PHYSICO CHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF *INDIGOFERA MYSORENSIS* ROTTLER EX DC.

B. Kavitha*, M. Sonia rani, M. Khajapeer.
1&2.Department of Botany, Rayalaseema University, Kurnool, Andhra Pradesh, India.
3. Department of Botany, S.V.University, Tirupati, Andhra Pradesh, India.

ABSTRACT:Plants have been a valuable source of natural products for maintaining human health. *Indigoferamysorensis*Rottl.exDC.Fabaceae, commonly known as KondaVempali is a glutinous shrub used for its antidiabetic activity in rural India.The aim of the present work is to evaluate the *in vitro*antimicrobial activity of aqueous and organic solvents extracts of leaves of *I.mysorensis*byagar well diffusion method using gram positive bacteria like *Bacillus subtilis* and gram negative bacteria like *Escherichia coli,Klebseallpneumonia, Proteus vulgaris;* fungal cultures are *Candida albicans* and *Aspergillusniger*.The antibacterial and antifungal activity indicatestheir effective inhibition in comparison to that of the control drugs*Ampicillin* and*Nystatin*at 10mg/well. Physicochemical analysis was also performed on the leaves of *I. mysorensis.*

KEYWORDS: Indigoferamysorensis,* Leaves, Physicochemical Analysis,Antimicrobial activity, MIC,*Ampicillin,*Nystatin.***

INTRODUCTION:

Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The pharmacological industries have produced a number of new antibiotics; against resistant microorganisms. In general, bacteria have the genetic ability to transmit and acquire resistance to synthetic drugs which are utilized as therapeutic agents (1). Plant-derived compounds have been used in different systems of traditional medicine since time immemorial. Interest in plants with antimicrobial properties has revived as a result of current problems associated with the use of antibiotics (2). Spread of drug-resistant pathogens which have become one of the most serious threats to successful treatment of microbial diseases (3).

Indigoferamysorensis is common in dry deciduous forests, especially on exposed rocky slopesMicrovave station and Japalitheertham in Tirumala way to waterfalls at Talakona, Punganur and border of Karnataka state.Glutinous shrub with apprised laterally attached hairs leaves simple, trifoliate, leaflets5 pairs, opposite, oblong elliptic, pubescent, entire, obtuse; bracts 1-3 lobed.Flower generally very small usually reddish or purple, in axillary raceme or spikes rarely solitary rarely panicled. Calyx minute campanulate, teeth sub equal or the lowest longest. Corolla more or less caduceus, standarad ovate or orbicular, sessile or slightly clawed; wings oblong slightly adherent to the keel; keel petal erect, obtuse with a downward spur on each side near the base stamens diadelphous the vexillary stamens free the other with connate filaments; anther uniform apiculate. Ovary sessile or subsessile 1-2 pod cylinder, pubscent, 3-5 in long 3-4 seeded. It is used for its anti-diabetic activity in rural India (4 & 5).

MATERIALS & METHODS:

Collection and identification of plant material:

The leaves material of ***Indigoferamysorensis*** werecollected during the September - December 2017 from the forest fields of Talakona, Andhra Pradesh, India. The taxonomic identification of the plant is confirmed by Prof. N. Yasodamma and the voucher specimen B.K-1 was deposited in the herbarium, Department of Botany, Rayalaseema University, Kurnool (RUK) for future reference as per standard methods (6). Leaves were thoroughly washed, further dried under shade at 28 ±2ºC for about 7 days andthe dried leaves were ground well into a fine powder in a mixer grinder and sieved to give particle size of 50-150mm. The powder was stored in air sealed polythene bags at room temperature.

Macroscopic / Morphological and Organoleptic characters:

Habit, morphology; colour, odour, taste, texture of leaves was observed (7).

Physicochemical analysis:

Solubility:

The solubility was carried out in eight solvents(Methanol, ethanol, ethyl acetate, chloroform, benzene, acetone, petroleum ether and aqueous) based on polarity gradience.

Extractive value determination:

Fifty grams of coarsely powdered air-dried material of leaves wasmacerated 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 105^0C for 6 hours, cool in a desiccators for 30 minutes and weigh it immediately. Calculate the content of extractable matter in % of air-dried material (8 & 9).

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 powder of leaves (10).

