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Assessment of pharmacognostic, phytochemical and antibacterial potential of fruit of *Nyctanthes Arbor-Tristis* Linn.

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ABSTRACT

Objective: To study the pharmacognostic, phytochemical and antibacterial potential of fruit of *Nyctanthes arbor-tristis* Linn. It is used for wide range of diseases in folk medicine. **Methods:** Macroscopical, microscopical, physico-chemical evaluation, florescence analysis, behavior of seed powder, preliminary phytochemical analysis by chromatographic method and antibacterial potential of extract against *Bacillus subtilis* (NCIM 2079), *Escherichia coli* (NCIM 2065) and *Pseudomonas aeruginosa* (NCIM 2200) were determined of various extracts of *N. arbor-tristis*. **Results:** microscopic study shows the general characteristic of seed. Physico-chemical investigation shows the total ash, acid insoluble ash; water soluble ash and sulphated ash values were 11.37 ± 0.04 % w/w, 2.16 ± 0.02 % w/w, 3.72 ± 0.02 % w/w and 5.56 ± 0.04 % w/w respectively. Methanolic and petroleum ether extract exhibit significant inhibition of microorganism. **Conclusion:** it can be conclude that these observations would be of immense value in the botanical identification and standardization of the drug in crude form.

Keywords: *Nyctanthes arbor-tristis* fruit, pharmacognostic study, physico-chemical evaluation, preliminary phytochemical assessment, antibacterial activity.

1. Introduction

Nyctanthes arbor-tristis Linn. (Nyctanthaceae) commonly called as night jasmine, a hardy large shrub or small tree widely distributed in outer Himalayan ranges from Chenab to Nepal, Assam, Burma, Bengal, Central India to Godavari, cultivated in many parts of India. It is also planted in Indian gardens for ornamental purpose due to its highly fragrant flowers [1-2]. It is a shrub or small tree up to 10 m in height with gray to greenish rough bark with stiff whitish hairs.

Leaves are opposite, ovate, acute or acuminate, entire or with few large distant teeth, short bulbous hairs rounded or slight cuneate. Flowers are small, delightful fragrant, sessile, slender, and hairy; corolla glabrous, orange colored and lobes are white. Fruits are a capsules of 1-2 m in diameter, long and broad, compressed, 2 celled separating into 2 flat one seeded carpels, reticular veined and glabrous [3,4].

The orange heart is used for dyeing silk and cotton, this practice was started with Buddhist monks whose orange robes were given their colour by this flower. The Parijata is regarded in Hindu mythology as one of the five wish-granting trees of Devaloka [5]. Different parts of *Nyctanthes arbor-tristis* are known to own for treatment of various ailments by tribal people of India especially Orissa and Bihar along with its use in Ayurveda, Siddha and Unani systems of medicines [6, 7]. The seeds are used as antihelmintic and in alopecia. It is antibilious and an expectorant, and is also useful in bilious pyrexia [8]. The powdered seeds are used to cure scurfy affections of scalp, piles and skin diseases [7]. Seeds, leaves and flower extract of this plant showed CNS depressant activity [9].

Phytochemical studies revealed the presence of tertiary alkaloids mainly 7-(α -anilino-p-nitrobenzyl)-8-quinolinol and quaternary alkaloids belonging to protoberberines and aporphines [10,11]. The leaves have been found to contain tannic acid, methyl salicylate, amorphous glucosides, mannitol, resin, ascorbic acid, carotene, and traces of a volatile oil. Flowers contain essential oils, coloring matter (nyctanthin), mannitol, tannin and glucose. Its roots are composed of alkaloids, tannins and glucosides [1,2,12].

Arbortristoside A and arbortristoside C isolated from plant showed antiviral activity [13]. As literature survey and scientific data revealed that a large number of indigenous drugs have already been investigated as regards their botany and chemistry is concerned, however a systematic standardization including pharmacognostical and physico-chemical study is still lacking. The present investigation *Nyctanthes arbor-tristis* (L.) of is therefore taken up to evaluate certain botanical and chemical standards which would help in crude drug identification as well as in checking adulteration, if any. Further the study will greatly help in quality assurance of finished product of herbal drugs.

2. Material and methods

2.1 Plant material

The plant material was obtained from Nasik district (M.S.) and authenticated by Dr. D. A. Patil, reader and the authorized plant identifier of Department of Botany, SSVPS College, North Maharashtra University, Dhule (M.S) India; a specimen is preserved in the college herbarium (KBHSS/PCG/2011/12).

