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PRELIMINARY PHARMACOLOGICAL STUDIES ON *NYCTANTHES ARBOR-TRISTIS* L. FLOWER EXTRACT

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ABSTRACT

Nyctanthes arbor-tristis belongs to *Oleaceae* family, which has various medicinal properties. Different parts of the plant are utilized in traditional treatment to cure various diseases like sciatica, chronic fever, skin related diseases. In the current research, the methanol and aqueous extracts of *Nyctanthes arbor-tristis* flowers were evaluated for phytochemical analysis, antioxidant, antibacterial and anti-inflammatory activities. The preliminary phytochemical analysis in the methanolic and aqueous extracts showed the presence of compounds such as alkaloids, glycosides, phenols, flavonoids, terpenoids, and tannins. The methanolic extract showed maximum anti-inflammatory activity than the aqueous extract. The antibacterial activity assessed using the methanolic extract of *N. arbor-tristis* flower extract showed significant zone of inhibition.

Keywords: *Nyctanthes arbor-tristis,* phytochemicals, antioxidant, antibacterial, anti-inflammatory activity.

1. INTRODUCTION

The medicinal herbs provide essential novel bioactive molecules. Major drugs used in the field of medicine are obtained from various plants. The drugs used from the plants extracts are non-toxic comparing with the chemicals used in pharmaceutical industries. The plants are a reservoir of potential phytochemicals which serve as drugs **(Kiew, 1984**). Medicinal plants are the important source of bioactive compounds such as aromatic components, phenolics, terpenoids, essential oils, sterols, alkaloids, polysaccharides and tannins **(Mollica *et al.*, 2015).** Natural phytoconstituents plays a significant role in drug discovery and in the development of novel therapeutics in the pharmaceutical field **(Mercy *et al*., 2018).** Plants have the necessary metabolites such as carbohydrates, lipids, and nucleic acids, the secondary metabolites such as alkaloids, terpenoids, and phenolic compounds. Plants possess biological properties **(Ksouri *et al*., 2007)** such as anti-allergic, anti-atherogenic, anti-inflammatory and hepato-protective properties

In recent years much attention has been shown on investigating the antioxidant and antibacterial activities of medicinal plants. It is proven that the antioxidant properties of medicinal plant products are employed as drugs in various fields. (**Nandhakumar *et al.*, 2013**). Around 70% of the rural population depend on medicinal herbs for their health benefits, India is one of the largest producer of medicinal based

products and is rightly called the "Botanical garden of the World" **(Ratnaraju *et al.*, 2014).** The uses of traditional medicine have become a norm in developing countries **(Aruneekumar *et al.*, 1990)** as therapeutic agents for the maintenance of good health care among the society.

In India the native people in rural area have their own way of using plants for their health care and practice their own culture, **(Sankara Rao *et al.*, 2015)** customs, and food habits. This knowledge on medicine is transferred from one generation to another among indigenous people.

N. arbor-tristis belong to the family *Oleaceae*, also known as Harsinger or Night jasmine, it is a well studied ornamental plant with many medicinal properties. It is a native plant of India, prominently distributed in Himalayan region and is found in Indian garden as ornamental plant **(Chetty *et al.*, 2008**). The native people use the whole plant for curing cancer, root and leaf for treating fever, bark as expectorant, diabetes and as anti-helmintic. Various extracts of the plant is used to treat arthritis **(Mathuram *et al.*, 1991**). The juice of the *N. arbor-tristis* leaves are used as digestives, antidote to reptile venom's, laxative, mild bitter tonic, diaphoretic and diuretic (**Nadkarni, 1982**).

The leaves of *N. arbor-tristis* has many pharmacological properties, extensive work have been done on *N. arbor-tristis* for exploring their pharmacological properties. Traditionally the stem bark is applied in a form of paste for rheumatic joint pains **(Kirtikar *et al.*, 2000).** The major medicinal value **(Sharma, 2003)** is due to presence of phytochemical like nyctantic acid, friedelin, beta-sitosterol and oleanolic acid which are present in leaves and responsible for antiviral activity. The present study is aimed to study the pharmacological properties of *N. arbor-tristis* flower extract.

2. Materials and Methods

2.1 Plant Collection

The fresh flower of *N. arbor-tristis* were collected from Avadi, Thiruvallur district, Tamil Nadu during the months of July and August. The flowers were washed thoroughly and allowed to dry. The flowers after drying were powdered using electrical blender.

2.2 Extraction of Plant Material

Cold percolation technique was employed wherein the powdered sample was soaked in methanol and aqueous for about 48hours. The extract was then filtered using Whatmann's filter paper. The extract after filtration was concentrated using rotary evaporator. The obtained extract was stored in air tight bottles for further experiments.

