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PHARMACOGNOSTICAL STUDIES AND ANTIMICROBIAL ACTIVITY OF THE LEAVES OF Sophora interrupta Bedd.

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ABSTRACT

Aim: The present study mainly focuses on the establishment of pharmacognostical studies and antimicrobial activity of Sophora interrupta leaves. It is a woody perennial shrub which grows endemically in Seshachalam hills of Thirumala, India. The plant was investigated and was found out to possess anti- cholesterolemic, anti-inflammatory, abortifacient, antibacterial, anti-spasmodic, diuretic, emetic, emollient, febrifuge, hypotensive, purgative, styptic and tonic properties. Methods: The powder of S. interrupta was successively extracted with aqueous, acetone, alcohol, benzene, chloroform, ethyl acetate, methanol and petroleum ether for pharmacognostical studies and antimicrobial activity. Results and Conclusion: Extractive values of leaf yielded highest amount in aqueous 20.80 w/w. Leaves yielded highest number of secondary metabolites as alkaloids, phenols, glycosides and saponins, tanins and lignins in most of the extracts. Antibacterial activity of leaf aqueous and methanol extracts showing more effective activity on E. coli with 29.50; 24.75 mm zone of Inhibition than other extracts. Minimum Inhibitory Concentrations with leaf extracts at 0.25 to 2.52 mg; compared to that of the 10 mg of Ampicillin. Antifungal activity of leaf benzene and chloroform extracts were more effective on C. albicans with 12.75; 12.50 mm zone of inhibition than A. niger when compared to Nystatin the control drug at 10 mg/well with 10.2 to 12.1 mm of zone of inhibition. From this study we can conclude that the traditional use of this plant for the treatment of infectious diseases is promising alternate supplement against pathogenic bacteria.

KEY WORDS

Antimicrobial activity, Minimum inhibitory concentration, Phytochemical studies, Sophora interrupta.

INTRODUCTION:

Medicinal plants have been an indispensable part of mankind. They have supported human populations from time Immemorial to combat various disease conditions and also for maintenance of health. The use of plant extracts in medicine, for example against microbial infections (1-4), is still very widespread and based on knowledge from traditional medicinal practice (5). Sophora interrupta Bedd. belongs to the family, Leguminosae is commonly called as Pili Girgoli. There are approximately 219 species in this genus Sophora. They were investigated to possess abortifacient,

antibacterial, anticholesterolemic, antiinflammatory, antispasmodic, diuretic, emetic, emollient, febrifuge, hypotensive, purgative, styptic, and tonic properties (6). *S. Interrupta* is one of such plants which is traditionally used in treatment of cancer. Assuming the anticancer compounds possesses the antioxidant activity and its family members investigated for presence of antioxidants (7). Several phytochemical researches, invivo and invitro experiments and clinical practices have demonstrated that *Sophora* contains many phytoconstituents like matrine, oxymatrine type of alkaloids (8, 9); flavonoids (10, 11); saponins and polysaccharides (12); which possess wide-reaching pharmacological



actions, including anti oxidant (13); anti cancer (14); anti asthamatic, anti neoplastic, antimicrobial, anti viral, antidote, anti pyretic, cardiotonic, anti inflammatory, diuretic and in the treatment of skin diseases like eczema, colitis and psoriasis (15).

MATERIALS AND METHODS:

PHARMACOGNOSTICAL STUDIES

Collection and identification of plant material:

The leaves of *Sophora interrupta* Bedd. were collected during September - December 2017 from Tirumala hills in Tirupati, Andhra Pradesh, India. The taxonomic identification of the plant is confirmed by Prof. N. Yasodamma. The voucher specimen BKSI:01 was deposited in the herbarium, Department of Botany, Rayalaseema University, Kurnool for future reference as per standard methods (16). The present work was carried out in the Department of Botany, Rayalaseema University, Kurnool. Plant materials were thoroughly washed and then dried under shade for one week. The dried parts were ground in a mixer grinder and sieved. The powders were stored in air sealed polythene bags at room temperature until further use.

MACROSCOPIC/MORPHOLOGICAL AND ORGANOLEPTIC CHARACTERS

Habit, morphology; colour, odour, taste, texture, of leaf was observed (17).