2.2 Macroscopic and microscopic examination

Macroscopic studies were done using simple microscope. The color, shape, size, taste and odour of fruit were determined. Microscopic study was carried out by preparing of thin hand section (longitudinal and transverse) of seed. The sections were cleared with chloral hydrate and stained with concentrated hydrochloric acid - phloroglucinol (1:1). Powdered drug was separately treated with concentrated hydrochloric acid - phloroglucinol, iodine solution, 60% sulphuric acid for identification of lignified elements, starch grains, calcium oxalate crystals in the powdered fruit by reported methods [14-16].

2.3 Physicochemical constant study

The Physicochemical parameters of the powdered drug such as total ash, water-soluble ash, acid-insoluble ash and sulphated ash were determined. Alcohol and water soluble extractives values were determined to find out amount of water and alcohol soluble components. The moisture content was detected by loss on drying method [17-19].

2.4 Fluorescence analysis

Powdered material was analyzed under visible light, short ultra-

violet light, long ultra- violet after treatment with various organic/inorganic reagents like NaOH, HCl, HNO₃ and H₂SO₄ was also carried out for the powder [20].

2.5 Behaviour of fruit powder

Behaviors of *N. arbor-tristis* fruit powder of with different chemical reagent were performed to detect the occurrence of phytoconstituents along with color changes under ordinary day light by standard method [21].

2.6 Preparation of extracts

The collected fruit was sun dried for 5 days and pulverized into a dry powder. The powder was subjected to extraction in soxhlet extractor using petroleum ether and methanol for 72 h coded as PEF and MEF respectively. The extracts were filter and each filtrate was evaporated by distillation under reduced pressure using rotary vacuum evaporator at 30 °C and stored [22].

2.7 Phytochemical screening

The obtained extracts were dried and weighed. The presence of various phytoconstituents viz. steroids and terpenoids (Liebermann Burchard test), alkaloids (Dragendorff's test), tannins and phenolics (Ferric chloride test), flavonoids (Shinoda test), Sugars (Fehling solution test), amino acids (Ninhydrin test), etc. was detected by usual methods prescribed in standard texts [21].

2.8 Thin layer chromatography

For the TLC fingerprint the petroleum ether extract and methanolic extract of fruit was subjected to thin layer chromatographic analysis, to find the presence of number of chemical constituents to support the chemical test. Analytical TLC plates were prepared by pouring the silica gel G slurry on the glass plates. Drying the thin layer plates, for 30 minutes in air and then in an oven at 110 °C for another 30 minutes. For qualitative work, spot was applied in a row along one side of plate, about 2cm from edge, by using capillary tubes. The range of sample volume was controlled, spreading not more than 0.5 cm. The plate was placed in previously saturated TLC chamber with mobile phase. The chromatographic conditions were described in table 1. The R_f values are compared with standard drug and colors are recorded [23,24].

Table 1: Chromatographic conditions for PEF and MEF

Extract	Stationary phase	Solvent system	Detection
PEF	Aluminium coated silica gel GF 254	Toluene: Ethyl acetate (9:1)	Sprayed with Vanillin-H ₂ SO ₄ after drying heated at 110 °C
MEF		Ethyl acetate: Methanol: Water(8.1:1.1: 0.8)	at 254 nm and Sprayed with anisaldehyde-H ₂ SO ₄ after drying heated at 110 °C

PEF: petroleum ether extract of fruit, MEF: methanolic extract of fruit

2.9 Antibacterial activity

Microorganisms: *Bacillus subtilis* (NCIM 2079), *Escherichia coli* (NCIM 2065) and *Pseudomonas aeruginosa* (NCIM 2200). The cultures were obtained from National Collection of Industrial Microorganism (NCIM) Pune, India. The cultures of these bacteria were grown in nutrient broth at 37 °C and maintained nutrient agar slants < 12 °C.

Chemical: Ciprofloxacin was procured from Ranbaxy research lab., Gurgaon, India. All the chemicals were of analytical grade and

used as received.

Preparation of Inoculum: Several colonies of a 48 hr culture of *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* were suspended in sterile saline solution (0.9%). Turbidity was adjusted to an absorbance of 0.18 to 0.25 at 625nm [25].