2.3 Qualitative Phytochemical Analysis

The methanol and aqueous extracts were tested for the presence of secondary metabolites. Standard procedures **(Harborne, 1973)** were used for identifying the phytoconstituents.

2.3.1 Test for Alkaloids

Dragendroff's test

2ml of the extract was taken in a 25ml test tube and about 2ml of the dragendorff's reagent was added to the extract. A prominent yellow colour precipitate indicates the presence of alkaloids in the extract.

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2.3.2 Test for Carbohydrates

Fehling's test

1ml of the filtrate was taken in a test tube and left to boil in a water bath for a few minutes.1ml of Fehling's solution A & B was added to the boiled filtrate. A red precipitate shows the presence of carbohydrates.

2.3.3 Test for Glycosides

50µl of the extract was hydrolysed with a few drops of concentrated HCl and the mixture was boiled in a water bath for a few minutes and then filtered.

Borntrager's test:

2ml of the filtrate was then taken and 3ml of chloroform was added and shaken, the chloroform layer separates and 10% ammonia solution was added. Pink colour indicates presence of glycosides.

2.3.4 Test for Phenolic Compounds

Ferric chloride test:

To 50mg of the extract, 5ml of distilled water was added and a few drops of neutral 5% ferric chloride was added to the mixture. A dark greenish colour indicates presence of phenolic compounds.

2.3.5 Test for Flavonoids

5ml of the extract was taken in a test tube and 2ml of ammonia solution was added to the extract. The change in the mixture to yellow indicates the presence of flavonoids.

2.3.6 Test for Terpenoids

Salkowski test

5ml of the extract was taken in a test tube and 2ml of chloroform was added to the extract. To this mixture few drops of concentrated sulphuric acid was added. The formations of a reddish brown layer at the interface of the two liquids indicate the presence of terpenoids.

2.3.7 Test for Saponins

Foam test

To 1mg of the plant extract add 2ml of distilled water and shake the mixture well. The persistent froth on the mixture confirms the presence of saponins.

2.3.8 Test for Steroids

Liebermann-Burchard test

0.2ml of chloroform was added to the extract in a test tube and then a few drops of acetic acid were poured and concentrated sulphuric acid is added to the mixture. Appearance of a mixture of blue and green colour indicates the presence of steroids.

2.3.9 Test for tannins

Braemer's test

To 1ml of the extract 2ml of distilled water and then 2 drops of ferric chloride was added. The change in the colour of the solution from green to blue green indicates the presence of tannins.

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2.4 Quantitative phytochemical analysis

2.4.1 Determination of flavonoid

The aluminium chloride colorimetric assay was employed for quantifying flavonoids in the crude extracts of methanol. One ml of each extract and 4ml of distilled water were taken into a 10ml volumetric flask. To this flask, 0.3 ml of 5% sodium nitrite was added and 0.3ml of 10% aluminium chloride was mixed, after 5min. Thereafter, 2ml of 1M sodium hydroxide was treated after 5min. and the content was diluted to 10ml with distilled water. Quercetin was used as the standard. The absorbance readings were recorded for test **(Hanane *et al*., 2010)** and standard solutions against blank at 510nm in UV/Visible spectrophotometer. The total flavonoid content was expressed as mg of QE/g of extract.

2.4.2 Determination of Phenol

The Folin - Ciocalteu spectrophotometric method was used to determine the total phenolic content in methanolic and aqueous extracts of *N.arbortristis* flower. To a 25ml volumetric flask, 1ml of extract and 9ml of distilled water was taken. One ml of Folin Ciocalteu phenol reagent was added **(Ghasemzadeh *et al*., 2010; Milan, 2011)** to this mixture and shaken well. After 5 mins, 10ml of 7% sodium carbonate solution was added to the mixture. The volume of the same was made to 25ml with distilled water. Gallic acid was taken as standard. It was then incubated for 90 mins at room temperature and the absorbance values for test and standard solutions were noted against blank at 550 nm with a UV /Visible Spectrophotometer. Total phenol content was expressed as mg of GAE/gm of extract

2.5 Antioxidant Activity

2.5.1 DPPH Assay

The ability of the methanolic and aqueous extract of *N. arbor-tristis* flower to scavenge free radical was determined according to the method described by **(Mensor *et al*., 2001)**. Sample stock solutions (1.0 mg/ml) were diluted to final concentrations of 250, 125, 50, 25, 10 and 5µg/ml in methanol. 1 ml of a 0.3mM DPPH methanol solution was added to 2.5ml solution of the extract or standard and allowed to react at room temperature for 30 mins. The absorbance of the resulting mixture was measured at 518 nm and converted to percentage antioxidant activity (AA %) using the formula:

$$\mathbf{AA\% = 100 - [(Abs\ sample - Abs\ blank) \times 100]/Abs\ control}$$

Methanol (1.0ml) plus extract solution (2.5ml) was used as a blank. 1 ml of 0.3 mM DPPH plus methanol (2.5ml) was used as a negative control. Solution of ascorbic acid served as positive control.

