IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

HISTOCHEMICAL AND PHARMACOGNOSTICAL STUDIES ON *NYCTANTHES ARBORTRISTIS* L.

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***Abstract*:** The present investigation on the various parts of *Nyctanthes* arbortristis L. has been undertaken mainly to understand the anatomy, tissue components, phytochemicals and antimicrobial activity. It has been achieved by adopting the basic techniques in anatomy and histochemistry. The main focus of this study was on the internal structure of stem, leaf and flower so as to understand the role of specialized cells (secretary cells) and pigments (carotenoids). These microscopic features play an important role in authentication of crude drug samples in pharmacognosy. In transactional view, stem shows four cortical bundles characterized as conjoint, collateral, open and exarch, present at the four ridged portion of the stem. This is due to the abnormality of stem which shows secondary growth at maturity. Midrib vascular bundle is crescentric showing cambial divisions, xylem and phloem. The essential oil of *N. arbortristis* L. has a pleasant fragrance and has been established as a potent antimicrobial agent. Presence of essential oil secreting cells was localized in stem, leaf and flower with Sudan III stain. Oil secreting cells appear bright orange - red. In the present study, tannins, starch, glycosides and carotenoids have been identified. Interestingly, tannins, starch, glycosides and carotenoids are seemed to be responsible for aroma and colour of the corolla tube. Interestingly, the results pertaining to the bioactive principles of flowers of *N. arbortristis* L. remain similar to that of jasmine. Further, the alkaloid and the glycosides extracted in the present investigation are known as nyctanthine and iridoid glycosides respectively. Besides, the significant antibacterial activity of the leaf powder extracted in butanol exhibited the maximum zone of inhibition (19mm) against Gram negative bacteria. Thus these properties of the plant *N. arbortristis* L., make it as a very promising / potential candidate species in both pharmaceutical and cosmetic industries as well.

Index Terms - *Nyctanthes arbortristis* L., *histochemistry, bioactive principles, antibacterial activity, pharmaceuticals*

I. INTRODUCTION

Medicinal plants are being employed in modern and traditional medicine so as to maintain health and / or to treat a specific illness. In 2002, the Food and Agriculture Organization estimated that more than 50,000 medicinal plants are being exploited globally. In 2016, the Royal Botanic Gardens, Kew, explored that about 17,810 plant species out of 30,000 plants have therapeutic use.

Plants employed in traditional medicine produce hundreds of chemical compounds for defense against insects, fungi, illnesses and herbivorous mammals. So far, the numerous phytochemicals are being discovered with proven biological functions. However, the consequences of taking a complete plant as medication are unknown because a single plant contains a large variety of phytochemicals. Furthermore, many plants with medicinal promise still lack comprehensive scientific research to determine their phytochemical components and pharmacological activities (Yudharaj *et al* ., 2017).

II. *NYCTANTHES ARBORTRISTIS* L.

This plant shall be grown on dry deciduous woods or rocky / loamy soils of dry hill shade and at 1500 m altitude above sea level with a variety of rainfall patterns. Because of the unique and pleasant aroma, it is being frequently grown in gardens (Bansal *et al*., 2015). It thrives well in a wide range of pH (5.6–7.5). Further, the light conditions require for this plant varies from direct sunlight to partial shade. Further, it is required to be watered on a regular basis. But still it should not be watered excessively (Kirtikar and Basu, 1935; Kiew and Baas, 1984; Rani *et al*., 2012)

III. MORPHOLOGICAL CHARACTERS

3.1 Stem and Bark

Being a big shrub, it has quadrangular branches. It can grow up to 10 meters length. Dark grey or brown coloured bark of *N. arbortristis* L., remains rough and strong. However, on scaling, the bark surface becomes dipped and patchy, with grey to brown colours. Whereas, the collapsed inner bark remains creamy white in colour and characterized with softness. Nevertheless, a discernible non-collapsed phloem zone has been identified in the inner bark.

3.2 Leaves

Leaves are opposite, simple, petiolate, exstipulate, 5 – 6.3 cm long, 2.5 – 6.3 cm wide, oblong, acute or acuminate, whole or serrated and with 6 cm long hairy petiole. While the upper surface shows dark greened speckles with glands, the pubescent lower side remains as pale green coloured. The venation of *N. arbortristis* L. is reticulate and unicostate.