2.9.1 Agar diffusion method:

All the experimentation was done in aseptic area under laminar air-flow cabinet. The agar diffusion method 10 was adopted for the

study. Broth cultures of the test isolates (0.1 ml) containing 1.0×10^5 CFU/ml of organism was introduced into a sterile petri dish and 15 ml of molten nutrient agar were added. The content was thoroughly mixed and then allowed to solidify. The extracts were dissolved in DMSO and used in concentrations 10, 20, 40 and 50 mg/ml. Ciprofloxacin ($5 \mu\text{g/ml}$) was used as standard for antibacterial activity. Holes were bored in the plates, using a standard sterile cork borer of 8 mm diameters and equal volumes of the plant extracts ($1000 \mu\text{l}$) were transferred into the wells with the aid of micropipette. The experiments were carried out in triplicate. The plates were kept for 1hr for pre-diffusion and incubated at $37^\circ\text{C}/24\text{hr}$ (plates containing bacterial cultures). At the end of incubation, zone of inhibition was measured in all the plates [26,27].

2.9.2 Minimum inhibitory concentration (MIC) method:

It was determined by tube dilution method (turbidimetric method) [28, 29]. 1 ml of the sterilized media was poured in the concentration of $50 \mu\text{g/ml}$ were used. The extracts were serially diluted to give a concentration of 25, 12.5, 6.25, 3.12 and $1.56 \mu\text{g/ml}$. In all the test tubes 0.1 ml of suspension of bacteria in saline was added and incubated at $37^\circ\text{C}/24 \text{hr}$. Post-incubation the plates were observed for turbidity.

3. Result

3.1 Macroscopic and microscopic examination

Macroscopic character of fruit: The fruit is flat, brown and heart cordate-shaped to rounded-capsule, around 2 cm in diameter with two celled opening transversely from the apex, each containing a single seed (fig.1).



Fig 1: Shows morphological character of *Nyctanthes arbor-tristis* fruit

Microscopically fruit showed typical character of fruit. In the epicarp epidermal cells were compactly arranged, polygonal cells with slightly anticlinal walls covered by a thin cuticle followed by 1-3 layers of collenchyma, Spongy parenchymatous tissue, sclerenchymatous fibres and oil gland (fig.2A). Mesocarp is composed of thin walled, oval to polygonal lignified parenchymatous cells, which showed presence of round to oval starch grains (Fig 2F). Pericyclic fibers are also observed in the mesocarp. Ovules are anatropous and bitegmic, the outer integument shows 3-5 layers and inner 3 layers, integument formed by cube shaped cells. The Micropyle channel is only formed by the exostome. The endostome is occupied by nucellar tissue. In chalaza

region a group of cell with thin wall between the integuments occurs. The provascular strands transverse the funicle and the raphe extending only up to the chalaza. The presence of endothelium which is single layer coordinates the development between the embryonic sac and the young endosperm. The exotegmen present in endosperm completely constituted by macrosclerides, which begin differentiation in the young seed (fig. 2D). The mucilage secretory cavity observed in the mesotegmen, white embryo occupies the entire seminal cavity and present a slightly curved hypocotyl- radical axis (fig. 2C). Endosperm and embryo are rich in lipid material and small quantity of starch grains observed on rapheal bundles (fig 2E).

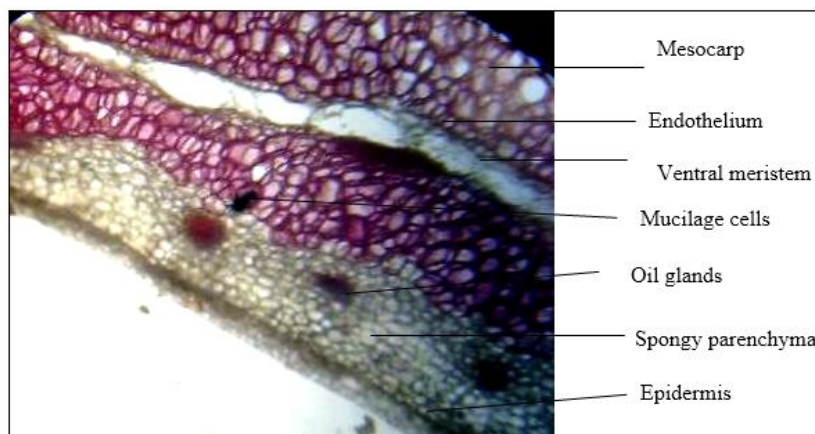


Fig 2A: Showed transverse section of *Nyctanthes arbor tristis* fruit after treated with phloroglucinol- Conc. HCl (1:1)

