SCREENING OF LEAF, FLOWER AND FRUIT EXTRACTS OF *NYCTANTHES ARBOR-TRISTIS* FOR ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY

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ABSTRACT

Ethanolic extracts of leaf, flower and fruit of *Nnyctanthes arbor-tristis* were screened phytochemicaly and found it was the source of glycoside, steroids and flavonoids. Antioxidant activity by DPPH assay method was showed gradual increasing percentage of inhibition with increasing concentration of extracts, with highest % of inhibition was found with ethanol extract of flower at 76%. Ethalolic leaf extract was significantly effective against both Gram positive and Gram negative bacteria and *Candida albicans but* less effective against *A. Niger*. Ethalolic flower extract was moderately effective against both Gram positive and Gram negative bacteria and *Candida albicans* but significantly effective against *A. Niger*. Fruit extract of *Nnyctanthes arbor-tristis* was less effective against all strains of micro- organism.

KEYWORDS: Antimicrobial, antioxidant, *Nyctanthes arbor-tristis*, DPPH-assay,

INTRODUCTION

The emergence of antibiotic resistance in bacterial pathogens in the community is a very serious development that threatens the end of the antibiotic era. In order to overcome this rapid development of drug resistance, new agents should preferably consist of chemical characteristics that differ from existing agents[1]. Some drugs are made from plant extracts, others are synthesized. Herbal medicinal product can offer alternative to conventional medicines in non-life-threatening condition, adequate quality and safety to suitable individuals[2]. The phytochemical are pharmacologically active with mineral, vitamins and trace elements and can exert therapeutic actions on the body.[3]

Very strong and pleasant fragrance emitting plant *Nyctanthus arbor-tristis* Linn belongs to Oleaceae is known as Night Jasmine or Harsinghar[4]. Essential oil, nyctanthin, d-mannitol, tannin, glucose and caratenoid were present in flower. Seeds are rich source of Arbortristoside A and B, glycerides oflinolelic, oleic, stearic, palmitic and myristic acids and nyctantic acid. Traditionally, hot infusion of flowers is used as a sedative in Sri Lanka. The inflorescence is used to treat scabies and other skin diseases[5], decoction of flowers ward off wind in the stomach, stimulate gastric secretions and improve expectoration from the lungs.[6] The leaf juice is useful to expul roundworms and threadworms in children, to treat loss of appetite, piles, liver disorders, biliary disorders, chronic fever, malarial fever, obstinate sciatica and rheumatism.[7] Pharmacologically, plant is reported for its Antioxidant Activity, Anticancer activity, Immuno-Stimulant activity, Hepatoprotective activity, Antiviral activity, Antimicrobial activity, Anti Allergy activity, Anti Diabetic activity.

MATERIALS AND METHODS

Collection and Size Reduction of Plant material

The leaves, flowers and fruit of *Nyctanthes arbor-tristis* was collected from Gwalior, Madhya Pradesh, India. The leaves, flowers and fruit of *Nyctanthes arbor-tristis* was dried under shade in college laboratory. All dried parts were pulverized into coarse powder by passed through sieve no. 18 to maintain uniformity and stored in cool and dry place in plastic bags for further study.

Physicochemical Screening of Powder

(A) Loss on Drying: About 10 gm. of the powdered drug of each part was weighed in a tarred Petridish. It was dried at 105°C for 1 hour in hot air oven and then reweighed. Loss on drying was determined from calculating the initial and final weight.

(B) Total Ash Value: About 5 gm. accurately weighed powdered drug was incinerated in a silica dish at a temperature not exceeding 450°C until free from carbon in muffle furnace. It was then cooled and weighed. The % w/w of ash with reference to the air-dried drug was calculated.

(C) Acid Insoluble Ash Value: Accurately weighed 1 gm. ash was boiled for 5 minute with 25ml hydrochloric acid by covering the crucible with a watch-glass on water bath then cooled. The watch-glass was rinsed with 5 ml of hydrochloric acid and this liquid was added in to the crucible. Then the content was filtered on a previously weighed Whattman filter paper and filtrate was dried and weighed. Acid insoluble ash value was determined by calculating the % content remaining after deducting the weight of filter paper.

