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COMPARATIVE STUDIES ON ANTIOXIDANT ACTIVITY OF FLOWERS, LEAVES, BARK, FRUITS AND HEARTWOOD OF THE NYCTANTHES ARBOR- *TRISTIS* L.

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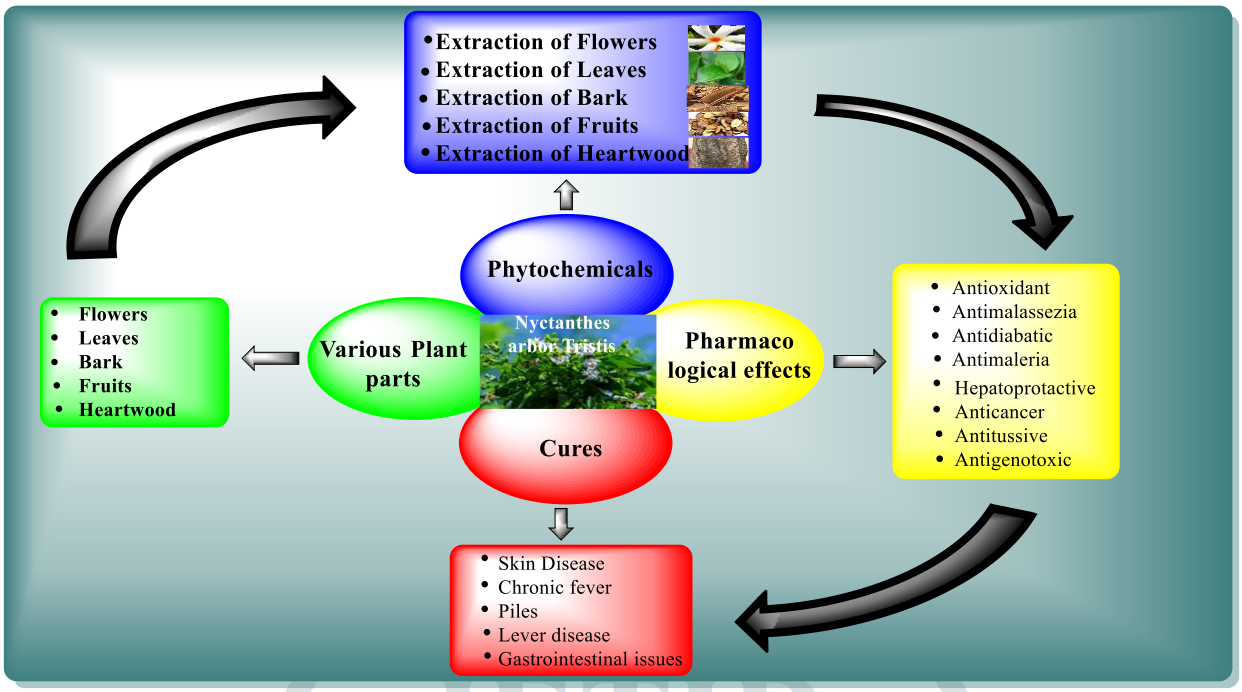
Abstract:
Antioxidants produced in high quantities by plants are pharmacologically powerful and have negligible to no negative effects when used therapeutically. The mythical plant Nyctanthes arbor- *tristis* L. (Oleaceae) has significant medicinal potential in Ayurveda and their extract to treat a variety of ailment, is one of the oldest medical systems. In Addition to being used as a laxative, for rheumatism, skin condition and as a sedative, it has a number of medicinal properties, including Antileishmaniasis, Antioxidant, Antifungal, Antipyretic, Antihistamic, Antimalerial, Antiinflammatory, Hepatoprotective and Antibecterial. The present study is focus on different parts like Flowers, Leaves, Bark, Fruits and Heartwood of Nyctanthes arbor- *tristis* L. plants were extracted in three different solvents such as ethanol, ethyl acetate and hexane and Antioxidant Activity were evaluated by in vitro assay DPPH Free radical scavenging method. Phytoconstituents likes phenolic and flavonoids content are present in Flowers, Leaves and Bark. The maximum Antioxidant activity was observed in ethanol, ethyl acetate extract of Flowers, Leaves and Bark as well as hexane extract of Bark, standard to Gallic acid. Thus, we observed that the ethanolic extract of Bark (96.37%), ethyl acetate extract of Bark (92.37%) and hexane extract of Bark (92.19%) exhibit higher antioxidant activity so we can firmly say that in all extracts of Bark shows greatest Antioxidant activity similarly ethanol extract of Leaves (95.82%) and flowers (96.55%) and ethyl acetate extract of Leaves (91.82%) and Flowers (95.46%) shows outstanding Antioxidant activity compared to all other sample. This is the novel finding that different extracts (ethanol, ethyl acetate and hexane) of Bark exhibited excellent Antioxidant activity.



Keywords: Antioxidant, Nyctanthes *arbor- tristis* L., DPPH* assay, Flowers, Leaves, Bark, Fruits and Heartwood extracts.