3.3 Flowers

Flowers are small with aromatic odour and bitter taste. Often they are found in clusters of 2–7 together (Jain and Pandey 2016). With sessile bracteate fascicles of 3–5 peduncles are positioned either at the terminals of branches or in the axils of leaves. Bracts are broadly oval or suborbicular, 6 –10 mm long, hairy on both sides and apiculate. Calyx is 6 – 8mm long, narrowly campanulate, hairy on the outside, glabrous on the inside and truncated or obscurely toothed / lobed. Thus the corolla consist of five white petals (limb) which measures about 1.4 –1.5 cm (length) and a bright orange coloured corolla tube. The margins of petals curl downwards and the tips are notched. While the corolla glabrous measures around 13 mm length, tube on the other hand measure 6 – 8 mm long. Interestingly, the throat of corolla shows orange centre. Androecium has been reported with two stamens inserted near the apex of corolla tube ; stigma splits into two parts by a cleft.

3.4 Fruits

Fruit is flat and brown coloured. It varies from heart shape to rounded capsule shape. It has two celled openings transversely from the apex. Each carries a single seed which roughly measures about 2 cm (diameter). Microscopically, epidermal cells were compactly packed in the epicarp with polygonal cells characterized by slightly anticlinical walls covered by a thin cuticle, followed by l-3 layers of collenchyma, spongy parenchymatous tissue, sclerenchymatous fibres and oil glands.

3.5 Seed

Seed is compacted into a single cell. Its count is seemed to be one per cell. The outer layer of seed remains transparent. The extremely vascularized seeds are exalbuminous and testa thick (Sasmal *et al*., 2007, Bhosale *et al*., 2009; Desai *et al*., 2016; Jain and Pandey, 2016: Rani *et al*., 2012).

IV. MEDICINAL USES

Almost every part of the plant has been exploited to treat a variety of ailments and pathologic conditions. As the leaves of this plant contain phytochemicals such as flavonoids and glycosides, they are expected to exhibit antifungal, anti-inflammatory, anti-pyretic and antibacterial properties as well. Flowers on the other hand are anti-filarial, antioxidant and diuretic in nature. Whereas the seeds are antifungal, antibacterial, anti-leishmanial and immunomodulatory. However, the stem seemed to be antipyretic and antioxidant. Nevertheless, the barks hold antimicrobial activity. Besides, the flower oil has found wide use in cosmetic industries as perfumes. Recently, very many researchers have explored the medicinal values of bioactive components of leaf, flower, fruit and seed of *N. arbortristis* L (Divya, 2015).

V. RESEARCH METHODOLOGY

5.1 Source

Ariel parts of *N.arbortristis* L. were collected from the plants maintained in the campus of The Standard Fireworks Rajaratnam College for Women, Sivakasi (**Latitude**: 9°46' N ; **Longitude**: 77°80’ W), Virudhunagar district, Tamil Nadu, India.

5.2 Free hand section

Thin sections of leaf, stem etc., were stained with safranin, TBO, Sudan III and I_2KI. The sections were then mounted with glycerine and observed under compound microscope.

VI. PREPARATION OF STAINS

6.1 Safranin

20g safranin dissolved in 100 ml water.

6.2 TBO (Toluidine Blue O method)

0.05% TBO in benzoate buffer (benzoic acid 0.25g & sodium benzoate 0.29g in 200ml water).

6.3 Potassium Iodide

4g iodine and 6g potassium iodide in 100 ml distilled water.

6.4 SUDAN III

0.7g Sudan III dissolved in 100 ml propylene or ethylene glycol at 10-100° C with gentle stirring.

VII. PRELIMINARY PHYTOCHEMICAL ANALYSIS

Dry powders .of stem, leaf and flower parts of *N. arbortristis* L., were extracted with the solvents like petroleum ether, chloroform, butanol, ethanol and water. The extracts were then subjected to the phytochemical analysis (Table 1).