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.

Antimicrobial activity:

Test organisms:s

Pure bacterial cultures of *Bacillus subtilis* (MTCC-441), *Escherichia coli* (MTCC-443), *Pseudomonas aeruginosa*(MTCC-741), *Klebseallaneumoniae*, *Proteus vulgaris* (Clinical isolates)and fungal cultures of *Candida albicans* (ATCC-10231) and *Aspergillusniger* (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 4^0C 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 40^0C 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 40^0C and used for further study.

Agar well diffusion method:

Antibacterial and antifungal activities of the leaves extracts were determined by using agar well diffusion method with slight modifications (11). 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.,*Ampicillin*10mg/well, *Nistatin*10mg/well. The plates were incubated at 37^0C for 24 for bacterial and 25^0C 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 drugs 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'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 (12 & 13). 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. Absorbance of the tubes is compared with the control *Ampicillin* (10 mg/ml) and minimum inhibitory concentration mg/ml was determined for bacteria and for fungal the absorbance was measured at 530 nm with a calorimeter. Absorbance of the tubes is compared with the control *Nystatin* (10 mg/ml) and minimum inhibitory concentration mg/ml was determined.

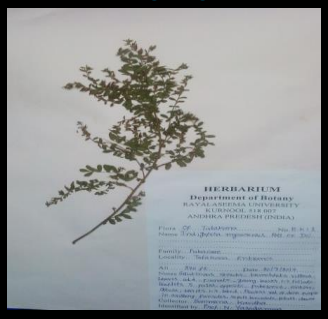
RESULTS & DISCUSSION:

PLATE :1
Morphological features of *I. mysorensis*

A. Habit

B. Twig with Flowering and Fruting stage

C. Leaves powder



D. Herbarium

CLASIFICATION

Division	:	Angiosperms
Class	:	Dicotyledons
Sub class	:	Polypetalae
Series	:	Calyciflorae
Order	:	Rosales
Family	:	Leguminosae
Sub Family	:	Fabaceae
Genus	:	*Indigofera*
Species	:	*mysorensis*

Table-1: Macroscopic / Organoleptic Studies:
Organoleptic Studies (Plate-1; Table-1):

S. No	Characters	Leaves
1	Colour	Green
2	Odour	Characteristic
3	Taste	Bitter
4	Taxture	Fine
5	Fracture	Smooth

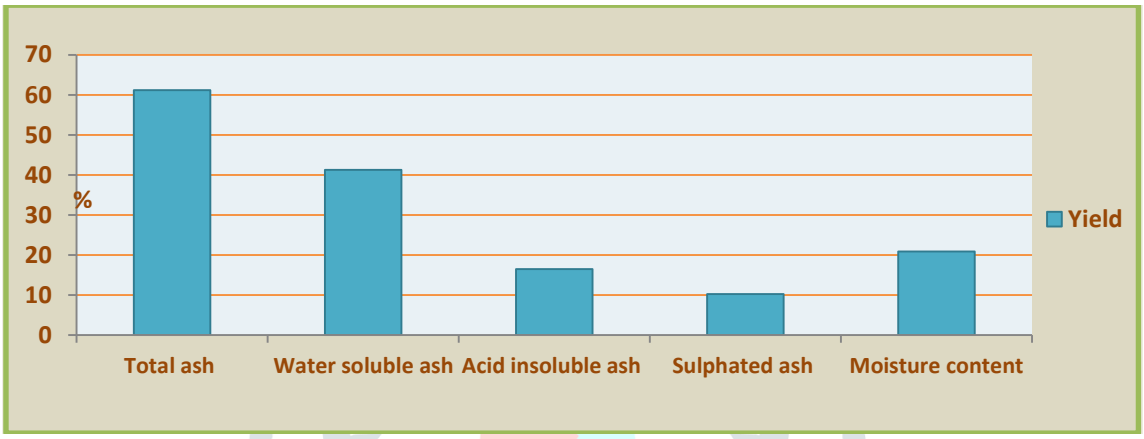
Color: Leavesgreen;**Odour**: Characteristic;**Taste**: Bitter;**Texture:** Fine; **Fracture:** Smooth.Morphological studies and physiochemical constants help in the standardization of the crude drugs. Study of Organoleptic characteristics provides firsthand information about the quality of raw material used for the study.