PHYSICOCHEMICAL ANALYSIS

Determination of ash values:

Ash values such as total ash, acid insoluble ash, water soluble ash, sulphated ash and moisture content/loose of weight on drying, values were determined with the leaf powder (18).

Total ash:

1g of air-dried powders was taken separately in a previously ignited and weighed silica crucible. The powder was spreaded in an even layer and ignited by gradually increasing the heat up to 500 - 600°C until it becomes white, indicating the absence of carbon. Then crucible was cooled in desiccator. The ash was weighed and percentage of total ash with reference to air dried powder was calculated.

% of Total Ash Value =
$$\frac{\text{Weight of the Ash}}{\text{Weight of the crude drug taken}} \times 100$$

Acid insoluble ash:

25ml of hydrochloric acid (70 g/l) was added to the crucible containing the total ash and boiled gently for 5 minutes. The insoluble matter was collected on the ash less filter paper and washed with hot water until the

filtrate is neutral. The filter paper was transferred to the original crucible and ignited to a constant weight. The residue was allowed to cool in a suitable dessicator for 30 min. The ash was weighed without delay and percentage of acid-insoluble ash with reference to air dried powder was calculated.

% of Acid inso lub le Ash = $\frac{\text{Weight of the acid inso lub le ash}}{\text{Weight of the crude drug taken}} \times 100$

Water soluble ash:

25 ml of water was added to the crucible containing the total ash and boiled for 5 min. The insoluble matter was collected on the ash less filter paper and washed with hot water. The filter paper was transferred to the original crucible and ignited to a constant weight at a temperature not exceeding 450°C. The residue was allowed to cool in a suitable desiccator for 30 minutes. The weight of the residue was subtracted from the weight of total ash. The ash was weighed without delay and percentage of water-soluble ash with reference to air dried powder was calculated.

% of Water Solube Ash Value = $\frac{\text{Weight of the Total Ash-Weight of the water insoluble ash}}{\text{Weight of the crude drug taken}} \times 100$

Sulphated ash:

A silica crucible was heated to red for 10 minutes and was allowed to cool in a desiccator and weighed. A gram of substance was accurately weighed and transferred to the crucible. It was ignited gently at first, until white fumes are no longer evolved and ignited at $800^{\circ}\text{C} \pm 25^{\circ}\text{C}$ until all black particles have disappeared. The ignition was conducted in a protected place from air currents. The crucible was allowed to cool. A few drops of concentrated sulphuric acid was added and heated. Ignited as before and was allowed to cool and weighed. The operation was repeated until two successive weighing do not differ by more than 0.5 mg.

% of Sulphated Ash Value =
$$\frac{\text{Weight of the Sulphated Ash}}{\text{Weight of the crude drug taken}} \times 100$$

Moisture content / Loss on drying:

Loss on drying is the loss of weight expressed as percentage w/w resulting from water and volatile matter can be driven off under specified conditions. 1 g of dried powder of leaves were accurately weighed and placed in a previously dried weighing bottle. The sample was heated at 100 - 105°C until two consecutive weighing does not differ by more than 5 mg. The loss of weight in mg material was calculated.

% of Moisture Content =
$$\frac{\text{Loss in weight of the sample}}{\text{Weight of the sample taken}} \times 100$$



Extractive value determination:

Fifty grams of coarsely powdered air-dried material of leaves macerated with 250 ml of each solvents, placed in a glass stoppered conical flask (Aqueous, Acetone, Alcohol, Benzene, Chloroform, Ethyl acetate, Methanol and Petroleum ether) shaking frequently, and then allowing it to stand for 18 hrs. Filter it rapidly through what man No.1 filter paper, taking care not to lose any solvent. Transfer 25 ml of filtrate to flat- bottom dish and evaporate the solvent on a water bath. Dry at 105°C for 6 hours, cool in a desiccator for 30 minutes and weigh it immediately. Calculate the content of extractable matter in % of air-dried material (19, 20).

Extraction yield (%) =
$$\frac{W_1}{W_2} \times 100$$

Where W_1 = Net weight of the extract in gm after extraction.

 W_2 = Weight of the total powder taken in gms.