Table 1: Preliminary phytochemical analysis

Tests	Experiment	Observation
Mayer's Test	Development of white precipitate on treatment of aqueous layer of plant extracts with 2N hydrochloric acid and one or two drops of Mayer's reagent	**Alkaloid**
Liebermann Burchard Test	Colour changes from purple to blue-green on treatment of one ml plant extract with 0.1 ml chloroform, 3 to 4 drops of acetic anhydride and one drop of sulphuric acid	**Steroids**
Lead Acetate Test	Appearance of yellow precipitate while adding 10% lead acetate to one ml extract	**Flavonoids**
Ferric Chloride Test	Appearance of blue-green precipitate with one ml plant extract and one ml 0.5% ferric chloride solution	**Tannins**
Fehling's Test	Formation of blue colour with Fehling A solution (Copper sulphate) and deep blue with Fehling B solution (Potassium sodium tartrate L and a strong alkali usually sodium hydroxide)	**Carbohydrate**
Biuret Test	Appearance of purple colour on the addition of few drops of biuret reagent to 2 ml plant extract	**Protein**
Ninhydrin Test	Formation of violet colour on heating one ml plant extract with one ml ninhydrin,	**Amino acid**
Foam Formation Test	Development of foam while shaking plant extract with water (1:1 ratio)	**Saponins**
Vanillin- Sulphuric Acid Test	Appearance of dark yellow colour on heating 1ml plant extract with few drops of sulphuric acid, in a water bath for 10 min and the subsequent process of cooling down for 15 min	**Triterpene**
Keller Killani Test	Development of brown ring between the layers on the addition of 3ml glacial acetic acid , few drops of 2% $FeCl_3$ and 1 ml Conc. H_2SO_4 to 10ml aqueous plant extract	**Glycosides**

VIII. ANTIMICROBIAL ACTIVITY

Antibacterial activity of the extracts of *N. arbortristis* L., prepared with various solvents *viz.*, petroleum ether, chloroform, butanol, ethanol and water were evaluated by agar well diffusion method (Bauer *et al*., 1966). 24 hrs broth cultures of bacteria were used for the assay. Accordingly, the bacterial suspension was then evenly spread over the Nutrient agar plate. Using a sterile borer, wells were then punched on the plates. The plant extracts were dispensed into each well with a sterile micropipette. Subsequently, the plates were then incubated at 37ºC for 24 hrs. Antibacterial activity of the extracts was determined by measuring the diameter of zone of inhibition (mm).

IX. PHOTOGRAPHY

The cytological preparations were photographed using Inverted tissue culture WTC-7500 WESWAX microscope. The pictures were developed / printed in commercial laboratories.

X. RESULTS AND DISCUSSION

N. arbortristis L is a small divine ornamental tree known for its fragrant white flowers (Fig. 1 &2). Flowers are being widely used to worship God across India. Further, the plant is well known in traditional Indian medicine and ayurveda as well. It has been attempted for various pharmacological actions such as anti-arthritic, antispasmodic, antibacterial, anti-inflammatory, immuno-stimulant, anti-diabetic, hepato-protective, antipyretic, anti-allergic and CNS depressant (Das *et al*., 2008 ; Suresh *et al*. , 2010). The present investigation has been designed to perform the anatomical study, histochemical localization, phytochemical analysis and antimicrobial activity of stem, leaf and flower of *Nyctanthes arbortristis* L.

Figure . 1- Habit of *N. arbortristis* L

Figure . 2- Flowers of *N. arbortristis* L.

STEM

Transverse section of the stem is more or less square in shape. The outermost layer is epidermis. Where in the rectangular cells with unicellular trichomes are being compactly arranged in a single layer (Fig. 3 & 4). A thick continuous layer of cuticle is present on the epidermis. Four layers of uniseriate, collenchymatous cells form a wavy ring just below the epidermis. A Few layers of parenchyma are present next to collenchyma. Many intercellular spaces are also being reported. Four cortical vascular bundles (conjoint, collateral, open & exarch), are being located at the four ridged portion of the stem. This is mainly due to the abnormality of stem and secondary growth at maturity. In contrast, the innermost layer is known as endodermis. It is seemed to be under developed. The pericycle in turn lies in a sclerenchymatous patch just below the endodermis. Further, the primary phloem is likely to be crushed and reported just below the pericycle in an irregular fashion. The secondary phloem in turn forms a continuous layer, which consists of thick walled, tracheids and vessels. Cambium on the other hand has been reported in between the xylem and phloem. Further, xylem is being characterized as endarch, where protoxylem faces towards pith (Shirsat et al., 2011).