(D) Water Soluble Ash Value: Accurately weighed 1 gm. ash was boiled for 5 minute with 25ml distilled water by covering the crucible with a watch-glass on water bath then cooled. The watch-glass was rinsed with 5 ml of distilled water and this liquid was added in to the crucible. The % of remaining content was deducted from initial % of ash taken (i.e. 100%) to determine the water soluble ash value.

(E) Foaming Index: About 1 gm. coarse powder was weighted and transferred to a 500 ml conical flask containing 100 ml of water. It was maintained at moderate boiling for 30 minute on water bath. It was cool and filtered in to a 100 ml volumetric flask. Volume was diluted by adding sufficient amount of water. The decoction was poured in test tube, and then shaken in a lengthwise motion for 15 seconds. They were allowed stand for 15 minutes and the height of foam was measured to determine the foaming index.

Preparation of *Nyctanthes arbor-tristis* extracts

Different selected parts of *Nyctanthes arbor-tristis* was extracted using ethanol as solvent by Soxhlet extraction method, while petroleum ether was used for defatting of the waxy materials.

Phytochemical analysis of crude extract

The crude extract was subjected to various qualitative tests with standard reported methods to detect the presence of common phytochemical constituents. All the chemicals and reagent used in phytochemical testing was of analytical grade.

Pharmacological Screening

Anti-oxidant activity by DPPH method

Protocol for estimation of DPPH scavenging activity

150µl DPPH solution was added to 3 ml methanol and absorbance was taken immediately at 517 nm for control reading. Diluted test sample with methanol up to 3 ml. 150µl DPPH solution was added to each test tube. Absorbance was taken at 517 nm in UV-visible spectrophotometer (Systronics 2203) after 15 min using methanol as a blank.

The free radical scavenging activity (FRSA) (% antiradical activity) was calculated using the following equation:

$$\%\ (\mathbf{FRSA}) = \frac{(\text{Absorbance of control} - \text{Absorbance of test sample})}{\text{Absorbance of control}} \times\ 100$$

Each experiment was carried out in triplicate and results are expressed as mean and percent antiradical activity.

Antimicrobial Screening of extracts

Evaluation of Antimicrobial Activity

A drug is considered as bacteriostatic or fungistatic when it inhibits the growth or multiplication of bacteria or fungi respectively and considered as bactericidal or fungicidal when it actually results in the death of bacteria or fungi. Several forms of disc diffusion methods have been advocated. Among this Kirby Bauer method is the official method of the USA Food & Drug Administration.

RESULT & DISCUSSION

Preliminary Work

Table No. 4:- Morphological characteristics of *Nyctanthes arbor-tristis*

S. No.	Character	Leaf	Flower	Fruit
1	Color	Green	White petals with orange red center	Green then dark brown
2	Odour	Odorless	Fragrant	Odorless
3	Shape	Ovate	5 to 8 lobed corolla	Flat heart shaped
4	Size	6-12 cm long 2-6.5cm broad	3 to 5 cm inn dia	2 cm in dia
5	Texture	Rough	Smooth	Rough

Table No. 5:- Physiochemical analysis of powder of *Nyctanthes arbor-tristis*

S. No.	Parameters	Leaf (%)	Flower (%)	Fruit (%)
1	Loss on drying	6.3	3	0.2
2	Total ash value	6	5	7
3	Acid insoluble ash value	2.3	1.9	3.7
4	Water soluble ash value	2.1	0.68	1.33
5	Foaming index	6(ml)	4(ml)	8 (ml)

Table No. 6:- Consistency and color of *Nyctanthes arbor-tristis* extract

Extract	Color	Consistency	Percentage yield
Ethanolic (Leaf)	Dark green	Semisolid	8.2%
Ethanolic (Flower)	Dark	Semisolid	4.7%
Ethalnolic (Fruit)	Dark brown	Semisolid	13.4%