Physico Chemical Studies:

Table-2: Ash values: Powered Drug: (%)

S.No	Parameters	Yield
1	Total ash	61.15
2	Water soluble ash	41.25
3	Acid insoluble ash	16.50
4	Sulphated ash	10.30
5	Moisture content	20.90

Figure-1: Ash values (%)



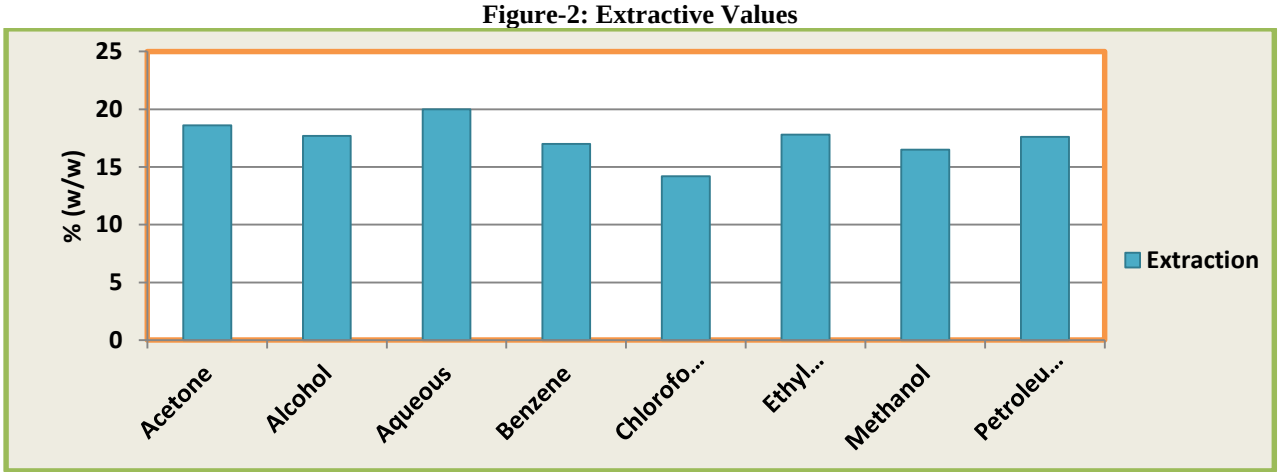
Powdered drug Ash values (%): (Table-2; Figure-1)

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** 61.15**%, Water soluble ash** 41.25%, **Acid insoluble ash** 16.50%**Sulphated ash** 10.30%and**Moisture content** 20.90% were reported.

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

Extracts	Extraction	Filtrate color	Extracts nature and colour
Acetone	18.60	Light green	Solid
Alcohol	17.68	Blackish green	Sticky
Aqueous	20.00	Blackish brown	Solid
Benzene	17.00	Blackish green	Sticky
Chloroform	14.20	Blackish green	Semi solid
Ethyl acetate	17.80	Blackish green	Sticky
Methanol	16.50	Light yellow	Semisolid
Petroleum ether	17.60	Light green	Solid

Figure-2: Extractive Values



Extractive Values (Table-3, Figure-2)

Extractive values in leaves yielded highest amount in aqueous**20w/w,**Filtrate color of leaves powder exhibitblackishbrown residue color and nature is solid. Lowest amount in chloroform **14.20w/w**, blackish green semi solid 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.