1. Introduction

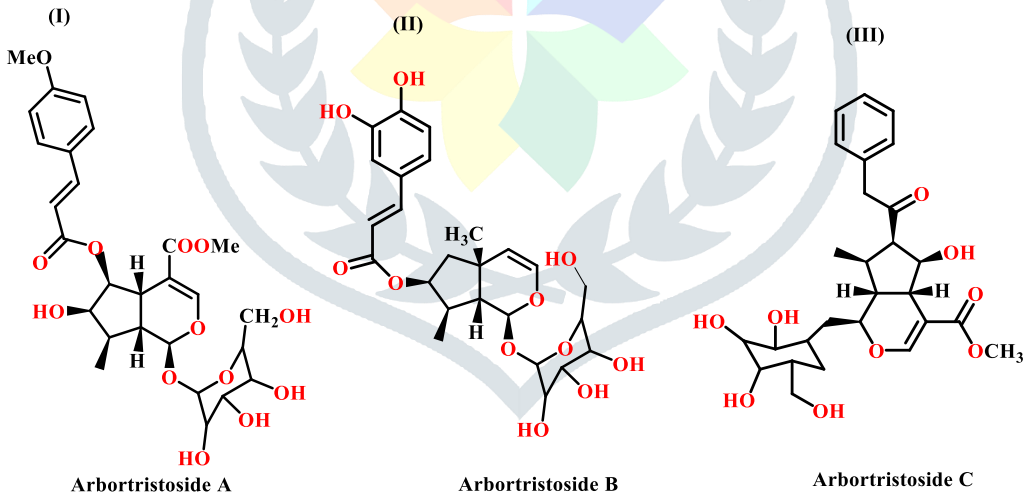
Free radicals and other reactive species produced by living cells are well known to play a significant role in the beginning of life and biological evolution. Lipid peroxidation can also be brought on by free radicals. Even though certain synthetic Antioxidants exist, such as butylated hydroxyanisole (BHA) and butylated hydroxy toluene (BHT), all of these have undesirable side effects. Most plant species have occasionally been looked into for their potential use as Antioxidants. Dietary Antioxidants with polyphenolic compounds, vitamins E and C, and carotenoids are thought to be potent anti-aging agents and aid in the prevention of illnesses linked to oxidative stress. Recently, attention in Antioxidant research has grown steadily (Pandey et al., 2014). An atom, ion or molecule with an unpaired electron in the outer orbit is referred to as a free radical. It becomes dangerous because, in its search for a matching electron, the free radical steals an electron from a neighboring stable molecule, turning it into a free radical in the chain reaction (Propagation step). As a result, the chain reaction can destroy tissues and impair their ability to function (Tendon et al., 2005). As by-products of biological reactions or as a result of foreign stimuli, reactive oxygen species (ROS), such as superoxide radicals, hydroxyl radicals, singlet oxygen, and hydrogen peroxide, are frequently produced (Cerutti., 1991). Some of these ROS have beneficial functions in vivo, including creation of physiologically significant chemicals, phagocytosis, control of cell development, and intercellular signaling (Halliwell., 1997). However, ROS can also contribute to the oxidation of lipids, which can damage membranes, reduce membrane fluidity, and even cause cancer by mutating DNA (Cerutti., 1994; Pietta., 2000). One potential defense against diseases caused by free radicals is a powerful scavenger of these ROS (Ames et al., 1995).

Nyctanthes arbor- *tristis* L. commonly known as night jasmine (Harsinghar), a well-known medicinal plant in India, is a member of Oleaceae family. It is one of the most adaptable therapeutic plants which commonly farmed in tropical and subtropical areas of the world and has a wide range of biological activity (Rani et al., 2012). In Ayurveda, different part of Nyctanthes arbor- *tristis* L. is used and has significant therapeutic potential for a number of formulations used to treat many diseases such as piles, gastrointestinal issues, insect venom, and Antirheumatic conditions. Asthma may be cured by the Fruit shell and Flowers (Selvakumar et al., 2017). It is a 10 m tall shrub or

small tree with flaking grey Bark. The Leaves are opposite, straightforward, 6–12 cm long, 2–6.5 cm wide, and have a complete edge (Saxena et al., 2002). The Seeds, Flowers, and Leaves have Antioxidant, Antileishmanial, Antiviral, Antifungal, Hepatoprotective and Immunostimulant properties (Puri et al., 1994). Also, the plant mentioned above has been investigated for Antimalerial (Das et al., 2021), Antiinflammatory, Amoebicious (Chitravanshi et al., 1992), Anthelmintic, Antitrypanosomal (Talakal et al., 2000) Antidepressant, Immunomodulatory, Anticancer, Antileishmania, Antiallergic and other activities.

The blooms can be used to make yellow clothes dye (Vankar et al., 2008). Pharmacological activity has been reported for crude extracts and their various fractions from Leaves, Bark, Flowers, Fruits, Root, Seeds and Oil. Numerous studies have been conducted on Nyctanthes arbor- *tristis* L. to investigate the pharmacological qualities of its Leaves, which have a variety of medicinal uses (Bhalakiya et al., 2019). The Leaves juice is used as a diuretic and to treat rheumatism, stubborn sciatica, chronic fever, piles, liver diseases, and loss of appetite (Banerjee et al., 2019). Scabies and other skin conditions are frequently treated with the inflorescence and Flowers because of their bitter flavor (Jain et al., 2005). The Seed powder is employed as an anthelmintic, a treatment for alopecia and for scalp scurvy (Chatterjee et al., 2007).