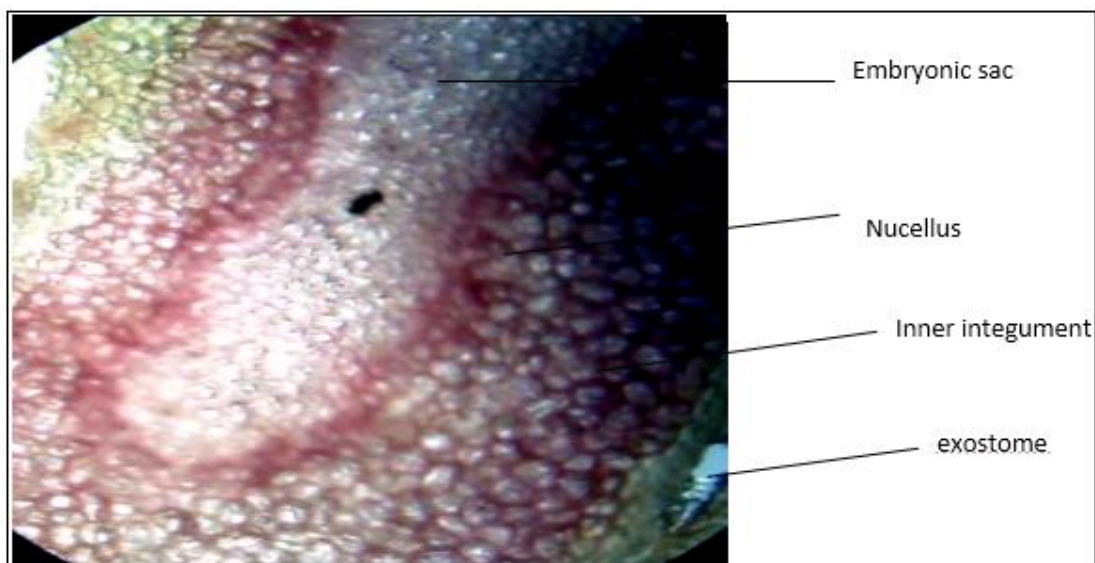


Fig 2B: shows epicarp of *Nyctanthes arbor tristis* fruit

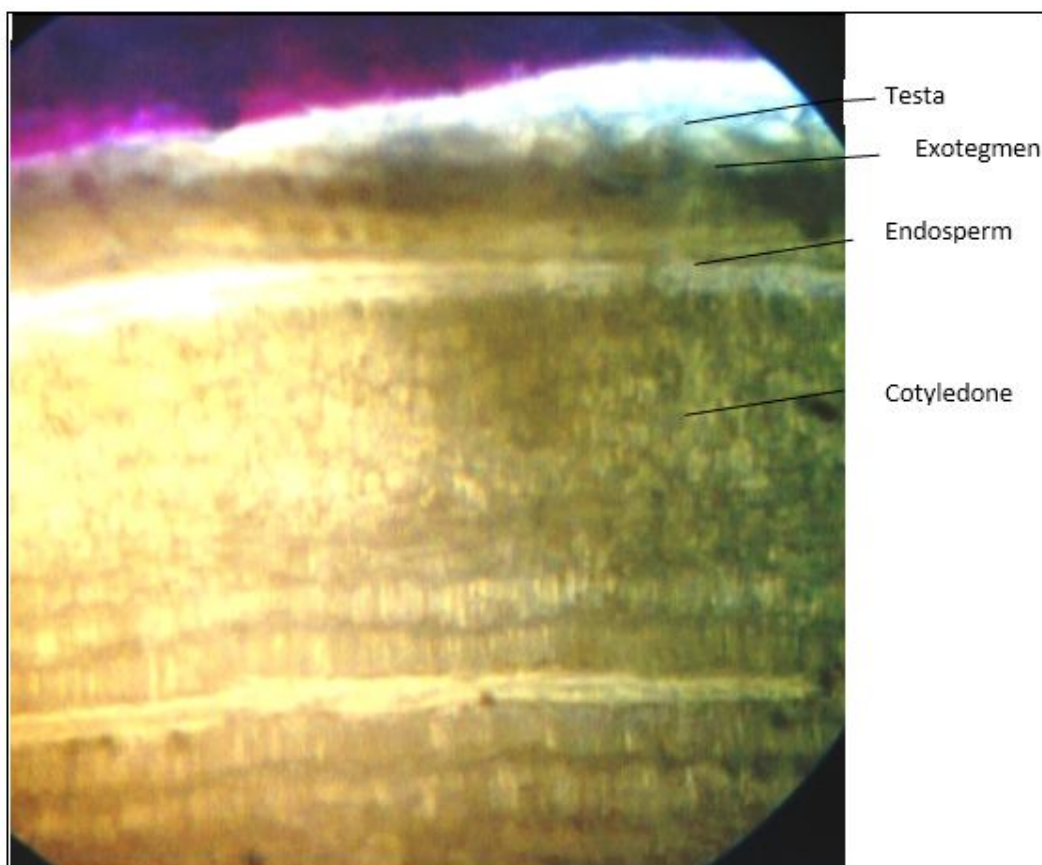


Fig 2C: Shows Endocarp



Fig 2D: Shows Parts of Endosperm

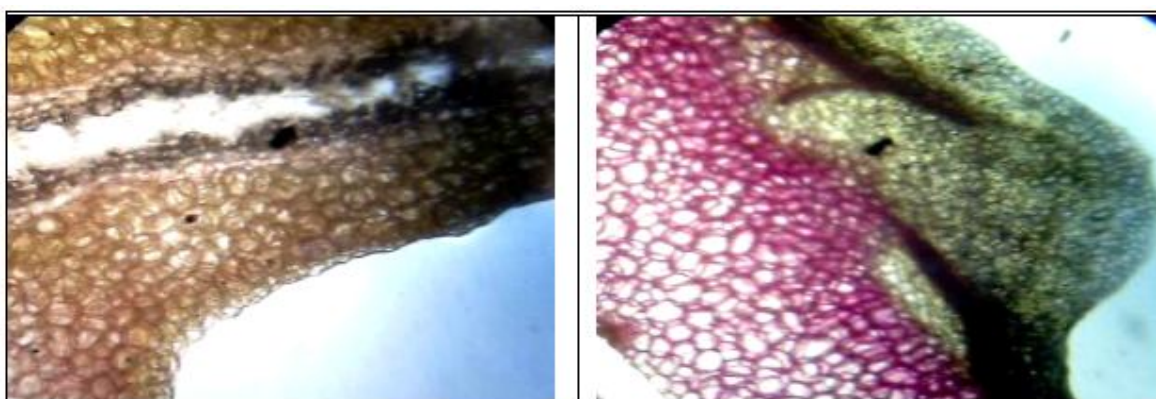


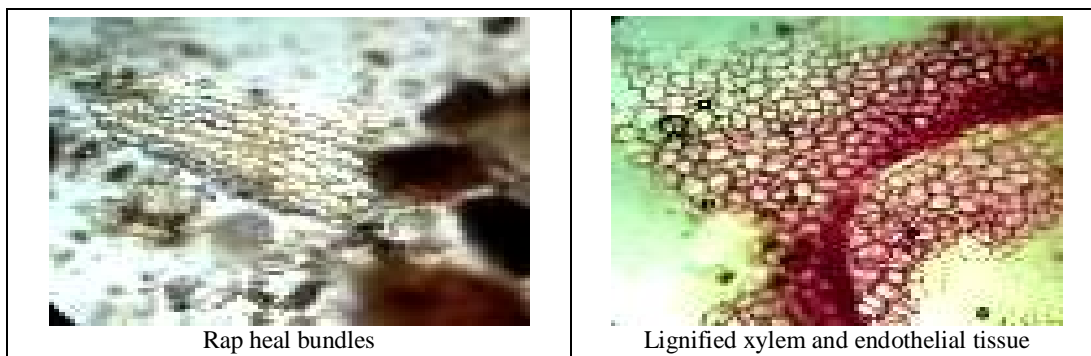
Fig 2E: Showed presence of starch grains on rapheal bundles **Fig 2F:** showed sclerenchymatous fibres and hypostase

Fig 2: Shows transverse section of *Nyctanthes arbor-tristis* fruit

3.2 Powder characteristics

Fruit powder evidenced the presence of multilayered rapheal bundles, lignified xylem and single layered oval shape endothelial tissue with thick wall, some fragments of endosperm tissue, oil

containing cells (glands), testa and thick walled lignified sclerenchymatous fibers with narrow lumen with sclerides tissues (fig. 3).