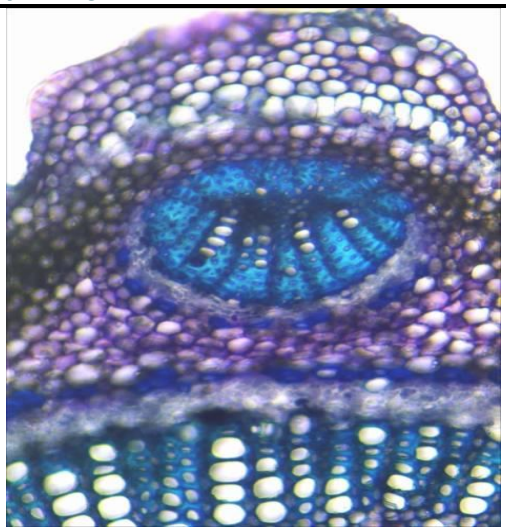

Figure : 3- Transverse section of stem

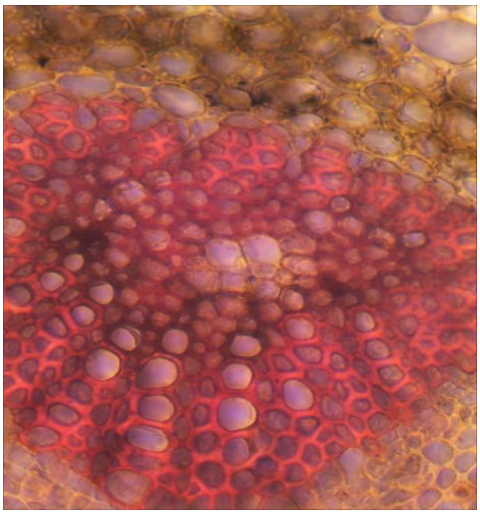

Figure 4- Cortical bundle

LEAF

Transverse section (Fig. 5) of a dorsiventral leaf has an outermost layer of epidermis which in turn exhibits uniseriate barrel shaped cells with sinuous anticlinal walls. This layer exhibits unicellular and glandular trichomes. Unicellular trichomes are larger than the lower epidermis, which have eight adjacent basal cell protruding from the surface. The glandular trichomes characterized with one stalk cell and four head cells are situated in a slight depression found in epidermis. Stoma confined to both sides is anomocytic. Mesophyll consists of 1– 2 layers of palisade cells and four to five layers of spongy cells (Fig. 6). Midrib vascular bundle is crescentric showing cambial divisions, xylem and phloem. Collenchyma cells in turn are seemed to be supporting the either side of crescentric vascular bundle (Shirsat *et al*., 2011)..

Figure : 5- Transverse section of leaf

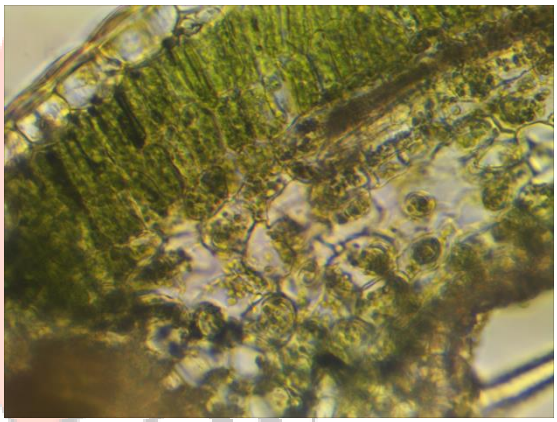

Figure : 6- Leaf lamina

FLOWER

Transverse section of flower shows parenchymatous cells both in the corolla and calyx (Fig. 7 &8). While the corolla shows four inversely oriented cortical bundles and 30 – 40 normal vascular bundles, calyx traces are not very distinct (Fotidar, 1939).

