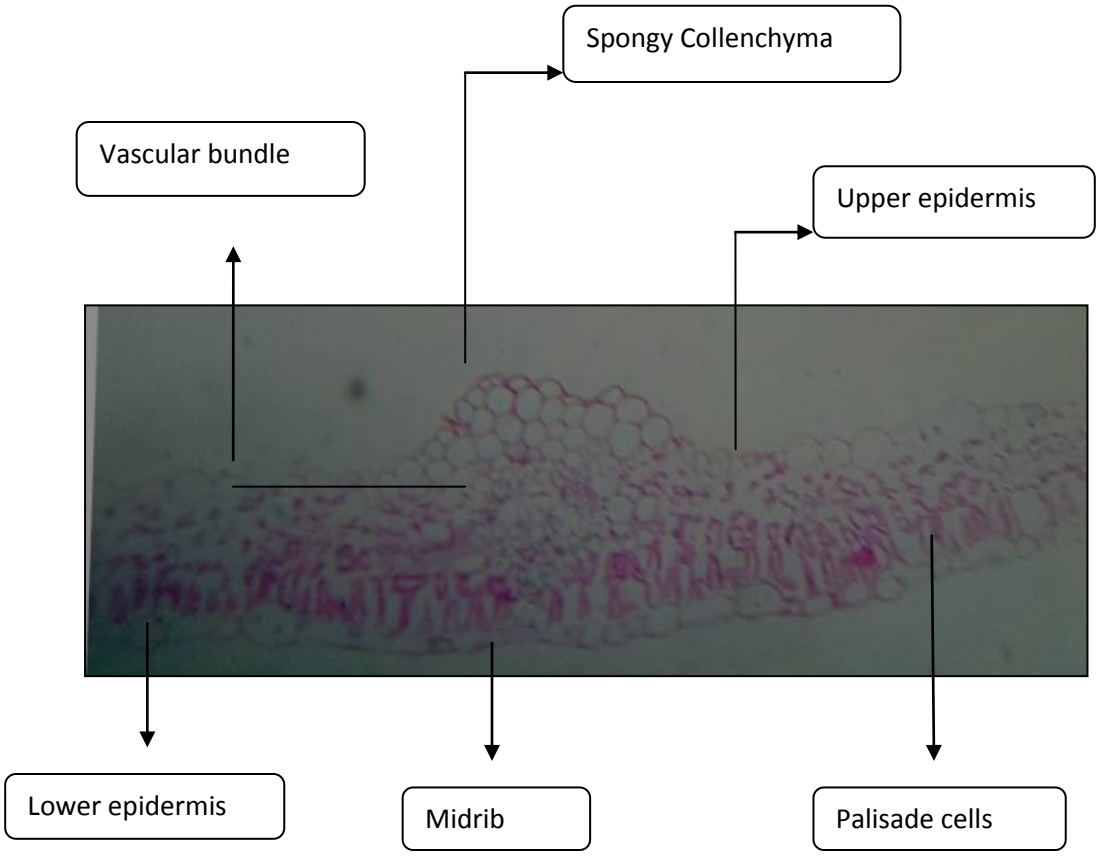
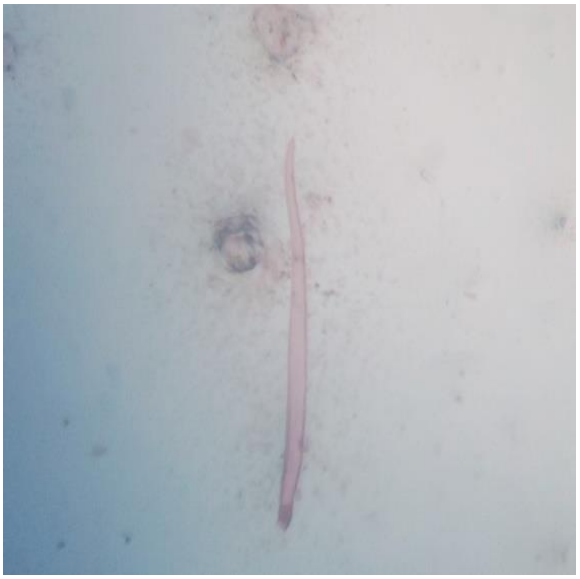
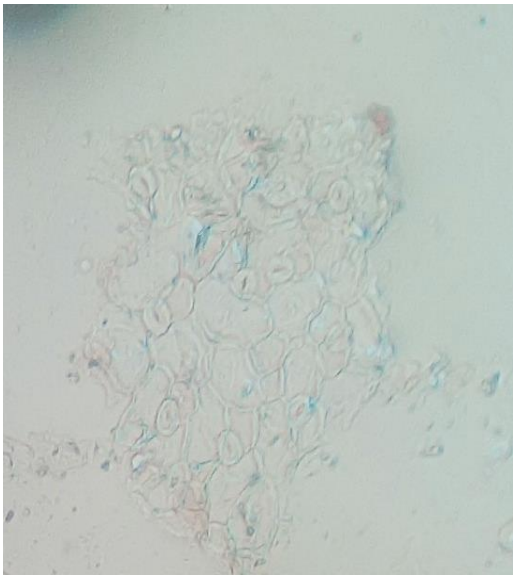