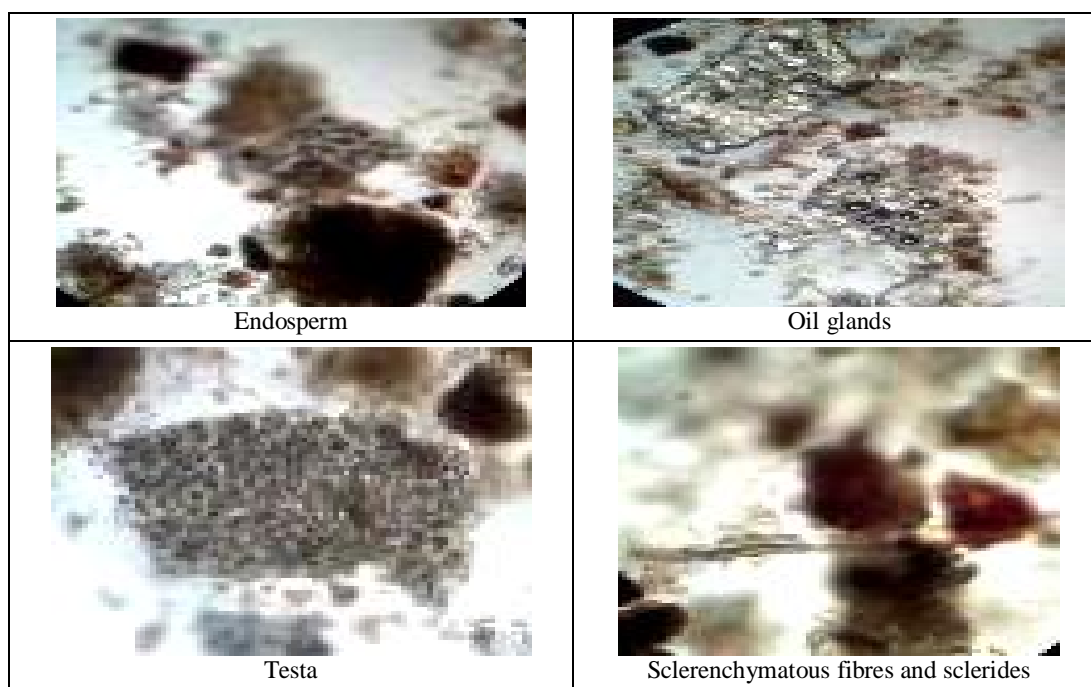


Fig 3: Shows powdered characteristics of *Nyctanthes arbor-tristis* fruit

3.3 Physicochemical constant

In physical constant study, the ash values, extractive values, moisture content of fruit were determined. The total ash, acid insoluble ash, water soluble ash and sulphated ash values were found to be 11.37 ± 0.04 % w/w, 2.16 ± 0.02 % w/w, 3.72 ± 0.02 % w/w and 5.56 ± 0.04 % w/w respectively. However, 11.73 ± 0.46 % w/w alcohol soluble and 8.56 ± 0.34 % w/w water soluble extractives were observed. The moisture content of fruit powder was nearly 8.70 ± 0.01 % w/w.

3.4 Fluorescence analysis

The fluorescence studies for the fruit powder by treating it with different chemical reagents and the results were reported (Table 2). Fluorescence characteristic of powdered fruit was observed in visible, short and long ultra- violet light for resolution of doubtful specimen.

Table 2: Fluorescence analysis of fruit powder

Treatment	Day light	UV light (254nm)
Powder as such	Pale brown	Light green
Powder + 1N HCL	Yellowish brown	Light green
Powder + Aq. 1N NaOH	Yellow	Dark green
Powder +Alcoholic 1N NaOH	Pale yellow	Yellowish green
Powder + 5% I ₂ solution	Bluish black	Dark brown
Powder + 50% HNO ₃	Yellowish orange	Light green
Powder + 50% H ₂ SO ₄	Yellowish orange	Light green
Powder + Methanol	Pale yellow	Emerald green
Powder + 5% FeCl ₃ solution	Red colour	Fluorescent green

3.5 Behavior of fruit against different chemical reagents

Behavior of fruit powder of *N. arbor tristis* with different chemical reagent showed presence of steroids, starch, tannins, flavonoids,

alkaloid, glycoside, carbohydrates (Table 3).

Table 3: Behavior of fruit powder with different chemicals

Reagent	Colour/precipitation	Constituents
Conc. Sulphuric acid	Reddish brown	Steroids present
Liebeman's bur chard test	Reddish green	Steroids / Triterpenoids present
Picric acid	Yellow ppt	Alkaloids present
Aqueous ferric chloride	Dark-blue	Tannins present
Iodine solution	Blue	Starch present
Aqueous mercuric chloride solution	Red	Alkaloids present
Magnesium – hydrochloric acid	Pink	Flavonoids present
Aqueous silver nitrate solution	White ppt	Protein present
Aqueous potassium hydroxide solution (5 %)	No change	Anthraquinone glycoside absent
Spot test	stain observed	Fixed oils present
Salvoski's test	A yellow ring at the junction	Steroids present
Frothing test	No change	Saponins absent
Molisch reagent	Purple colour at the junction	Carbohydrate present
Aq. lead acetate	White precipitation	Tannins present
Dragendorff's reagent	Reddish precipitation	Alkaloids present
Aqueous NaOH	Yellow	Flavonoid present

3.6 Phytochemical investigation and extractive values

Preliminary phytochemical analysis revealed the presence of flavonoids, terpenoids, tannins, saponins, steroids, carbohydrates, phenolic compounds, carbohydrates and proteins (Table 4). The

extractive values of PEF and MEF were found to be 3.28 w/w (dark green), 11.29 w/w (yellowish brown).