Chemical components found in Nyctanthes arbor- *tristis* L. include polysaccharides, iridoid glycosides, flavonol glycosides, astragalin, phenylpropanoid glycoside (NyctosideA), ß-sitosterol, ß-amyrin, hentri-acontane, benzoic acid, glycosides, nyctanthoside-a iridoid, nyctanthic acid, Arbortristosides-A (I) , Arbortristosides-B (II), Arbortristosides-C (III), Arbortristosides- D (IV) and Arbortristosides- E (V), Nyctanthoside (VI), Arborside C (VII) and Arborside D (VIII) as well as 6-ß-hydroxyloganin (IX) (Jansen et al., 2002) and 4-hydroxyhexahydrobenzofuran-7-one tertiary alkaloids, mostly 7- (alpha-anilino-p-nitrobenzyl) Additionally, quarternary protoberberine and aporphine alkaloids, including -8-quinolinol, have been identified from this plant (Kanan et al., 2010) that shown in **(Fig.1).**



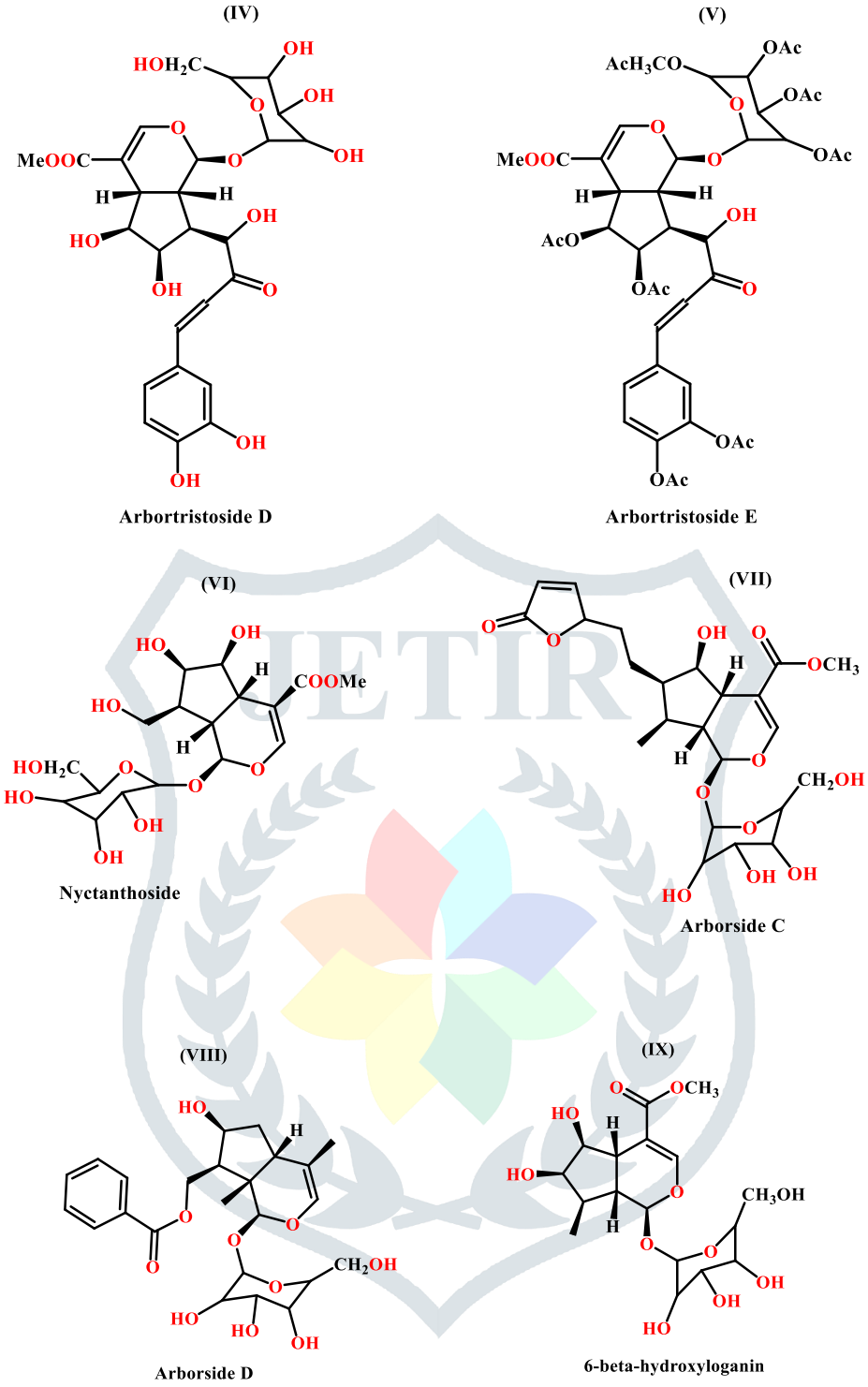


Fig.1. Phytoconstituents from *Nyctanthes arbor- tristis* L.