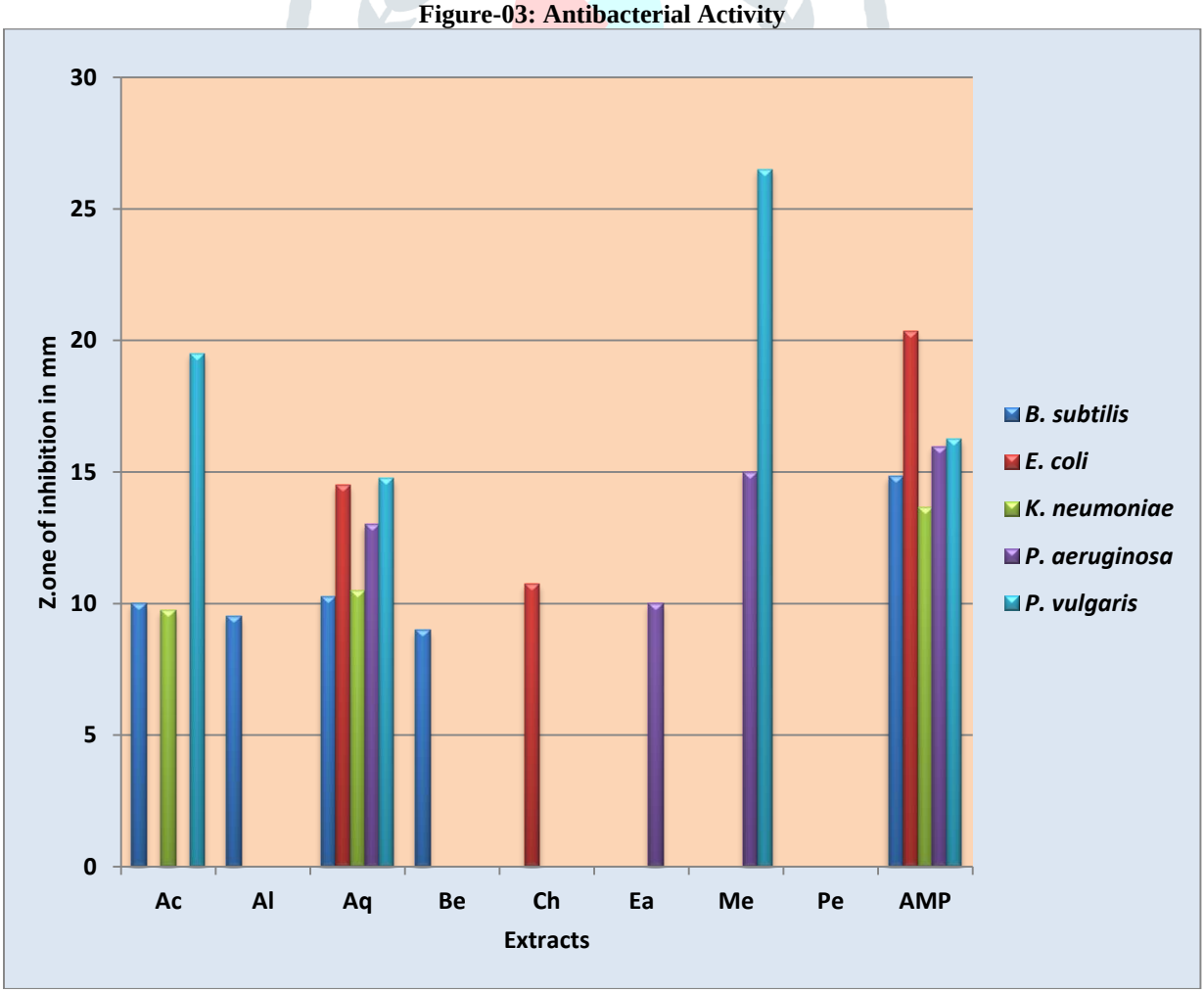
Table-04: Antibacterial Activity (Zone of Inhibition in mm)