Figure No: 5 Transverse section of *Sesbania sesban (L) merr* leaves with stain

Figure No:6 POWDER MICROSCOPY OF *SESBANIA SESBAN (L) MERR* LEAVES

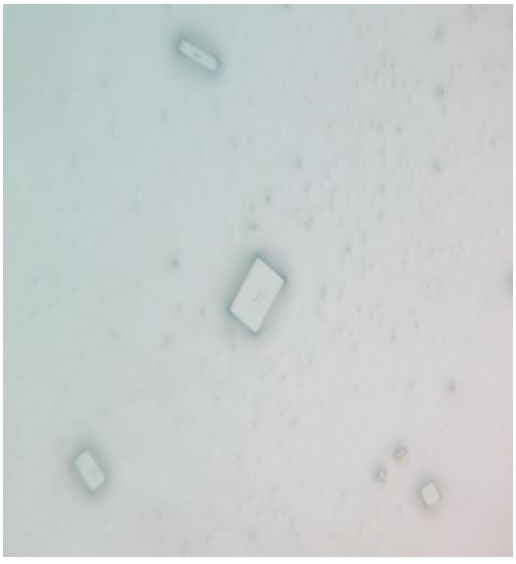


Stained with covering trichome



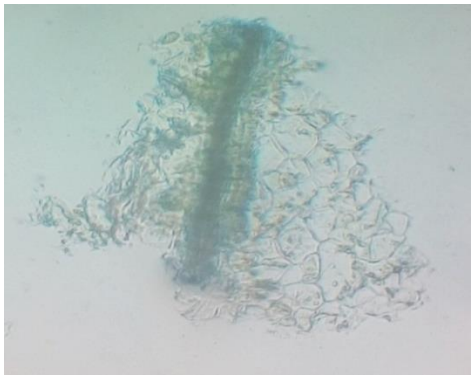
Anisocytic stomata

Bronchiole vein

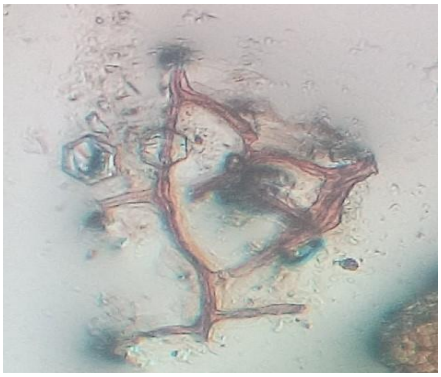


Calcium oxalate crystal

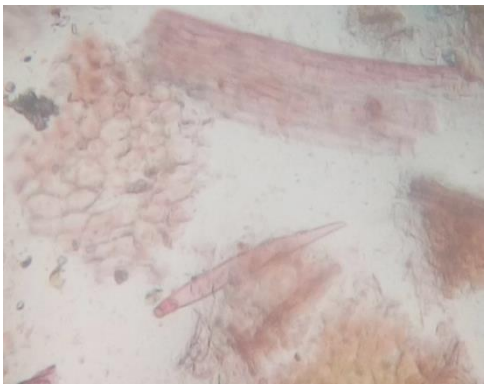
Figure No:7 POWDER MICROSCOPY OF *SESBANIA SESBAN (L) MERR* LEAVES