In recent year, the determination of In-vitro Antioxidant activity of Nyctanthes arbor- *tristis* L. ethanolic extracts of Flower and Leaves by Ferric chloride reducing ability test have investigated. Conclusively, all the reported ingredients show an increasing Antioxidant activity with increases the concentration (Saubhagya et al., 2020). Similarly, determination of total Antioxidant activity of Nyctanthes arbor- *tristis* L. extract was evaluated by phosphomolybdenum method, and also Antioxidant activity of Nyctanthas arbor- *tristis* L. of Flowers are determind by DPPH assay this study reveals that the Antioxidant activity of Nyctanthes arbor- *tristis* L. extract are increases with increasing the concentration of extract (Mary at al., 2021). Comparative Antioxidant study in different Flower extracts (ethanol, ethyl acetate and aqueous) of Nyctanthes arbor- *tristis* L. has been studies extensively. Conclusively, aqueous extract of Flowers has low free radical scavenging activity in comparison to ethanol and ethyl acetate extract of Flowers (Sasikumar et al., 2010). In this regard we are investigated comparative studies on

Antioxidant activity of different parts i.e.,Flowers, Leaves, Bark, Fruits and Heartwood of Nyctanthes arbor- *tristis* L. in ethanol, hexane and ethyl acetate extracts using standard procedures.

2. Materials and Methods

1, 1- diphenyl-2-picrylhydrazyl (DPPH*) was purchased from HiMedia Pvt. Ltd. Mumbai, India and Methanol AR was purchased from Loba Chemie Pvt. Ltd. Mumbai, India. The absorbance of all sample was recorded by UV-Visible spectrophotometer (Systronic, Model No. 119). The experiments were performed in the Department of Chemistry, CMP Degree College (Constituent College of University of Allahabad) during March 2022 to July 2022.

3. Collection and Authentication of Plant

Flowers, Leaves, Bark, Fruits and Heartwood of the plant of Nyctanthes arbor- *tritis* L. were collected from the Company Garden, Prayagraj, U.P., India, during the month of October. Taxonomically identified and verified by Botanical survey of India, Prayagraj and herbarium specimen was prepared and depicted in the department herbarium.

3.1. Preparation of extract

Initially, Flowers, Leaves, Bark, Fruits and Heartwood of the plant of Nyctanthes arbor-*tristis* L. were dried separately at room temperature for seven days and powdered them with grinder. The powder of the Flowers, Leaves, Bark, Fruits and Heartwood of the plant of Nyctanthes arbor-*tristis* L. (100 gm) separately were exhaustively extracted with 500 ml each of ethanol, hexane and ethyl acetate using soxhlet apparatus. Then, the extracts were centrifuged thrice and the supernatants were combined. The combined supernatants were filtered over Whatman No. 1 filter paper. The extracts were then evaporated to dryness by rotary flash evaporator (Buchi type Rotavapor). Different concentrations of extracts were prepared from the resultant crude ethanol, hexane and ethyl acetate extracts of Flowers, Leaves, Bark, Fruits and Heartwood of the plants to determine in vitro Antioxidant assays.

3.2. DPPH* (1,1- Diphenyl-2-picrylhydrazyl) radical scavenging activity

The DPPH* radical scavenging method was used to evaluate the Antioxidant capabilities. The ability of the various extracts to donate hydrogen or scavenge radicals was assessed using a stable radical called DPPH*. Gallic acid was used as standard. As a reagent for our spectrophotometric test, we are using the stable radical DPPH*. The process entails measuring the decline in DPPH* absorbance at its 517 nm absorption maximum. Concentration of DPPH* was 0.002 percent in methanol.

The stock solutions of the extracts were prepared in methanol (1.0 mg/10 ml). Different volume (2.0, 1.5, 1.0, 0.5, 0.25, and 0.125 ml) of extracts were taken in separate test tube and volume was made up to 2 ml with methanol. Now 2ml of DPPH* solution was added in each test tube and kept in dark for 30 minutes. The same procedure was followed for Gallic acid as well. Later optical absorbance was recorded at 517 nm using UV- Visible spectrophotometer.

DPPH* and Methanol was used as a control. All the samples were tested in triplicate. The formula used for the calculation is:

% Inhibition of DPPH* activity = (Ac - As / Ac) X 100

Where Ac = Absorbance of control, As = Absorbance of sample.

4. Results

The Antioxidant value of ethanol, hexane and ethyl acetate extracts of Flowers, Leaves, Bark, Fruits and Heartwood of the Nyctanthes arbor- *tristis L.*, were compared and evaluated by DPPH* assay (Table 1).