PHYTOCHEMICAL STUDY

Preliminary Phytochemical Screening:

To detect the different classes of secondary metabolites in the leaf crude extracts of *S. interrupta* a preliminary phytochemical analysis was undertaken by adopting standard qualitative methods (21).

Crude drug preparation of aqueous and organic solvent extracts:

Dried leaf powder (50 g in 250 ml) was extracted with aqueous, acetone, alcohol, benzene, chloroform, ethyl acetate, methanol and petroleum ether. The drug was soaked for 72 hrs. and the filtered extract was dried on water bath stored at 4° C in refrigerator.

ANTIMICROBIAL ACTIVITY

Test organisms:

Pure bacterial cultures of *Bacillus subtilis* (MTCC-441), *Escherichia coli* (MTCC-443), *Pseudomonas aeruginosa* (MTCC-741), *Klebsealla neumoniae*, *Proteus vulgaris* (Clinical isolates) and fungal cultures of *Candida albicans* (ATCC-10231) and *Aspergillus niger* (ATCC-16404) were procured from department of Microbiology, S.V. University and Sri Venkateswara Institute of Medical Sciences, Tirupati. These were further maintained on nutrient agar slants at 4°C until further use.

Preparation of the bacterial medium:

To prepare 1 lit of nutrient agar medium 5 gm of beef extract, 3 gm of Sodium chloride, 3g of peptone, 15 gm of agar were accurately weighed using digital electronic balance and dissolved in 1 liter of distilled water before

the addition of agar, the P^H of the medium was adjusted to 7.2 by adding few drops of 0.1N NaOH/HCl using digital P^H meter. Later this medium was transferred to conical flasks and plugged with non-absorbent cotton. These were then sterilized by autoclaving at 15 lbs for 20 minutes, cooled to 40° C and used for the study.

Preparation of the fungal medium:

To prepare 1 lit of potato dextrose sugar medium 200 g of potato slices were boiled with distilled water. The potato infusion was used as water source of media preparation. 20 g of dextrose was mixed with potato infusion. 20 grams of agar was added as a solidifying agent. These constituents were mixed and autoclaved at 15 lbs for 20 minutes cooled to 40°C and used for further study.

Agar well diffusion method:

Antibacterial and antifungal activities of the leaf extracts were determined by using agar well diffusion method with slight modifications (22). Nutrient agar was inoculated with the selected microorganisms by spreading the bacterial and fungal inoculums on the media. Four agar wells (9 mm, diameter) were made in each plate equidistantly by cutting out the media using sterile broad end (8.5 mm) of micropipette tip, in order to load test solutions and are filled with 10 mg/well of the extracts in quadruplicates. Control wells containing pure solvents (negative control) or standard antibiotic (positive control) viz., Ampicillin10 mg/well, Nistatin 10 mg/well. The plates were incubated at 37°C for 24 hrs for bacterial and 25°C for 48 hours for fungal activity. The antimicrobial activity was assessed by measuring the diameter of the zone of inhibition for the respective drug. The relative antimicrobial activity was calculated by comparing its zone of inhibition with that of the standard drug. The data of crud drug activity is given the mean of quadruplicates along with the standard error.

Statistical analysis:

The results were analyzed for statistical significance using **One-way ANOVA** followed by Dunnet^{t'}s test. The p < 0.01 and p < 0.05 was considered significant.

Evaluation of minimum inhibitory concentration (MIC): Minimum Inhibitory Concentration was determined by broth dilution method (23, 24). Extracts to be tested were taken ranging from 10 mg/ml. It involves a series of nine tubes for each test extract against each strain. To the first assay tube 4 ml of broth was transferred and then 4 ml of test extracts of 10 mg/4 ml was added and mixed thoroughly. To the remaining nine assay tubes, from the first tube 4 ml of the content was pipette out



into second test tube and this was mixed thoroughly. This twofold serial dilution was repeated up to ninth tube. 0.2 ml of the inoculums was added to all test tubes and also to the control tubes were taken aseptically and incubated for 24 hrs. Next day the absorbance was measured by calorimeter at 600 nm Bacterial and 530 nm fungal. Bacterial MIC was compared with the control *Ampicillin* (10 mg/ml) for fungal was compared with the control *Nystatin* (10 mg/ml) and minimum inhibitory concentration mg/ml was determined.