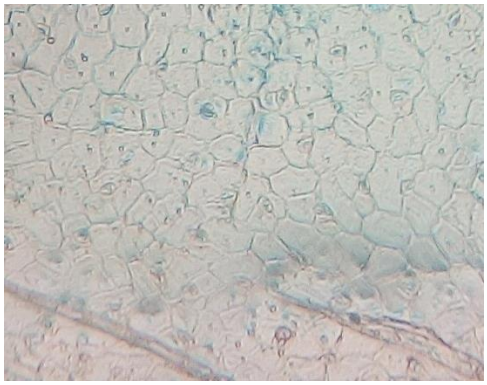
Xylem vessels



vien islets



Spiral vessels with covering trichomes



Anisocytic Stomata with trichomes

Table No: 02 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	Yellowishbrown	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: 3 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

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Keywords:

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

Table No: 4 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

Table:5 Antitubercular activity of COMP-1

S- Sensitive (anti TB activity)

R- Resistance (no anti TB activity).

S.no	Sample	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
1	COMP-1	S	S	S	S	S	R	R	R

S.NO	COMPOUND	Minimum Inhibitory Concentration (MIC,µg/ml)
1.	COMP-1	3.5
2.	PYZ	3.125
3.	CPF	6.25

Table No. 6 MIC of COMP-1 and Standard drugs

4.	STR	3.125

Figure No. 8 Graph representing MIC of COMPT-1 with that of std P,C,S and synthetic (S1-S3)

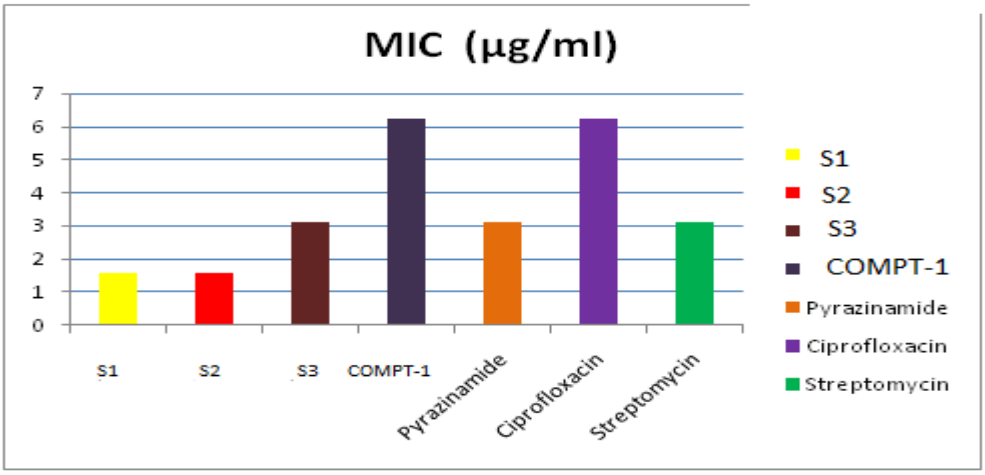
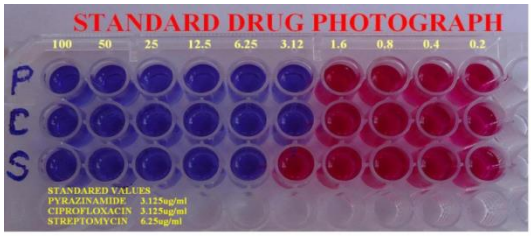
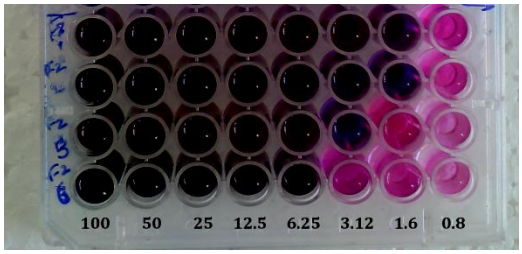


Figure No: 9 Photography of Standard and COMT Anti-TB activity of Micro Plate almar blue assay method





Discussion

Present study includes Pharmacognostic and Phytochemical investigations of the leaves of *Sesbania sesban (L) merr,* and evaluation of the same for Antidiabetic activity.