		% Antioxidant activity (by DPPH* assay)				
S.No.	Solvent/Extract	Flower	Leaves	Bark	Fruit	Heartwood
1.	Ethanol	96.55	95.82	96.37	52.87	17.60
2.	Ethyl acetate	95.46	91.83	92.37	70.23	31.21
3.	Hexane	56.26	84.74	92.19	84.39	26.86

Gallic acid(Stand.): 95.66%

The Flowers, Leaves, Bark, Fruits and Heartwood of Nyctanthes arbor- *tristis* L., exhibited Antioxidant potential of ethanol, ethyl acetate, hexane extract. The ethanol and ethyl acetate extract of Flowers exhibited with inhibition as 96.55%, 95.46% respectively which is approximately similar to Gallic acid (inhibition 95.66%). On the other hand hexane extract shows 56.26% Antioxidant activity as compared to Gallic acid (standard) shown in **(fig.2)**, similarly Leaves shows Antioxidant potential of ethanol, ethyl acetate, hexane extract with 95.82%, 91.83%, 84.74% respectively shown in **(fig.3)**, By moving forward we observed that Bark exhibited 96.37%, 92.37%, 92.19% Antioxidant potential of ethanol, ethyl acetate, hexane extract shown in **(fig.4)** further more Fruit shows ethyl acetate and hexane extract with 70.23%, 84.39% respectively, while ethanol extract shows 56.26% Antioxidant activity shown in **(fig.5)** and like as Heartwood exhibited ethanol, ethyl acetate, hexane extract with 17.60% 31.21%, 26.86 % respectively, Antioxidant potential shown in **(fig.6)**.

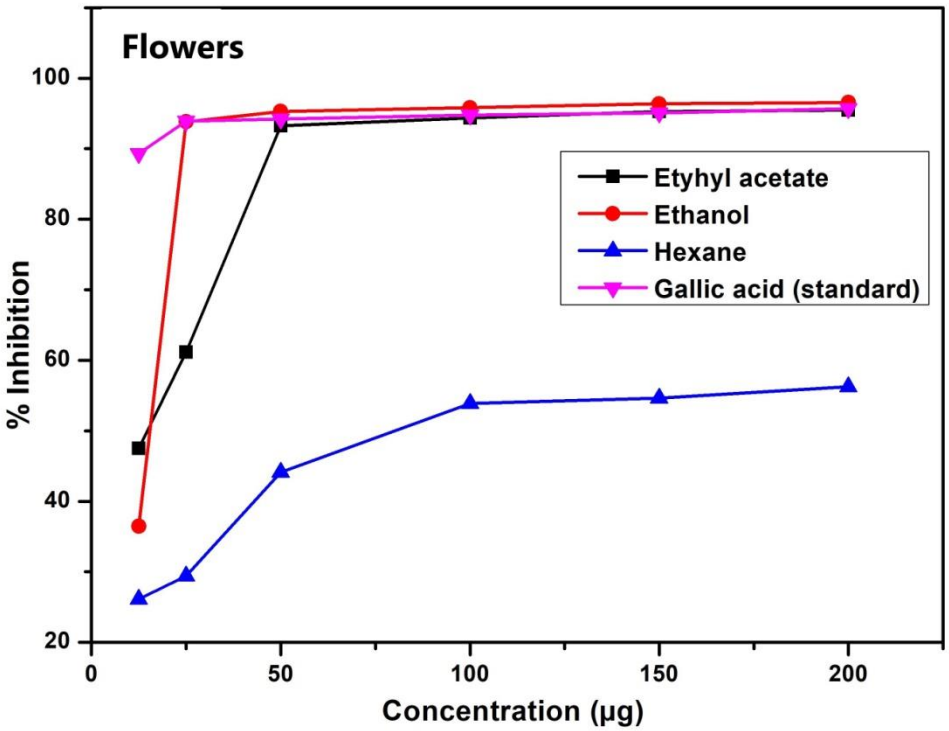


Fig.2. Ethyl acetate, Ethanol, Hexane extraction of Flower of Nyctanthes arbor- *tristis* L.

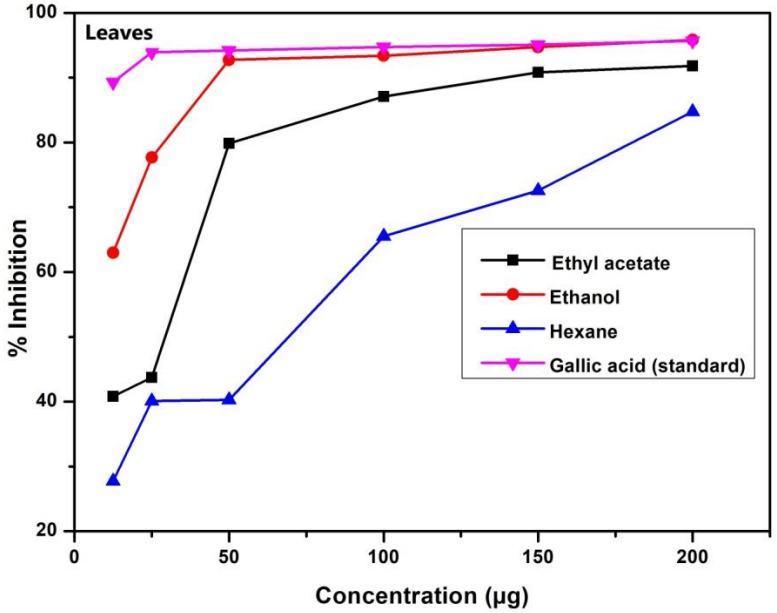


Fig. 3. Ethyl acetate, Ethanol, Hexane extraction of Leaves of Nyctanthes arbor- *tristis* L.