7.2 Phytochemical Screening of Ethanolic Extracts of *Nyctanthes arbor-tristis* extract

Table No.7: Phytochemical screening of ethanolic extract of leaf flower and fruit of ***Nyctanthes arbor-tristis*** **extract**

S. No.	Name of Chemical Tests	Leaf Extract	Flower Extract	Fruit Extract
1	**Carbohydrates** i) Molisch's Test ii) Fehling's Test iii) Benedict's test	 (+) (-) (-)	 (-) (-) (-)	 (+) (+) (-)
2	**Tannins** i) with 5%ferric chloride solution ii) with 10% aqueous Potassium dichromate solution iii) with 10% lead acetate solution	 (-) (+) (+)	 (-) (-) (-)	 (-) (+) (+)
3	**Alkaloids** i)Dragendorff's Test ii) Mayer's Test iii) Hager's Test	 (-) (-) (-)	 (-) (-) (-)	 (-) (-) (+)
4	**Glycosides** i) Borntrager's Test ii) Legal Test iii) Baljet Test	 (+) (+) (+)	 (+) (+) (+)	 (+) (+) (+)
5	**Flavonoids** i) Shinoda's Test ii) Alkaline reagent test iii) Lead test	 (+) (+) (+)	 (+) (+) (+)	 (+) (+) (+)
6	**Steroids and Sterols** i) Libermann-Burchard Test ii) Salkowski Test	 (+) (+)	 (+) (+)	 (+) (+)
7	**Proteins and Amino Acids** i) Biuret Test ii) Ninhydrin Test iv) Millon's Test	 (-) (-) (-)	 (-) (-) (-)	 (-) (-) (-)

(+) = Present, (–) = Absent

7.3 Pharmacological screening

7.3.1 Evaluation of antioxidant activity of *Nyctanthes arbor-tristis*

Table No.8:- Antioxidant activity by DPPH assay

Sample	Antioxidant activity results					Mean	% Inhibition
	Conc. µg/ml	Absorbance at 517 nm					
		Control	1	2	3		
Standard (Ascorbic acid)	100	0.488	0.265	0.266	0.267	0.266	45.49
	200	0.488	0.202	0.203	0.204	0.203	56.90
	300	0.488	0.165	0.166	0.167	0.166	58.40
	400	0.488	0.155	0.154	0.153	0.153	68.64
	500	0.488	0.112	0.111	0.113	0.112	77.04
Ethanolic extract Leaf	100	0.488	0.392	0.394	0.396	0.394	19.26
	200	0.488	0.326	0.326	0.326	0.326	33.19
	300	0.488	0.278	0.277	0.277	0.277	43.23
	400	0.488	0.233	0.234	0.233	0.233	52.25
	500	0.488	0.182	0.183	0.184	0.183	62.50
Ethanolic extract Flower	100	0.488	0.354	0.354	0.352	0.353	27.66
	200	0.488	0.312	0.314	0.316	0.314	35.66
	300	0.488	0.274	0.275	0.277	0.275	43.65
	400	0.488	0.221	0.221	0.221	0.221	54.71
	500	0.488	0.115	0.118	0.119	0.117	76.02
Ethanolic extract fruits	100	0.488	0.373	0.375	0.374	0.374	23.36
	200	0.488	0.301	0.309	0.313	0.308	36.89
	300	0.488	0.274	0.276	0.279	0.276	43.44
	400	0.488	0.202	0.203	0.212	0.218	55.33
	500	0.488	0.163	0.165	0.160	0.163	66.59

7.3.2 Evaluation of antimicrobial activity of *Nyctanthes arbor-tristis*

Table No.9: Antibacterial activity of *Nyctanthes arbor-tristis*

Sample applied	Diameter of zone of inhibition (mm)	
	B. subtilis	*E. coli*
Extract leaf (ELf)	20(6)[a]	26(12.5)
Extract Flower(EFl)	16(12.5)	17(12.5)
Extract Fruit (EF)	14(6)	13(6)
Control	-	-
Ciproflaxacin	22(6)	26(12.5)

[a] Values in brackets are MIC values (µg mL^{-1}).