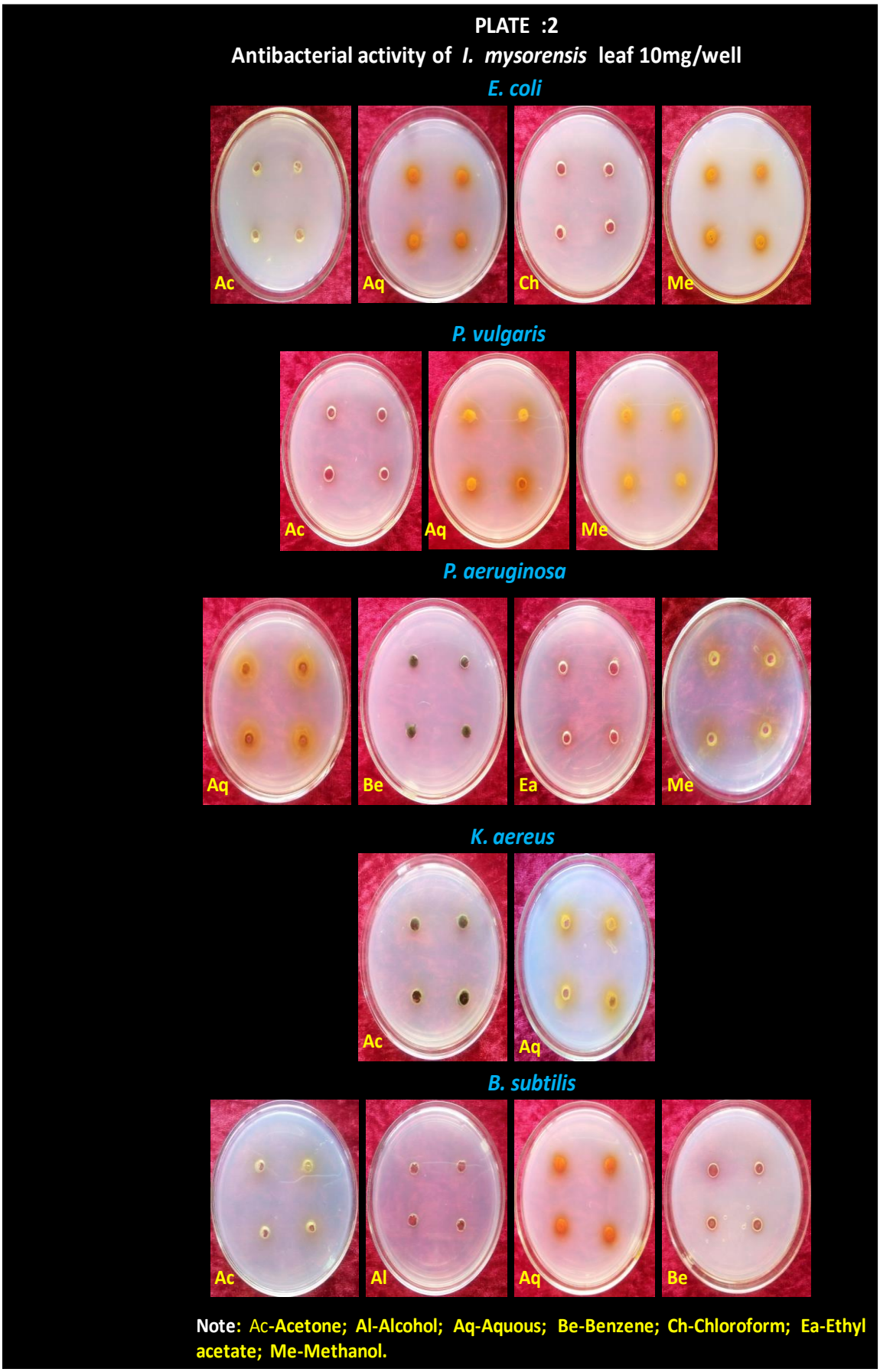
Extracts	***B. subtilis***	***E. coli***	***K. neumoniae***	***P. aeruginosa***	***P. vulgaris***
Ac	10.0±0.00**	0.0±0.00	9.75±0.43**	0.0±0.00	19.5±0.50**
Al	9.50±0.50**	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
Aq	10.25±0.50**	14.5±0.50**	10.50±0.50**	13.00±0.00	14.75±0.43*
Be	9.00±0.00**	13.5±0.50**	0.0±0.00	14.75±0.43**	0.0±0.00
Ch	12.0±0.00**	10.75±0.43**	0.0±0.00	0.0±0.00	0.0±0.00
Ea	0.0±0.00	0.0±0.00	0.0±0.00	10.00±0.00**	0.0±0.00
Me	0.0±0.00	0.0±0.00	0.0±0.00	15.00±0.00**	26.5±1.50**
Pe	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±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