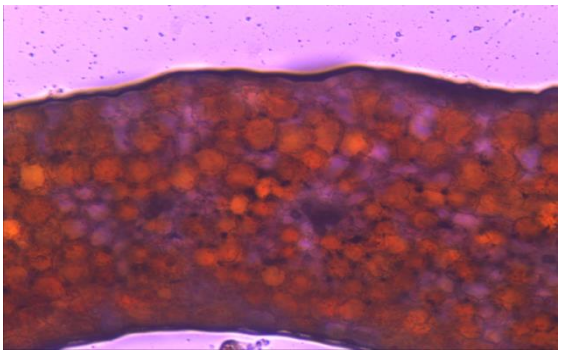

Figure: 7. Transverse section of corolla

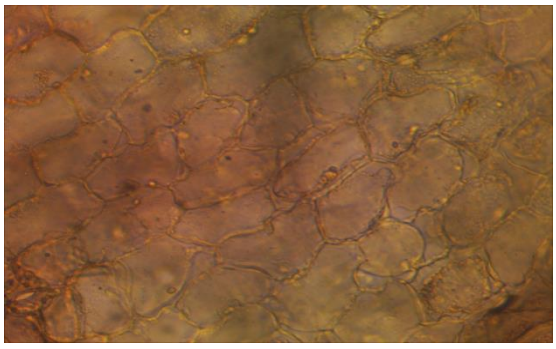

Figure:8. Transverse section of calyx

HISTOCHEMICAL STUDY

Histochemical studies were carried out in the extracts of stem, leaf and flower of *N.arbortristis* L. While starch has been localized by staining with I_2KI, invariably all the above three extracts showed purple colour. Interestingly, as stems remain green in colour they are able to perform photosynthesis and accumulate starch in the cells surrounding vascular bundles (Fig. 11). Thick walled cells in turn have been identified with TBO stain. Further, the sites of synthesis and accumulation of essential oil have also been recognized histochemically with Sudan III. Oil secreting cells appear bright orange – red (Fig. 10). Thus the oil bodies are being localized in the epidermal cells of stem, calyx and corolla. The commercially important essential oil of *N. arbortristis* L with pleasant fragrance gets accumulated in secretary cells and cavities. Like the essential oil of low molecular mass terpenes of jasmine, it tends to evaporate upon exposure to air. Interestingly, the oil has been proved as a potent

anti-microbial agent (Fig. 18). Floral scent emission is one of the crucial strategies which many plants employ to attract pollinators to assure productivity. The bright orange corolla tubes of the flowers contain a pigment, nyctanthin which probably occurs as a glucoside in a concentration of 0.1% (Fig. 9).