RESULTS AND DISCUSSION:

ABOUT THE SELECTED MEDICINAL PLANT Distribution:

Selected medicinal plant *Sophora interrupta* Bedd. (Fabaceae) common name is adavibillu. There are approximately 219 species in genus *Sophora*. *S. interrupta* is a woody perennial shrub which grows endemically in Seshatheertham of Seshachalam hill ranges, and Kumaradharatheertham Seshachalam hill ranges of Thirumala, India.

Description:

Large shrubs, leaves imparipinnate, leaflets 2.5 -3 × 1.2-1.5cm sub opposite,12-16 pairs, broadly ovate, emarginate, mucranate at apex, obtuse at base, glabrous above, pubescent below. Flowers are 3 ×1 cm bright yellow in auxiliary and terminal racemes. Pods 10 cm long, 3-6 seeded, 4-winged constricted between the seeds (25).

Medicinal uses:

It has multifarious medicinal properties. The plant was investigated and was found out to possess abortifacient, antibacterial, anti- cholesterolemic, anti- inflammatory, anti-spasmodic, diuretic, emetic, emollient, febrifuge, hypotensive, purgative, styptic and tonic properties (Poretz and Barth, 1976). The literature survey reveals that *S. interrupta* is used to treat various types of gastrointestinal problems. Therefore, an attempt has been made to evaluate antimicrobial activity of leaf.

PHARMACOGNOSTICAL STUDIES

Macroscopic / Organoleptic Studies: (Plate-1, Table-1)
Color: Leaves green; Odour: Characteristic; Taste:
Bitter; Texture: Fine; Fracture: Smooth. Morphological studies and physiochemical constants help in the standardization of the crude drugs. Study of Organoleptic characteristics provides firsthand information about the quality of raw material used for the study.







Table-1: Macroscopic / Organoleptic Studies

Characters	Leaf
Colour	Green
Odour	Characteristic
Taste	Bitter
Texture	Fine
Fracture	Smooth
Fracture	Smooth

PHYSICO CHEMICAL STUDIES

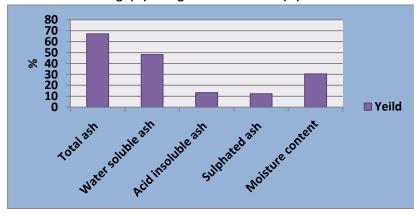
Powdered drug Ash values (%): (Table-2)

Ash values of any crude drug gives an idea about the presence of earthy matter and /or inorganic composition and /or other impurities present along with

the crude drug in the present study **Total ash** 67.15%, **Water soluble ash** 48.25%, **Acid insoluble ash** 13.35% **Sulphated ash** 12.25% and **Moisture content** 30.50% were reported.

Table -2: Ash values of Powdered Drug: (%) Figure-1: Ash values (%)

S.No.	Parameters	Yield
1	Total ash	67.15
2	Water	48.25
	soluble ash	
3	Acid	13.35
	insoluble	
	ash	
4	Sulphated	12.25
	ash	
5	Moisture	30.50
	content	



Extractive Values (Table-3, Figure-2)

Extractive values of leaf yielded highest amount in aqueous 20.80w/w, Filtrate color of leaf powder exhibit blackish green residue color and nature is Smooth green. Lowest in chloroform 14.20w/w, Blackish green

semi solid with blackish green extract. Extractive values represented the presence of compounds in polar and non-polar solvents. It is useful for the diversity of chemical nature and property of drug contents.

Table-3: Extractive Values (%w/w)

Extracts	Extraction	Filtrate color	Extract nature and colour
Ac	18.60	Green	Solid & Blackish green
Al	16.68	Blackish green	Sticky & green
Aq	20.80	Blackish green	Soomth& Green
Ве	16.80	Blackish green	Sticky & Blackish green
Ch	14.20	Blackish green	Semisolid & Blackish green
Ea	17.80	Blackish green	Sticky& Blackish green
Me	18.50	Light yellow	Semisolid &Light yellow
Pe	16.60	Light green	Solid & Light green