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

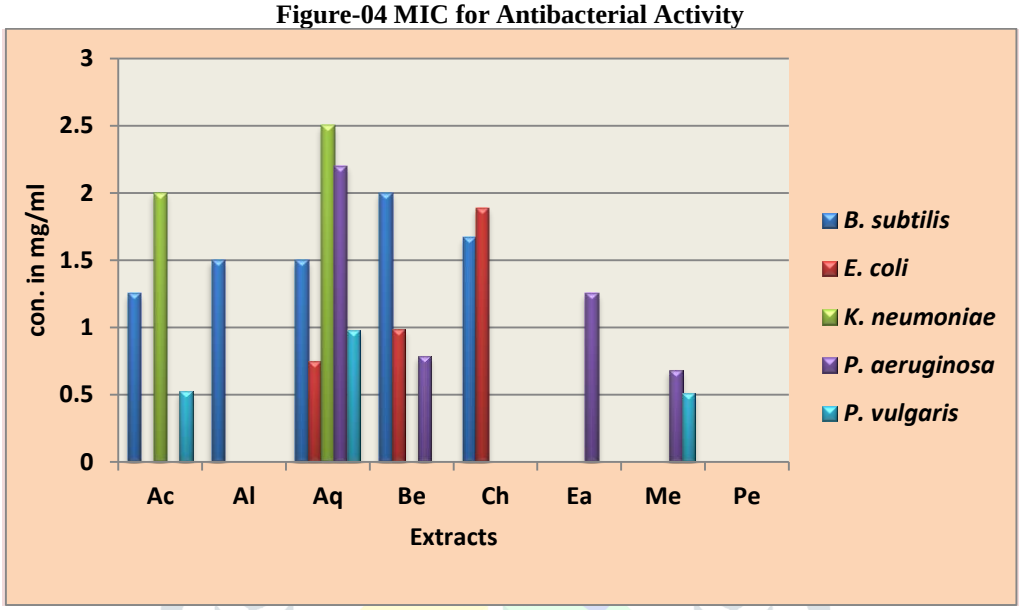
Antibacterial activity of leaves methanol extracts showing more effective activity on ***P. vulgaris*** with 26.5±1.50mm zone of Inhibition than other extracts. It is also observed that there is no activity in petroleum ether extracts on all organisms. It is also observed that ***P. vulgaris*** is more susceptible and ***B. subtilis*** and ***E. coli*** is least susceptible.

Table-06: MIC for Antibacterial Activity (mg)

Extracts	*B. subtilis*	*E. coli*	*K. neumoniae*	*P. aeruginosa*	*P. vulgaris*
Ac	1.25	-	2.00	-	0.525
Al	1.50	-	-	-	-
Aq	1.50	0.750	2.50	2.20	0.980
Be	2.00	0.985	-	0.785	-
Ch	1.67	1.89	-	-	-
Ea	-	-	-	1.25	-
Me	-	-	-	0.68	0.512
Pe	-	-	-	-	-

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

Figure-04 MIC for Antibacterial Activity



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

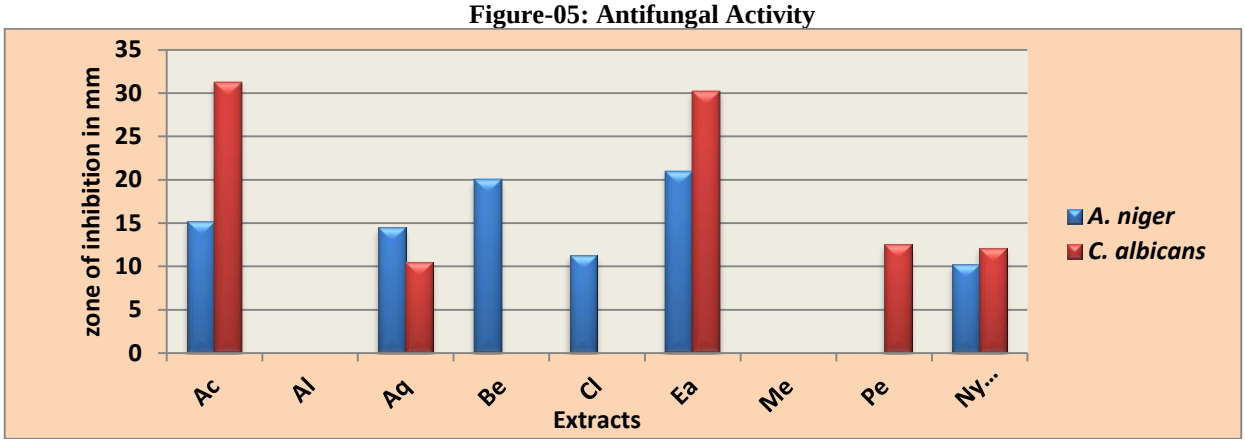
Minimum Inhibitory Concentrations with leaves extracts at 0.512 to 2.50 mg; compared to that of the 10 mg of *Ampicillin*.