Table No.10: Antifungal activity of *Nyctanthes arbor-tristis*

Sample applied	Diameter of zone of inhibition (mm)	
	C. Albicans	*A. Niger*
Extract leaf (ELf)	22(6)[a]	10(12.5)
Extract Flower(EFl)	20(12.5)	12(12.5)
Extract Fruit (EF)	20(6)	18(6)
Control	-	-
Ciproflaxacin	22(6)	27(12.5)

[a] Values in brackets are MIC values (µg mL^{-1}).

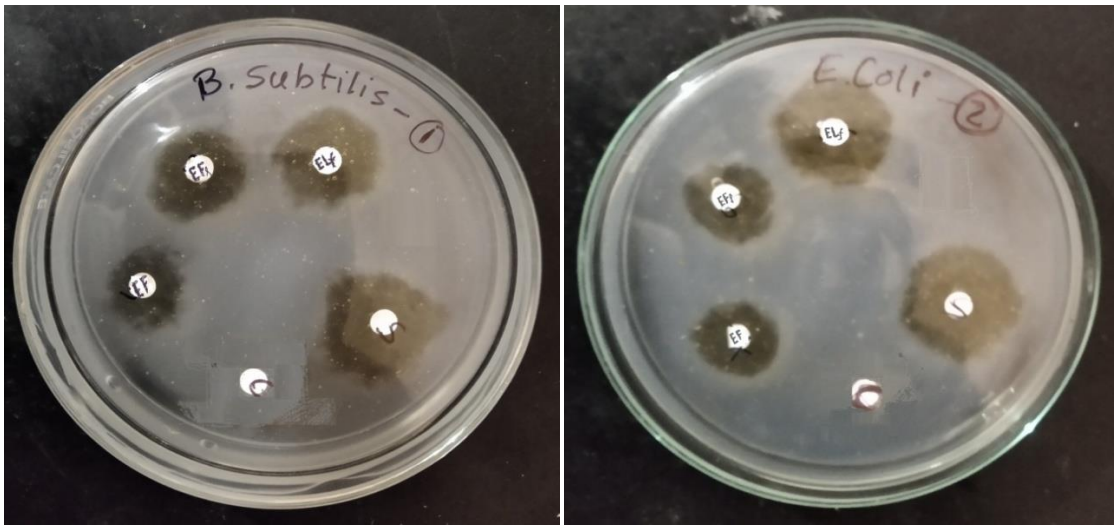