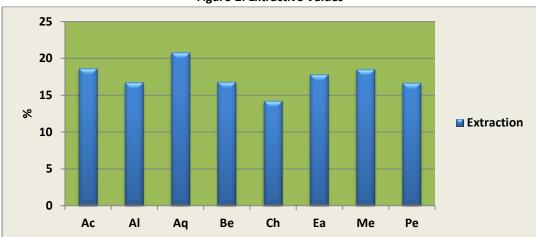


Figure-2: Extractive Values

PHYTOCHEMICAL STUDIES

Preliminary Phytochemical screening: (Table-4)

Leaves yielded highest amounts and more number of secondary metabolites as alkaloids, phenols, glycosides

and saponins, tanins and lignins in most of the extracts. Alkaloids are abundendent in most extracts. Low quantities of phyto-constituents are flavonoids and quinones.

Table-04: Preliminary Phytochemical screening

Test	Ac	Αq	Αl	Be	Ch	Ea	Me	Pe
Alkaloids								
Mayers	-	++	++	+	++	++	-	-
Wagner's	-	++	++	++	++	++	++	++
Flavonoids								
Shin dons	-	-	+	-	-	-	-	-
FeCl₃	-	-	-	-	+	-	-	-
Phenols								
FeCl ₃	+	-	+	+	+	++	-	++
Ellagic acid	-	+	-	-	++	-	-	-
Glycosides								
Keller –Kilani	+	+ -	-	++	+	+	_	-
Tannins								
FeCl₃	-	++	-	-	++	-	-	-
Steroids								
Salkowski	-	++	-	-	+	-	+	+
Quinones	-	-	+	-	-	-	-	-
Lignins								
Labat test	-	-	-	-	++	++	-	-
Saponins	-	++	-	++	++	-	+	++

"++" indicates - Abundant presence; "+" indicates - (Slightly presence); "- "indicates - Absent

Ac: Acetone, Al: Alcohol, Aq: Aqueous, Be: Benzene, Ch: Chloroform, Ea: Ethyl acetate, Me: Methanol, Pe: Petroleum ether

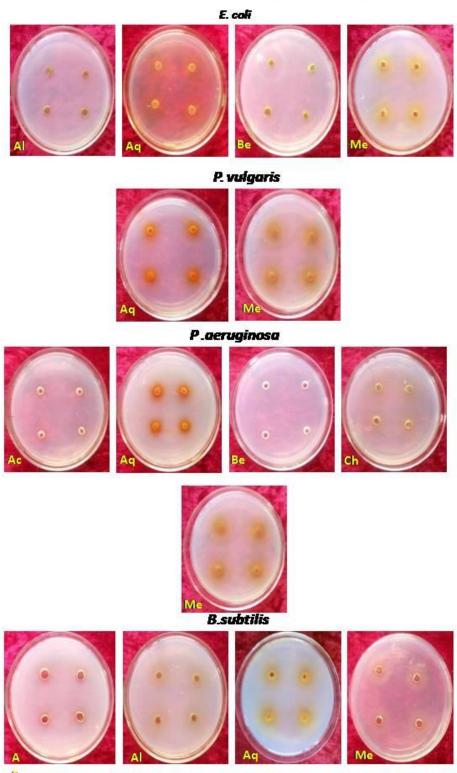
Antibacterial Activity (Zone of inhibition in mm) (Plate-02; Table-05; Figure-03)

Antibacterial activity of leaf aqueous and methanol extracts showing more effective activity on *E. coli* with 29.50; 24.75mm zone of Inhibition than other extracts.

Alcohol extract shows lowest zone of inhibition 8.50mm. It is also observed that there is no activity in petroleum ether extracts on all organisms. It is also observed that *P. vulgaris* is more resistance and *B. subtilis* and *E. coli* is least susceptible.



PLATE :2
Antibacterial activity of *S. interrupta* leaf 10mg/well



Note: Ac-Acetone; Al-Alcohol; Aq-aquous; Be-Benzene; Ch-Chloroform; Ea-Ethyl acetate; Me-Methanol.