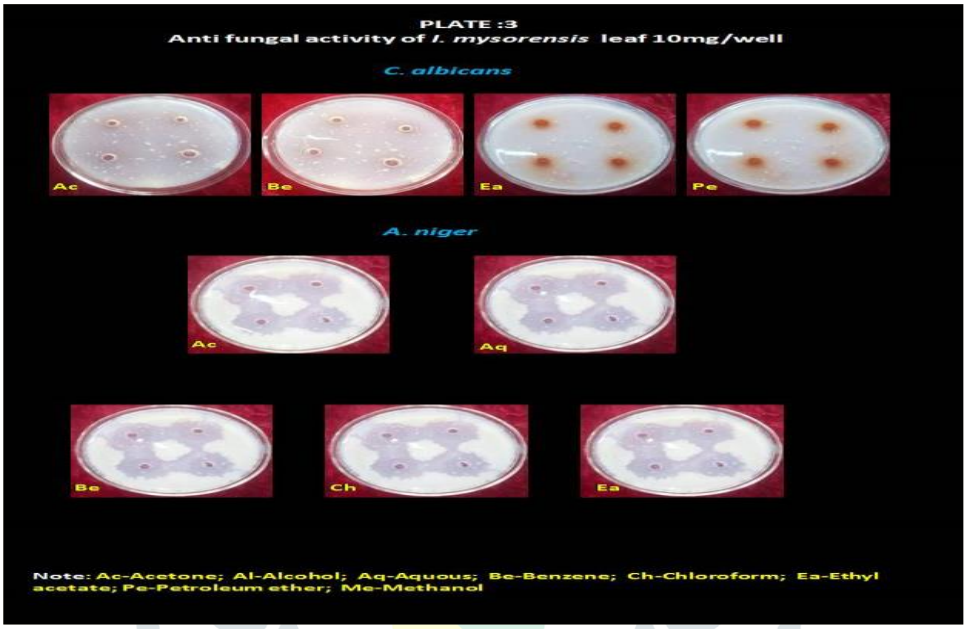
Table-07: Antifungal Activity (Zone of Inhibition in mm)

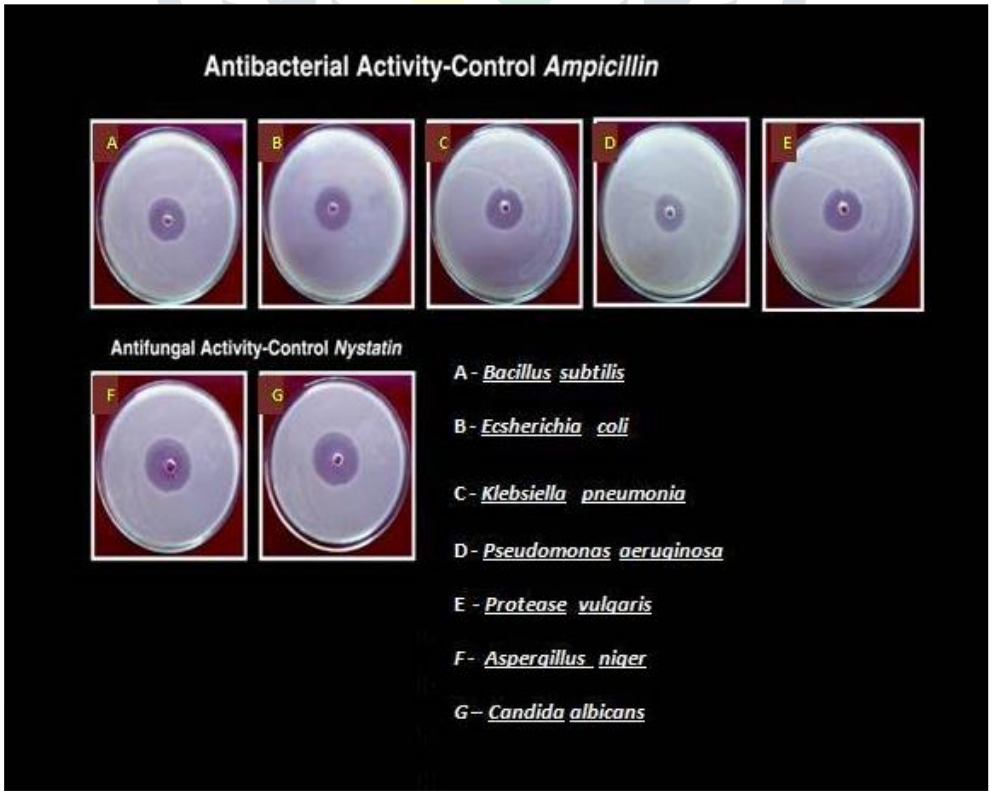
Extracts	*A. niger*	*C. albicans*
Acetone	15.25±0.43**	31.25±0.82**
Alcohol	0.00±0.00	0.00±0.00
Aqueous	14.5±0.58**	10.50±0.50
Benzene	20.0±0.00**	0.00±0.00
Chloroform	11.25±0.43	0.00±0.00
Ethyl acetate	21.0±0.70**	30.25±0.43*
Methanol	0.00±0.00	0.00±0.00
Petroleum ether	0.00±0.00	12.5±0.50
***Nystatin*(Control)**	10.20±0.20	12.10±0.16