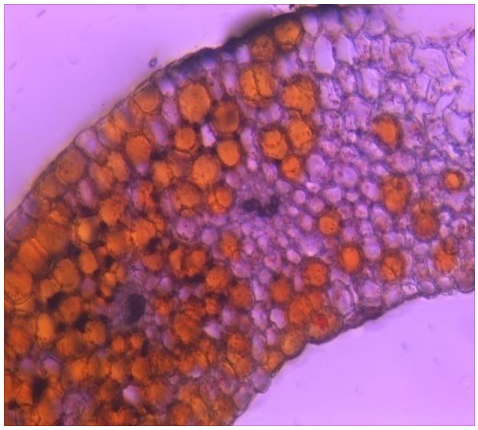

Figure :9. Localization of carotenoids

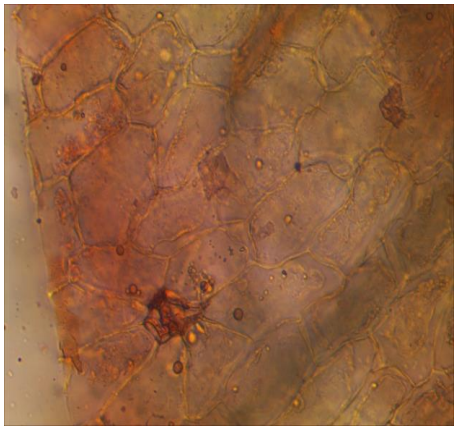

Figure:10. Oil granules in calyx

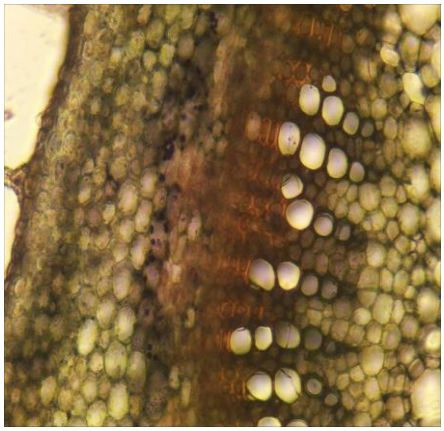

Figure : 11. Starch in endodermis

PHYTOCHEMICAL STUDIES

Results (Table 2, 3 &4) of preliminary phytochemical analysis for the presence of some bioactive principles carried out in the powdered leaf, flower and stem of *N. arbortristis* L. reveal that the leaf powder extracted in chloroform contain almost all components (cholesterol, saponins tannin triterpene alkaloids, amino acids, flavonoid and carbohydrates) except protein. Besides tannin has been reported not only with the extracts of leaf powder but also with flower extracts obtained with all the solvents (petroleum ether, chloroform, ethanol, butanol and water) studied. As far as the phytochemical analysis of stem powder extract is concerned alkaloids and amino acids have been reported with almost all solvents experimented in the present investigation except water. Similar such situation has also been warranted for alkaloids in flower extracts. Further, the alkaloid and the glycosides extracted in the present investigation are known as nyctanthine and iridoid glycosides respectively. Interestingly, the results pertaining to the bioactive principles of flowers of *N. arbortristis* L. remain similar to that of jasmine.

Table:2. Phytochemical components of leaf extract of *N. arbortristis* L.

Test	Petroleum ether	Chloroform	Ethanol	Butanol	Water
Cholesterol	++	++	-	++	-
Saponins	-	++	++	-	-
Tannin	++	++	++	++	++
Triterpene	-	++	++	++	-
Alkaloids	++	++	-	++	++
Amino acid	-	++	-	++	++
Flavonoid	++	++	-	++	++
Carbohydrate	-	++	-	-	-
Protein	++	-	++	++	++

Table :3. Phytochemical components of flower extract of *N. arbortristis* L.

Test	Petroleum ether	Chloroform	Ethanol	Butanol	Water
Cholesterol	++	-	-	++	++
Saponins	++	-	-	-	-
Tannin	++	++	++	++	++
Triterpene	-	++	++	-	-
Alkaloids	++	++	++	++	-
Amino acid	++	++	-	-	++
Flavonoids	++	++	++	++	-
Carbohydrates	-	++	-	-	++
Protein	-	-	++	++	-

Table :4.Phytochemical components of stem extract of *N. arbortristis* L.

Test	Petroleum ether	Chloroform	Ethanol	Butanol	Water
Cholesterol	++	++	++	-	++
Saponins	-	++	++	-	-
Tannin	++	-	-	-	++
Triterpene	-	++	-	-	++
Alkaloids	++	++	++	++	-
Amino acid	++	++	++	++	-
Flavonoids	-	-	++	-	-
Carbohydrates	++	-	++	++	++
Protein	++	-	-	-	++

ANTIBACTERIAL ACTIVITY

Study on antibacterial activity of dry powder extracts of stem, leaf and flower of *N. arbortristis* L. against *Escherichia coli* a Gram negative bacterium (Table 5) reveals the maximum (1.6mm) zone of inhibition in flower powder extracted with butanol when compared to ethanol (15mm). Similar such findings are also being reported with the stem extracts (butanol 17mm; ethanol 14mm). Whereas the leaf exhibits the maximum (1.9mm) zone of inhibition when extracted in ethanol and nil value with butanol (Fig. 11). These results are in line with the study made by Priya and Deepak (2007).While they have evaluated the potential of various parts of *N. arbortristis L.* extracted in ethanol and chloroform against both the Gram negative and Gram positive bacteria, significant antibacterial activity has been reported with gram negative bacteria.