Table 4: Preliminary phytochemical analysis of PEF and MEF

Phytoconstituents	PEF	MEF
Alkaloids	-	+
Glycosides	-	+
Saponin	-	-
Carbohydrates	-	+
Tannins and phenolic compound	-	-
Flavonoids	-	+
Phytosterols	+	-
Proteins and amino acids	-	+
Triterpenoids	+	-
Fixed oils and fats	+	-
Gums and mucilage	-	-

+: present, -: absent, PEF: petroleum ether extract of fruit, MEF: methanolic extract of fruit

3.7 Thin layer chromatography of fruit extract

The plate was developed in respective mobile phase upto 80% and sprayed with respective spraying reagent. PEF showed light blue, violet and yellowish color spot for phytosterols and

terpenoids respectively compared with available literature. MEF also showed grey, orange, yellow color spots (figure 4-5 and table 5).

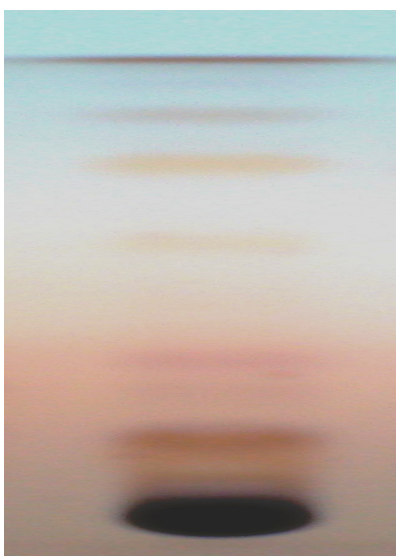


Fig 4: Showed phytoconstituents pattern of PEF on TLC plate.

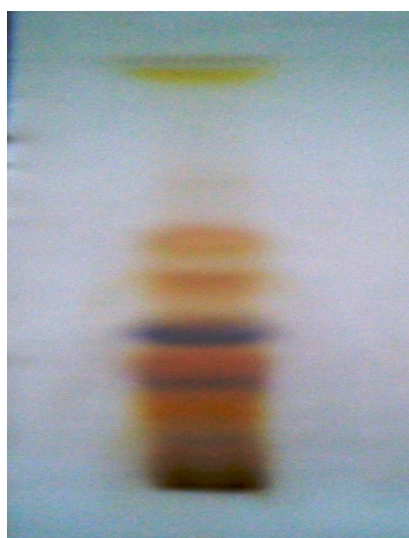


Fig 5: Showed phytoconstituents pattern of MEF on TLC plate.

Table 5: Thin layer chromatographic profile of PEF and MEF

Extract	Number of spots observed	Color of spots	Observed Rf values
PEF	06	Orange, pink, violet, yellow, yellow and brownish yellow	0.21, 0.34, 0.39, 0.67, 0.78, 0.89 respectively.
MEF	07	Grey, dark blue, orange, blue, yellow, yellow, greenish yellow	0.20, 0.38, 0.42, 0.48, 0.56, 0.6, 0.97 respectively.

PEF: petroleum ether extract of fruit, MEF: methanolic extract of fruit

3.8 Antibacterial study

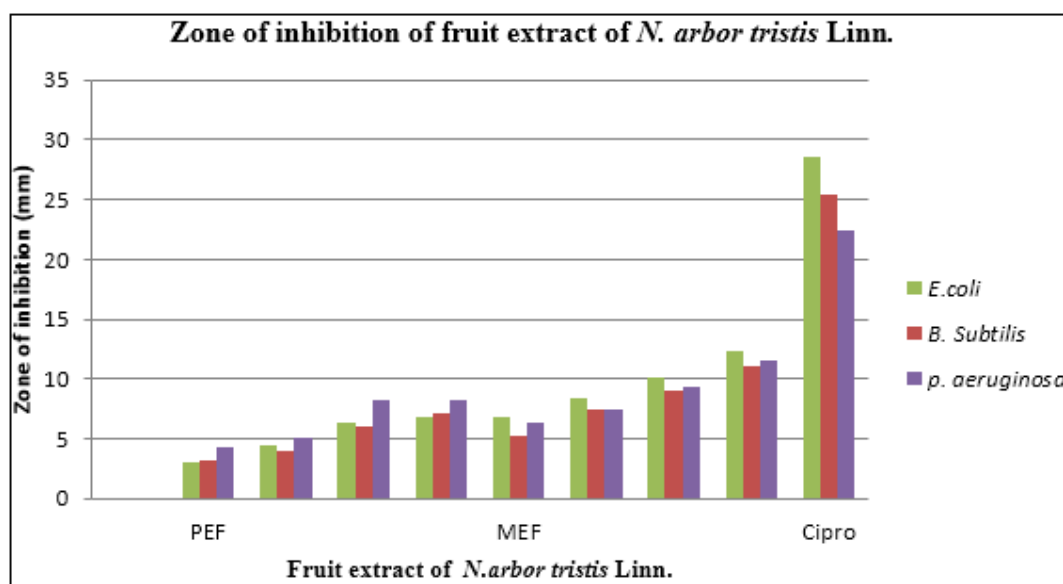
Table 6 shows the results of antimicrobial activity against the tested microorganisms. Graph 1 shows the comparison of the PEF