Table-05: Antibacterial Activity (Zone of Inhibition in mm)

Extracts	B. subtilis	E. coli	K. neumoniae	P. aeruginosa	P. vulgaris
Ac	29.50±0.50**	0.00±0.00	11.75±0.43**	19.00±0.00**	0.00±0.00
Al	11.25±0.43**	8.50±0.50**	0.00±0.00	0.00±0.00	0.00±0.00
Aq	20.00±0.00**	29.50±0.50**	14.50±0.50	20.25±0.43**	19.50±0.50
Be	0.00±0.00	10.50±0.50**	0.00±0.00	10.00±0.00**	0.00 ± 0.00
Ch	18.50±0.50**	17.75±0.43**	0.00±0.00	10.75±0.43**	0.00±0.00
Ea	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Me	20.25±0.43**	24.75±0.43**	12.50±0.50**	19.50±0.50**	13.35±0.50
Pe	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
AMP	14.83±0.11	20.33±0.23	13.66±0.06	15.96±0.23	16.25±0.24

Ac: Acetone, Al: Alcohol, Aq: Aqueous, Be: Benzene, Ch: Chloroform, Ea: Ethyl acetate, Me: Methanol, Pe: Petroleum ether, **AMP: Ampicillin**

All the data are expressed as mean ±S EM: **p<0.01, * p<0.05 as compared to control group, n=4: (One -way ANOVA followed by Dunnett's test).

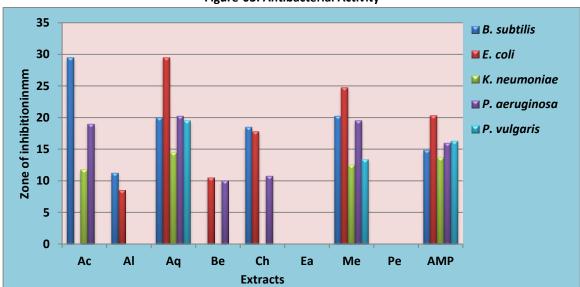


Figure-03: Antibacterial Activity

MIC for Antibacterial Activity: (Table-06, Figure-04)

Minimum Inhibitory Concentrations with leaf extracts at 0.25 to 2.52 mg; compared to that of the 10 mg of Ampicillin.

B. subtilis **Extracts** E. coli K. neumoniae P. aeruginosa P. vulgaris Ac 0.61 2.00 1.00 2.00 Αl 1.80 0.91 0.75 2.50 0.80 0.98 Αq Be 1.25 2.25 Ch 0.67 0.98 2.20 Ea Me 0.68 0.25 2.25 0.98 1.51

Table-06: MIC for Antibacterial Activity (mg)

Ac: Acetone, Al: Alcohol, Aq: Aqueous, Be: Benzene, Ch: Chloroform, Ea: Ethyl acetate, Me: Methanol, Pe: Petroleum ether



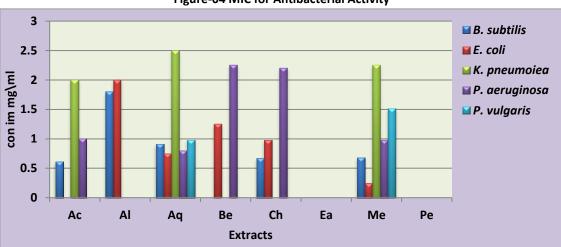


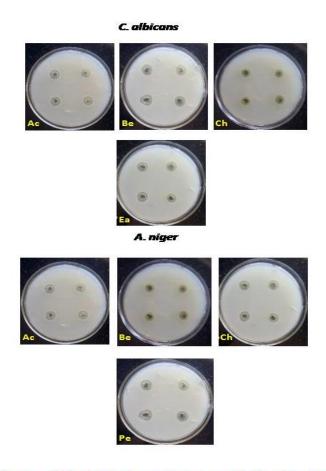
Figure-04 MIC for Antibacterial Activity

Antifungal Activity: (Plate-03, Table-07, Figure-05)

Antifungal activity of leaf benzene and chloroform extracts were more effective on *C. albicans* 12.75; 12.50mm with zone of inhibition than *A. niger* when compared to *Nystatin* the control drug at 10mg/well with 10.2 to 12.1 mm of zone of inhibition. Benzene shows low activity. Aqueous, alcohol and methanol extracts have not shown any antifungal activity on both organisms.

PLATE:3

Anti fungal activity of S. interrupta leaf 10mg/well



Note: Ac-Acetone; Al-Alcohol; Aq-Aquous; Be-benzene; Ch-Chloroform; Ea-ethyl acetate; Me-Methanol.