Fig. no. 5: Antibacterial activity of *Nyctanthes arbor-tristis*

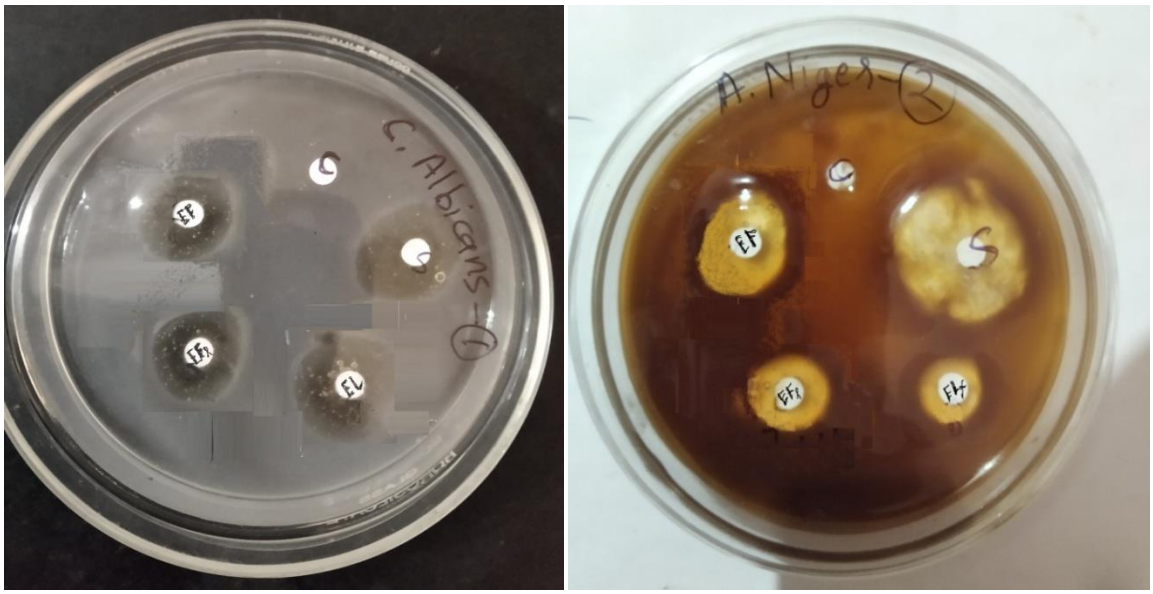

Fig. no. 6: Antifungal activity of *Nyctanthes arbor-tristis*

7.4 Statistical representation of pharmacological evaluation data

7.4.1 Antioxidant activity by DPPH assay

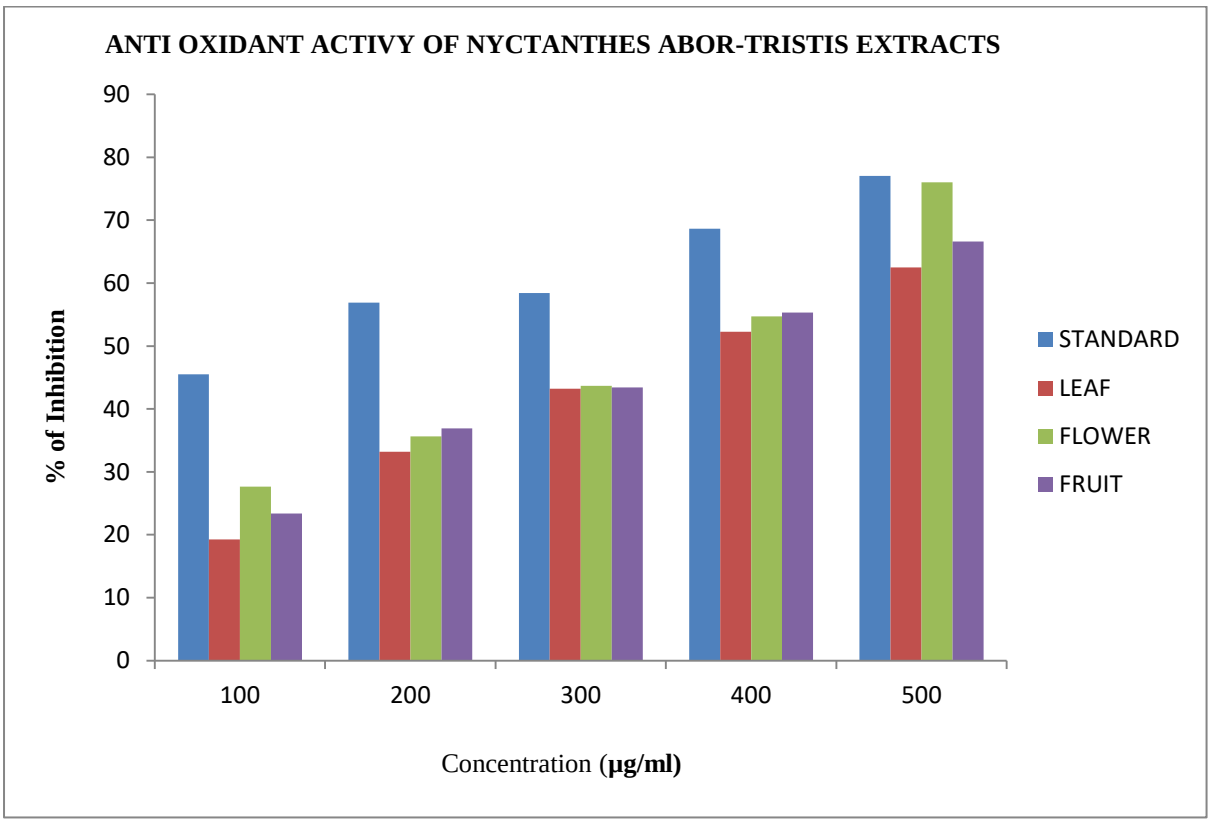

Fig. no. 7: Antioxidant activity of extracts of Nnyctanthes arbor-tristis by DPPH assay