and MEF extracts at a concentration of 50mg/ml with the standard drugs. The Minimum Inhibitory Concentration (MIC) values of the extracts against tested microorganisms were shown in Table 7.

Table 6: Antibacterial activity (Zone of inhibition) of fruit extract of *N. arbor-tristis* Linn.

Extracts/ standards	PEF				MEF				Cipro
	10	20	40	50	10	20	40	50	
Microorganisms									
<i>B. subtilis</i>	3.23±0.66	4.04±0.3 3	6.07±0.3 3	7.12±0.3 3	5.39±0. 44	7.42±0.22	9.12±0.3 3	11.1±0.22	25.4±0.33
<i>E. coli</i>	3.12±0.66	4.54±0.6 6	6.43±0.5 5	6.34±0.2 2	6.87±0. 33	8.43±0.33	10.1±0.3 3	12.4±0.33	28.6±0.33
<i>P. aeruginosa</i>	4.34±0.33	5.12±0.3 3	7.21±0.3 3	8.31± 0.33	6.45±0. 66	7.45±0.66	9.45±0.0 0	12.5±0.33	22.5±0.66

PEF: petroleum ether extract of fruit, MEF: methanolic extract of fruit, Cipro: Ciprofloxacin

**Graph 1:** Antimicrobial activity (zone of inhibition) of fruit extracts of *N. arbortristis* Linn. at a concentration 50mg/ml.**Table 7:** Minimum inhibitory concentration of fruit extract of *N. arbor-tristis* Linn.

Microorganism	MIC ($\mu\text{g/ml}$)		
	PEF	MEF	Cipro.
<i>B. subtilis</i>	12.5	6.25	0.625
<i>E. coli</i>	6.25	3.12	0.312
<i>P. aeruginosa</i>	3.12	3.12	0.312

PEF: petroleum ether extract of fruit, MEF: methanolic extract of fruit, Cipro: Ciprofloxacin

4. Discussion

A maximum increase in length and diameter of fruit was observed with maturity and the colour also changes from green to golden yellow. The macroscopical characters of the fruit can serve as diagnostic parameters. The microscopical studies of the transverse section showed presence of two superior ovaries, two locule: each locule two ovules, two lobed stigmas, and capsule shaped fruit with straight embryo, with endosperm; radical curved upward or downward which is characteristic of the family Oleaceae. The presence of large oval lysigenous formed cavities as seen in the

transverse section of fruit were the distinguishing features and can be used as anatomical markers. The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent. In the present investigation water-soluble and alcohol soluble extractive values decreased with maturity of fruit. Preliminary phytochemical analysis indicated presence of phytosterol, triterpenoids, tannins, alkaloids and flavonoids. All extracts showed varying degrees of inhibition against all the bacterial stains. It showed that MIC for *Pseudomonas aeruginosa*

is found to be less followed by *Escherichia coli* as compared with other tested microorganisms. In general, methanolic extract of the fruit of *N. arbortristis* Linn. was exhibited considerable antibacterial activity.

5. Conclusion

In last four decades the scientists are keen to evaluate many plant drugs used in medicinal folk lore. It is due to their specific healing properties, healthy action and non-toxic effects. In this dimension pharmacognostic studies on *Nyctanthes arbor-tristis* L. fruit is a substantial step and it further requires a long term study to evaluate pharmacological action as well as therapeutic efficacy and toxicity of fruit to establish as the drug. The pharmacognostic study of the *Nyctanthes arbor-tristis* L. fruit has been carried out for the first time. This could also serve in the identification and preparation of a monograph on the plant.

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