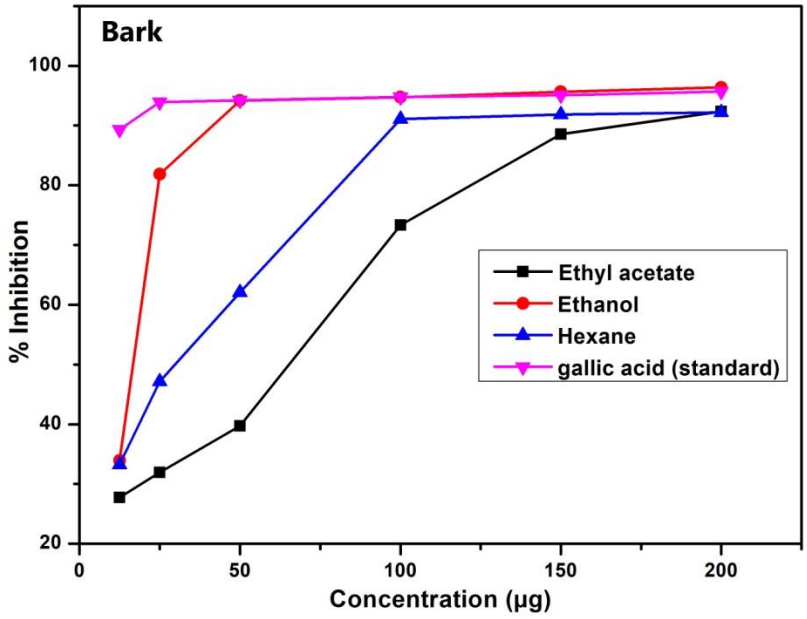


Fig. 4. Ethyl acetate, Ethanol, Hexane extraction of Bark of Nyctanthes arbor- *tristis* L.

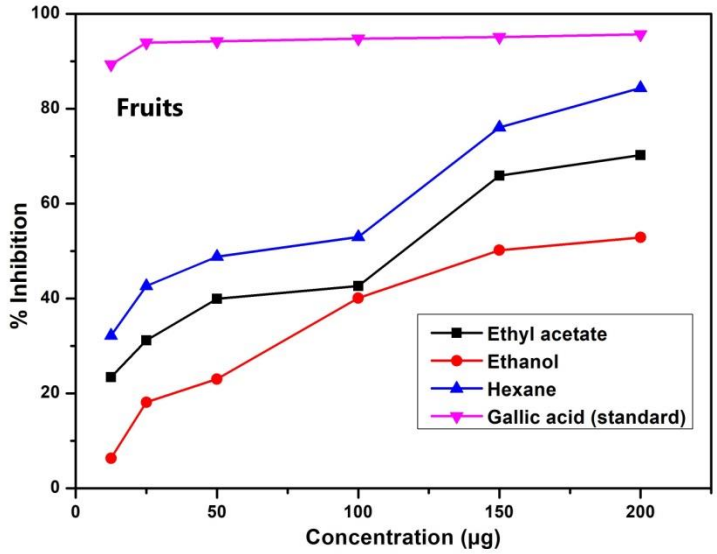


Fig. 5. Ethyl acetate, Ethanol, Hexane extraction of Fruits of Nyctanthes arbor- *tristis* L.

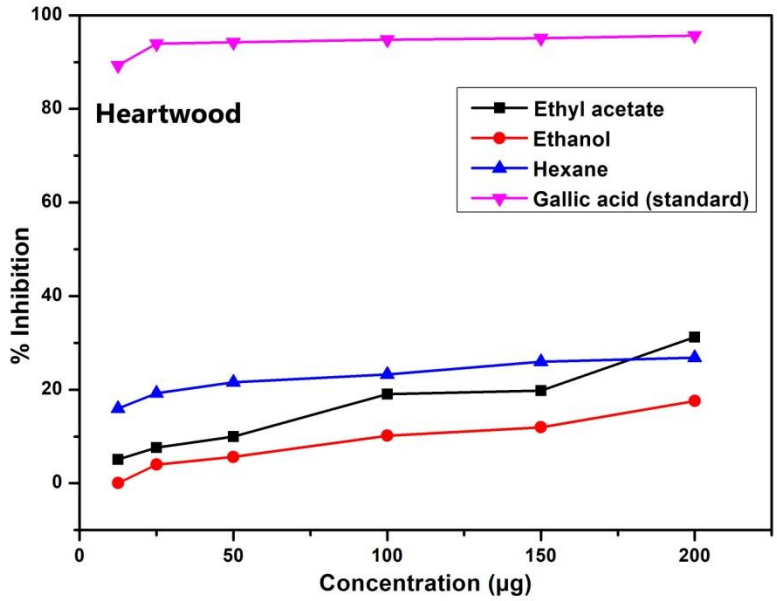


Fig. 6. Ethyl acetate, Ethanol, Hexane extraction of Heart wood of Nyctanthes arbor- *tristis* L.