7.4.2 Antimicrobial Activity

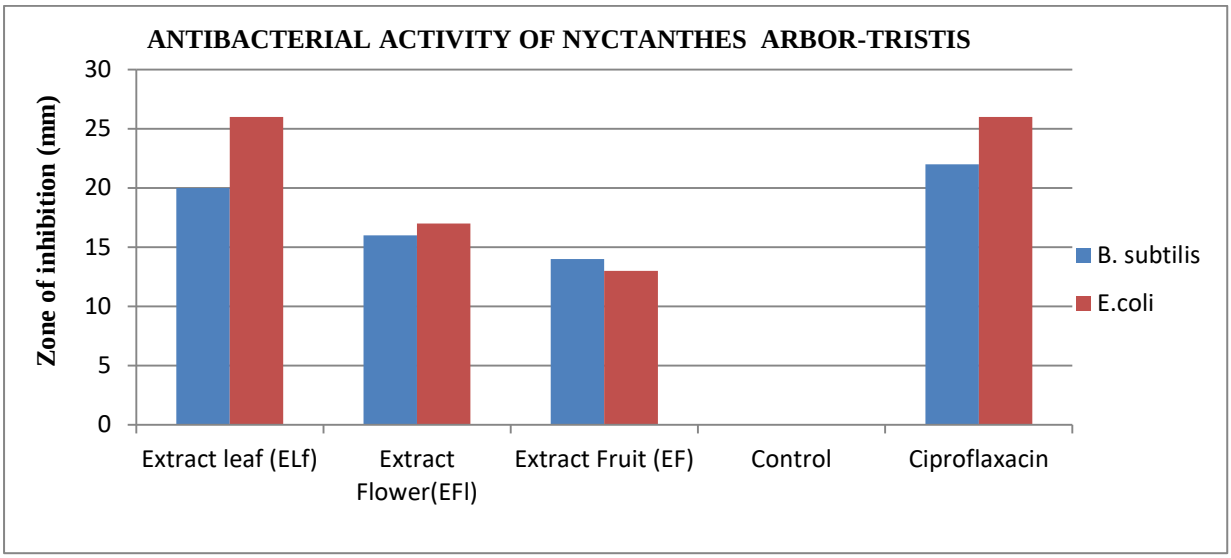

Fig. no. 8: Antibacterial activity of extracts of *Nyctanthes arbor-tristis*

7.4.3 Antifungal Activity

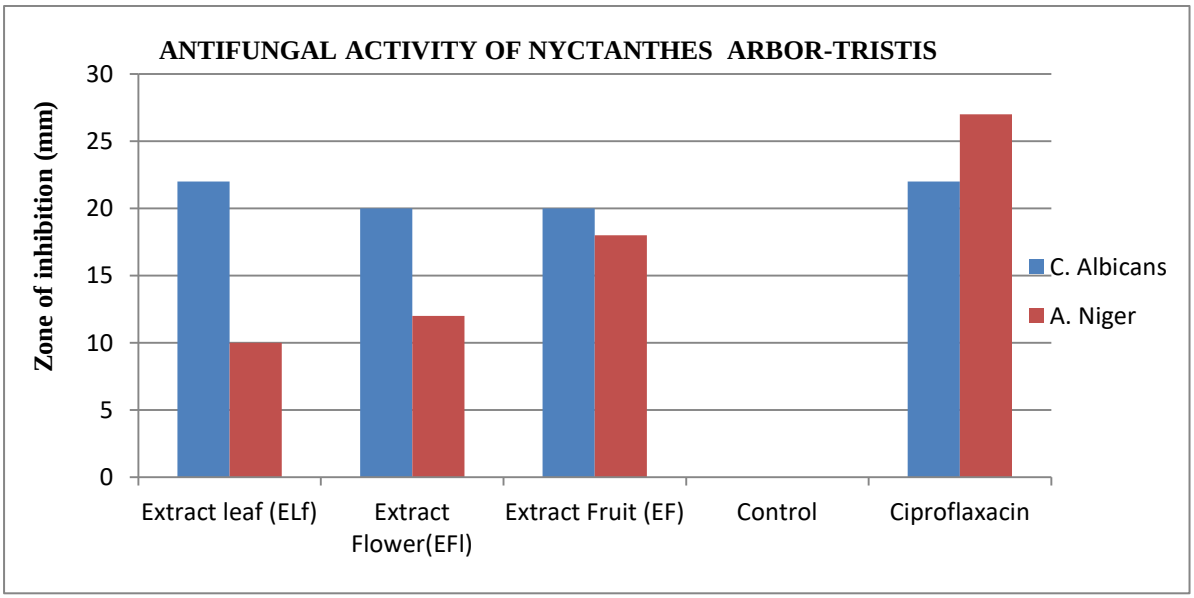

Fig. no. 9: Antifungal activity of extracts of *Nyctanthes arbor-tristis*

DISCUSSION

Various parts, leaves, flowers and fruits of plant nyctanthes arbor-tristis were collected and dried under shade. Dried plant parts pulverized and screened to get uniform sized. Powdered plant

materials were subjected for extraction, after the extraction, phrmacognostical evaluation was done including determination of Ash value in which percentage of Water soluble ash, Acid insoluble ash, Total ash and moisture content, foaming index were determined. Extract was subjected to various phytochemical tests for preliminary identification of various phytoconstituents. The leaf extract contains carbohydrates, tennins, flavonoids, glycosides, steroids. Flower extract contains flavonoids, glycosides, steroids. Fruit extract contains carbohydrates, tennins, flavonoids, glycosides steroids and alkaloids. Antioxidant reacts with DPPH, which is a nitrogen-centered radical with a characteristic absorption at 517 nm and convert it to 1,1,-diphenyl-2-picryl hydrazine, due to its hydrogen donating ability at a very rapid rate. Extracts showed gradual increasing percentage inhibition with increasing concentration at 517 nm in spectrophotometer as antioxidant by DPPH assay. Ascorbic acid showed gradual increase in % inhibition with increasing concentration as standard antioxidant by DPPH assay.

In this study, effects of ethanolic extract of *Nyctanthes arbor-tristis* was evaluated against Gram positive (*B. subtilis*) and Gram negative bacteria (*E. coli*) and strain of fungus (*C. Albicans and A. niger*). Antimicrobial activities were estimated by disk diffusion method. Firstly, minimum inhibitory concentration (MIC) values obtained. Antimicrobial activities of ethanolic extracts of leaf, flower and fruit of *Nyctanthes arbortristis* was evaluated in the term of zone of inhibition as described in tables. It observed that the ethalolic leaf extract of *Nnyctanthes arbor-tristis* was significantly effective against both Gram positive and Gram negative bacteria and *Candida albican but* less effective against *A. Niger.* Ethalolic flower extract of *Nnyctanthes arbor-tristis* was moderately effective against both both Gram positive and Gram negative bacteria and *Candida albicans* but significantly effective against *A. Niger*. Fruit extract of *Nnyctanthes arbor-tristis* was less effective against all strains of micro- organism.

ACKNOWLEDGEMENTS

The authors would like to thank Gurukul Institute of Pharmaceutical Science and Research, Gwalior, (M.P.) India, for their support and providing us laboratory to perform the work of the M. Pharmacy thesis.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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