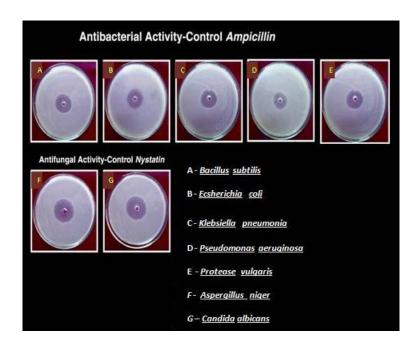
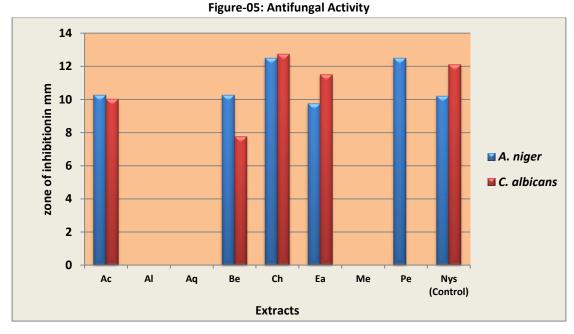


Table-07: Antifungal Activity (Zone of Inhibition in mm)

Extracts	A. niger	C. albicans
Ac	10.25±0.43**	10.00±0.00**
Al	0.00±0.00	0.00±0.00
Aq	0.00±0.00	0.00±0.00
Be	10.25±0.43**	7.75±0.43**
Ch	12.5±0.50**	12.75±0.43*
Ea	9.75±0.43**	11.5±0.50*
Me	0.00±0.00	0.00±0.00
Pe	12.5±0.50*	0.00±0.00
Nys (Control)	10.20±0.20**	12.10±0.16**

All the data are expressed as mean \pm SEM: **p<0.01,* p<0.05 as compared to control group, n=4: (One –way ANOVA followed by Dunnett-s test).

Ac: Acetone, Al: Alcohol, Aq: Aqueous, Be: Benzene, Ch: Chloroform, Ea: Ethyl acetate, Me: Methanol, Pe: Petroleum ether





MIC for Antifungal Activity: (Table-08, Figure-06)

Fungal Minimum Inhibitory Concentrations on both organisms with different leaf extracts ranges from 2.00 mg to 1.31 mg compared to 10 mg of *Nystatin*.

Table-08: MIC for Antifungal Activity (mg)

Extracts	A. niger	C. albicans
Ac	1.61	1.31
Al	-	-
Aq	-	1.25
Be	1.95	2.00
Ch	1.35	1.98
Ea	1.62	1.32
Me	-	-
Pe	1.98	-

Ac: Acetone, Al: Alcohol, Aq: Aqueous, Be: Benzene, Ch: Chloroform, Ea: Ethyl acetate, Me: Methanol, Pe: Petroleum ether

2.5 2 Con .in mg/ml A. niger **■** C. albicans 0.5 0 Ac Αl Aq Be Ch Ea Me Pe **Extracts**

Figure-06: MIC for Antifungal Activity

CONCLUSION:

Morphological studies of the leaves give the standard values for future studies. Physicochemical analysis resulted in the highest water-soluble ash content which helps in the drug designing. Moisture content is very less which proved long life and storage of the crude drug. Extractive values in leaves yielded highest amount in aqueous extract. Preliminary phytochemical screening results reveled that Leaves yielded highest amounts alkaloids, phenols, glycosides and saponins, tanins and lignins. Low quantities of phyto-constituents are flavonoids and quinones. The results show that the Aqueous and Methanol extracts are more effective zone of inhibition on E. coli than the standard drug Ampicillin. It is also observed that P. vulgaris is more resistance and B. subtilis and E. coli is least susceptible. Minimum Inhibitory Concentrations with leaves extracts at 0.25 to

2.52 mg; compared to that of the 10 mg of *Ampicillin*. Antifungal activity reveals that benzene and chloroform extract on *C. albicans* showed effective zone of inhibition. Fungal Minimum Inhibitory Concentrations on both organisms with different leaves extracts ranges from 2.00 mg to 1.31 mg compared to 10 mg of *Nystatin*. Further studies need to establish the mechanisms of action are required.

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