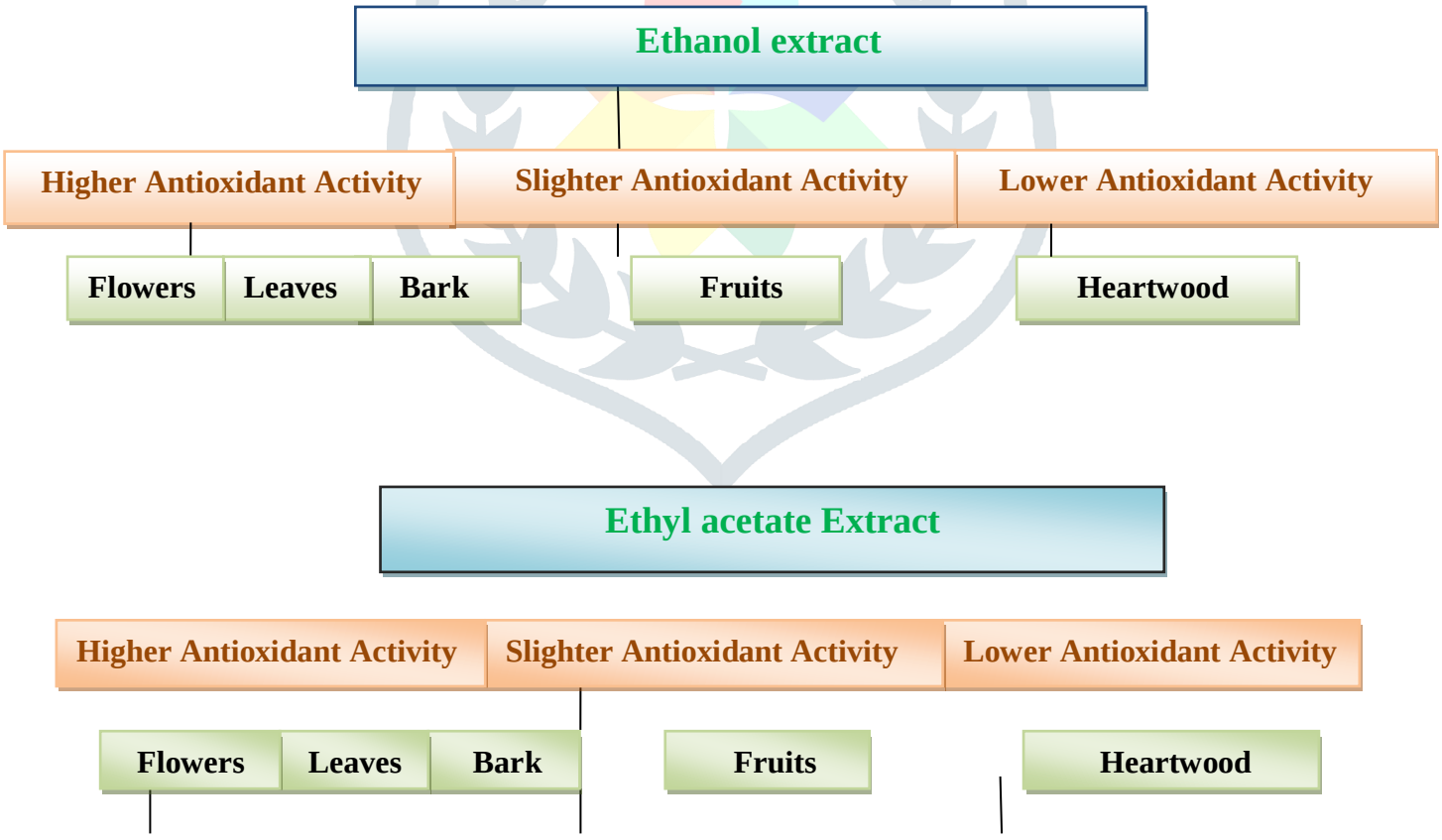
5. Discussion

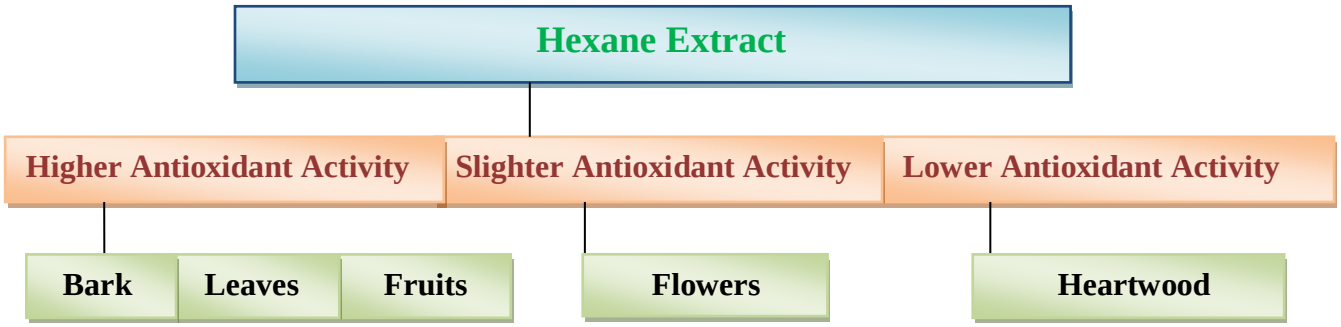
The ethanol extract of Flowers, Leaves and Bark showed higher Antioxidant activity with 96.55, 95.82, 96.37% inhibition in comparison to ethanol extract of Fruit and Heartwood with DPPH* % inhibition of 52.87 and 17.29%. It is very interesting that the ethanol extract of Leaves and Bark has higher Antioxidant potential as compare to standard (Gallic acid). Similarly, the ethyl acetate extract of Flowers, Leaves and Bark shows higher Antioxidant potential in comparison to ethyl acetate extract of Fruits and Heartwood but when we see that ethyl acetate extract

of Fruits and Heartwood, Fruits showed slightly more Antioxidant potential. When compared to Hexane extract of Flowers, Leaves, Bark then we see that Flowers shows slightly less Antioxidant potential in comparison to Leaves and Bark like as Hexane extract of Fruits and Heartwood, Fruits shows higher Antioxidant activity than the Heartwood in comparison to Gallic acid so the order of Hexane extract Antioxidant potential are: Bark> Leaves> Fruits> Flowers> Heartwood.

When we see that ethanol, ethyl acetate and hexane extract of Flowers then we observed that the ethanol extract of Flowers (96.55%) and ethyl acetate extract of Flowers (95.46%) shows higher Antioxidant potential than the hexane extract of Flowers (56.26%). In the case of ethanol, ethyl acetate and hexane extract of Leaves then we observed that ethanol extract of Leaves (95.82%) and ethyl acetate extract of Leaves (91.83%) shows higher Antioxidant potential than the hexane extract of Leaves (84.74%). Additionally in case of ethanol, ethyl acetate and hexane extract of Bark then we observed that the ethanol extract of Bark (96.37%), ethyl acetate extract of Bark (92.37%) and hexane extract of Bark (92.19%) exhibit higher Antioxidant activity so we can firmly say that in all extracts of Bark shows greatest Antioxidant activity. When we are looking forward towards ethanol, ethyl acetate and hexane extract of Fruits then we observed that the hexane extract of Fruits (84.39%) and ethyl acetate extract of Fruits (70.23%) shows good Antioxidant potential than ethanol extract of Fruits (52.87%). Similarly, ethanol, ethyl acetate and hexane extracts of Heartwood, we observed that the ethyl acetate extract of Heartwood (31.21%) and hexane extract of Heartwood (26.86%) exhibit acceptably Antioxidant potential than the ethanol extract of Heartwood (17.60%) so we can say that in all extract of Heartwood exhibited lower Antioxidant activity.