PHARMACOGNOSTIC INVESTIGATIONS:

Histological studies of the leaves showed the arrangement of tissues in the lamina and midrib region as follows: 1-2 layered upper and lower epidermis consists of rectangular epidermal cells covered with cuticle the dorsiventral leaf epidermal layer consists of anisocytic type of stomata and covering and glandular type of trichomes. Mesophyll region is differentiated into palisade cell and spongy parenchyma cells with starch grains, pericycle. In the midrib region, vascular bundles are well developed, collateral type and show both lignified xylem and phloem.

Examination of diagnostic characters of powdered leaves indicated the presence of fibres, (limited number present) epidermal cells, stomata, glandular and covering trichomes, xylem vessels, bronchiole vein, calcium oxalate crystals, vein islets, spiral vessels and starch grains.

The determination of average covering trichomes length and width of leaf powder diameter of *Sesbania sesban (L) merr* in microns average length of trichomes is (614.51) micron, and average width of trichomes is (33.325) micron,

Leaves surface data like stomatal index of upper epidermis (26.03) and lower epidermis (24.86), vein islet termination (88.8) and vein termination number (33.3), the palisade ratio *of Sesbania sesban leaf* is (1:7.5) respectively.

PROXIMATE VALUES:

Various proximate values for the leaves of *Sesbania sesban (L) merr* are as follows, Alcohol soluble extractive value (8.8 %), ether soluble extractive value (3%), Water soluble extract value (28%), Moisture content (5.5%), Total ash (11), Acid insoluble ash (1.5%), Water soluble ash (5%) and Sulphated ash (4%). These values are average values after performing experiment in triplicate.

Phytochemical investigations:

Preliminary phytochemical analysis of various extracts revealed the presence of flavonoids, phenolic compounds, steroids, Carbohydrates, Alkaloids, Glycosides, triterpinoids and tannins. 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 were further characterized by physicochemical tests, chromatography and spectral analysis such as, UV, FT-IR, NMR and Mass spectroscopy.

FOR THE ISOLATED COMP-I:

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.

^{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.

The invitro anti-TB results of COMP-1 revealed that it possessed good promising activity when compared to respective standards.

Conclusion

The overall phytochemical investigations and screening for diabetic activity of leaves *Sesbania sesban (L) merr* exhibited results to conclude as follows:

Morphological evidences to identify and authenticate the drug are as follows:

Microscopy of leaves of *Sesbania sesban (L) merr* exhibited prominent histological features like cuticle, upper and lower epidermis, spongy parenchyma, xylem, and phloem and collenchyma tissue.

Preliminary phytochemical investigations have revealed the presence of flavonoids, phenolic compounds, glycosides, steroid, alkaloids, tannins and triterpinoids.

The isolated COMP-I of alcoholic extract was characterized by UV, FT-IR, H^1-NMR, ^{13}C-NMRand Mass spectroscopy which can be claimed as member of natural flavonoids.

In vitro methods of anti-TB activity used alcoholic and chloroform extract of *Sesbania sesban (L) merr* Leaves has shown equipotent activity when compared to standard pyrizinamide, ciprofloxacin and streptomycin respectively. We can conclude that it may be due to synergistic effect of several phytoconstituents present in the extracts.From the results we can conclude the alcoholic extracts have shown comparatively good result with that of chloroform extract.

conclude that it may be due to synergistic effect of several phytoconstituents present in the extracts. Collectively these natural flavonoids and sterols are promising antidiabetics.

Histopathological study revealed restoration of normal cellular size of Isets of Langerhans when treated with alcoholic extract (200 mg/kg) The contribution of this investigation justify the folkloric practice of *Sesbania sesban (L) merr* leaves for the treatment of diabetes and the plant is worth for further isolation of more bioactive molecules.

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