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-05: Antifungal Activity







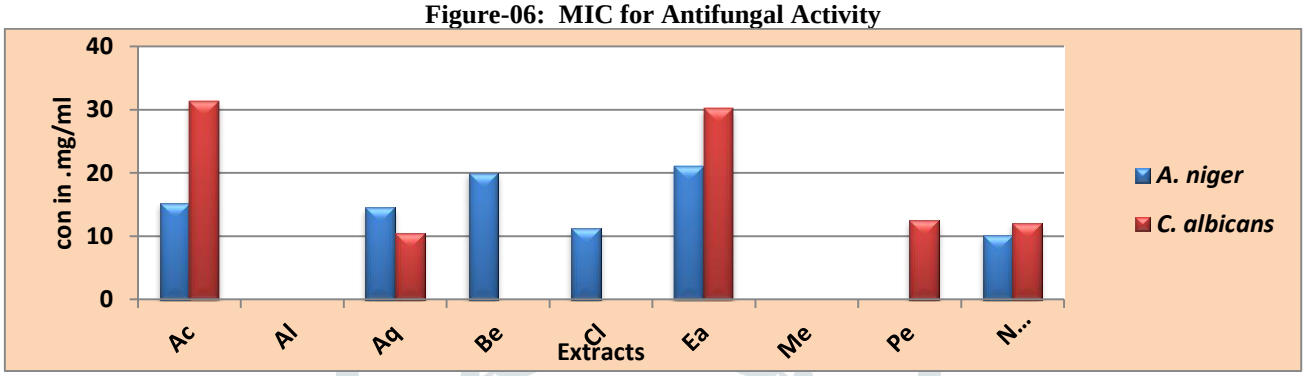
Antifungal Activity (Plate-03, Table-07; Figure-05)

Antifungal activity of leaves acetone and ethyl acetate extracts were more effective on ***C. albicans*** 31.25±0.82 and 30.25±0.43mm 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. Methanol and alcohol extracts have not shown antifungal activity on both organisms.

Table-08: MIC for Antifungal Activity (mg)

Extracts	*A. niger*	*C. albicans*
Acetone	0.612	0.31
Alcohol	-	-
Aqueous	0.85	1.25
Benzene	0.95	-
Chloroform	1.35	-
Ethyl acetate	0.62	0.32
Methanol	-	-
Petroleum ether	-	1.60

Figure-06: MIC for Antifungal Activity



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

Fungal Minimum Inhibitory Concentrations on both organisms with different leaves extracts ranges from 1.75 mg to 0.31 mg compared to 10 mg of *Nystatin*.

CONCLUSION:

These results indicate thatvarious crude extractsshowed effective antimicrobial activity on seven different microorganisms. From this finding the leaves of *I.mysorensis*is more effective in the formulation of medicine for the treatment of diseases such as gastrointestinal tract, pathogenesis, skin infection and Candida sis.

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