When compared the all parts of Nyctanthes arbor- *tristis* L. plant (i.e., Flowers, Leaves, Bark, Fruits and Heartwood) in different extracts, (i.e., ethanol, ethyl acetate, hexane extract) we observed that the order:





We can confidently state that the phenolic and flavanoids components present in the extracts' Antioxidant potential, because phenolic compounds are composed of one or more aromatic rings holding one or more hydroxyl groups, which have the capacity to quench free radicals, their structure is directly related to their Antioxidant effects.

6. Conclusion

Medicinal plants are regarded as abundant sources of components that can be employed in the manufacture of pharmaceuticals, whether they are pharmacopoeial, non-pharmacopoeial, or synthetic. A side from that, these plants is essential to the growth of human cultures all across the world. Additionally, some plants are advised for their medicinal benefits since they are regarded as vital sources of nutrition. The use of medicinal herbs is seen to be quite safe because there are rarely any negative side effects. The major benefit is that these treatments work in harmony with nature.The usage of herbal remedies can benefit people of all ages and genders, which is a key fact.

We observed that medicinal potential of the Nyctanthes arbor- *tristis* L. plant extracts chosen for Antioxidant activity has not been thoroughly investigated. The goal of the current study was to screen for phytochemicals present in various extracts of different part of plant Nyctanthes arbor-*tristis* L. i.e. Flowers, Leaves, Bark, Fruits and Heartwood extract and examine its Antioxidant potential.

Ethanol, ethyl acetate, hexane extracts of Flowers, Leaves and Fruits of Nyctanthes arbor- *tristis* L. plant has good Antioxidant activity. Except the ethanol, ethyl acetate and hexane extract of Heartwood. The most significant activity has been observed in the ethanol extract of Flowers, Leaves and Bark as well as ethyl acetate extracts of Flowers, Leaves and Bark and hexane extract of Bark as compared to all others samples. The newly investigated that in different extracts (Ethanol, ethyl acetate, hexane) of Bark shows outstanding Antioxidant activity. This research would be very helpful in treating various kinds of oxidative stress and disease.

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