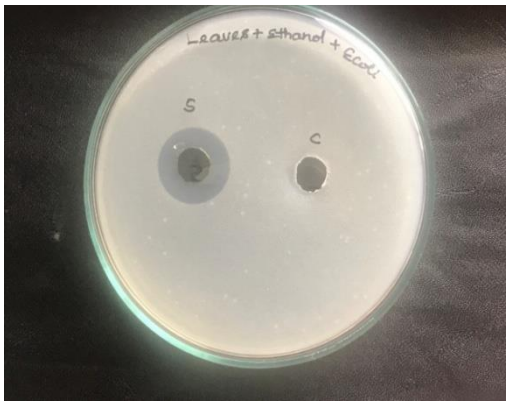

Figure. 12- Maximum inhibition in leaf extract

Table:5 antimicrobial activity of various plant parts of *N.arbortristis* L

Plant parts	Extract in butanol	Extract in ethanol
Flower	16mm	15mm
stem	17mm	14mm
leaves	-	19mm

XI. REFERENCEs

[1] Bansal, G., Suri, K.A. and Grover, P. 2015. A Comprehensive review on *Nyctanthes arbortristis* , Int. J.Drug Dev. and Res.7(1):183-193.

[2] Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Thuruck, M. 1966. Antibiotic Susceptibility Testing by a Standardized Single Disc Method, Am J Clin Pathol 44 : 493-496.

[3] Bhosale, A.V., Abhyankar, M.M., Pawar, S. J., Khan, S. and Patil, N. 2009. *Nyctanthes arbortristis*- A Pharmacognostic Review, Research J. Pharmacognosy and Phytochemistry, 1(2):91-97

[4] Das, S., Sasmal, D. and Basu, S.P. 2008. Evaluation of CNS Depressant Activity of Different Plant parts of *Nyctanthes arbortristis* Linn. Indian Journal of Pharmaceutical Sciences, 70(6): 803-806.

[5] Desai, S.V., Dhumal, A.S. and Chauhan, P.S. 2016. Literature review on *Nyctanthes arbortristis*, International Journal o,f Pharmacy and Technology, 1: 3611-362

[6] Divya, P. 2015. Phytochemical Analysis of leave extract of *Nyctanthes arbortristis*, J Environmental Science, Toxicology and food Tech, 1(3):39-42.

[7] Fotidar, A.N.1939. The primary vascular system of the stem of *Nyctanthes arbortristis* L.J. Indian Bot. Soc., 18: 43-45.

[8] Jain, P, K, and Pandey, A. 2016. The wonder of Ayurvedic medicine- *Nyctanthes arbortristis*, International Journal of Herbal Medicine, 4(4):09-17.

[9] Kiew, R. and Baas, P. 1984. Nyctanthes a member of Oleaceae, Proceeding Indian Academic Science, 3:349-58.

[10] Kirtikar, K.R. and Basu, B.D. 1935. *Nyctanthes arbortristis* in medicinal plants, edited by Blatter E, Cains JF, Mhaskar KS, LM Basu Publishers, Allahabad, India, 5: 36 - 46.

[11] Priya K. and Deepak, G. 2007. Antibacterial activities and phytochemical analysis of different plant parts of *Nyctanthes arbor*-tristis (Linn.), Research Journal of Phytochemistry,1:61-67.

[12] Rani C., Chawala S.,Mangat, S., Mangal, A.K., Kajla, S. and Dhawan, A. K. 2012. *Nyctanthes arbortristis* Linn(Night Jasmine):A Scared Ornament Plant With Immense Medicinal Potentials, Indian Journal of Traditional Knowledge.11(3): 427-435.

[13] Sasmal, D., Das, S. and Basu, S.P. 2007. Phytoconstituents and therapeutic potential of *Nyctanthes arbor-tristis* Linn. Phcog. Rev. 1: 344-349.

[14] Shirsat, M. K., Gupta, S.K., Vaya, R., Dwivedi, J., Singhvi, I. J., Mahatma, O. P. and Bale, D. 2011. Histological Study of Different Part of *Nyctanthes Arbortristis* Linn. Asian Journal of Biochemical and Pharmaceutical Research, ,2 :1, 292-297.

[15] Suresh V., Arunachalam V. and Jaikumar, S. 2010. Anticancer activity of ethanol extract of stem bark of *Nyctanthes arbor*-tristis Linn. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 1(4): 306-311.

[16] Yudharaj, P, M. , Shankar, R., Sowjanya, B., Sireesha, E. , Ashok, N. and Jasmine Priyadhrshini, R. 2017. Importance and uses of medicinal plants- An overview. International Journal of Preclinical & Pharmaceutical Research, 7(2) : 67-73.