2.5.2 ABTS assay

The ABTS assay was used to study the antioxidant capacity as described by **(Re *et al*., 1999)** with slight modification. 7mM ABTS and 2.45 mM of potassium persulphate as the oxidative agent were used as stock solution. The working solution was prepared by diluting the stock solution in 99.9% ethanol. 200µl of the methanolic and aqueous extract were mixed with 1800µl of ABTS solution separately. The absorbance

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was taken at 734nm at 30°C after 10mins after mixing of solution. The percentage of inhibition of the test sample and control was calculated using the following formula.

$$\% \text{ inhibition} = ((A\circ - A)/A\circ) \times 100$$

Where A_0 is the absorbance of blank and A is the absorbance of sample. The percentage inhibition of radical scavenging activity was expressed as Trolox equivalent antioxidant capacity TEAC (µmol Trolox /g sample).

2.6 Anti-inflammatory activity

The anti-inflammatory activity of methanolic and aqueous extracts of *N. arbor-tristis* flower were studied by using inhibition of albumin denaturation technique by (**Mizushima *et al*., 1968 and Sakat *et al*., 2010)** followed with minor modifications. The reaction mixture consisted of both the extracts of *N. arbor-tristis* and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted by adding small amount of 1N HCl. The sample extracts were incubated at 37ºC for 20 mins and then heated to 51ºC for 20 mins, after cooling the samples the turbidity was measured at 660 nm. The experiment was performed in triplicate. The Percentage inhibition of protein denaturation was calculated as follows

Percentage inhibition = (Abs Control –Abs Sample) X 100/ Abs control

2.7 Antibacterial activity

The bacterial strains were maintained on nutrient agar. The cultured bacteria were prepared by transferring **(Adnan *et al*., 2017)** a single colony into a fresh medium and grown overnight at 37◦C. Turbidity of the culture was adjusted with sterile saline solution

2.7.1 Agar well diffusion method

Antibacterial activity of methanolic extract of *N. arbor-tristis* flower was analyzed by agar well diffusion method against *E.coli*, *Klebsiella pneumoniae* and *Pseudomonas*. A well was made on the plate with the help of gel puncture and 50 µl of methanolic extract of *N. arbor-tristis* (20%, 40%, 60%, 80%, and 100%) were inoculated into the well and plates (**Adnan *et al*., 2018**) were incubated at 37˚C for 24 hours. On the next day, zone of inhibitions were observed. Gentamycin standard antibiotic was used as the positive control. The diameters of the inhibition zones were measured in millimeters

3 Results and Discussion

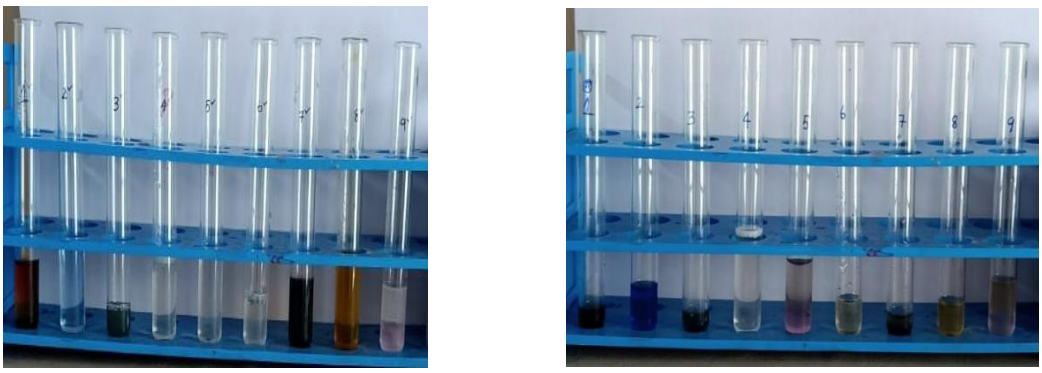

Fig. 1 Phytochemical analysis of methanolic and aqueous extract

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Phytoconstituents	Name of the test	Methanol	Aqueous
Alkaloids	Dragendroff's test	+	+
Carbohydrates	Fehling's test	-	-
Glycosides	Borntrager's test	-	+
Phenolic compounds	Ferric chloride	++	-
Flavonoid	Aluminium chloride test	++	-
Terpenoid	Salkowski test	+	-
Saponins	Foam test	-	-
Steroid	Liebermann-Burchard test	+	+
Tannin	Braemer's test	+	-

Table. 1 Qualitative phytochemical analysis of N. *arbor-tristis* flower extracts

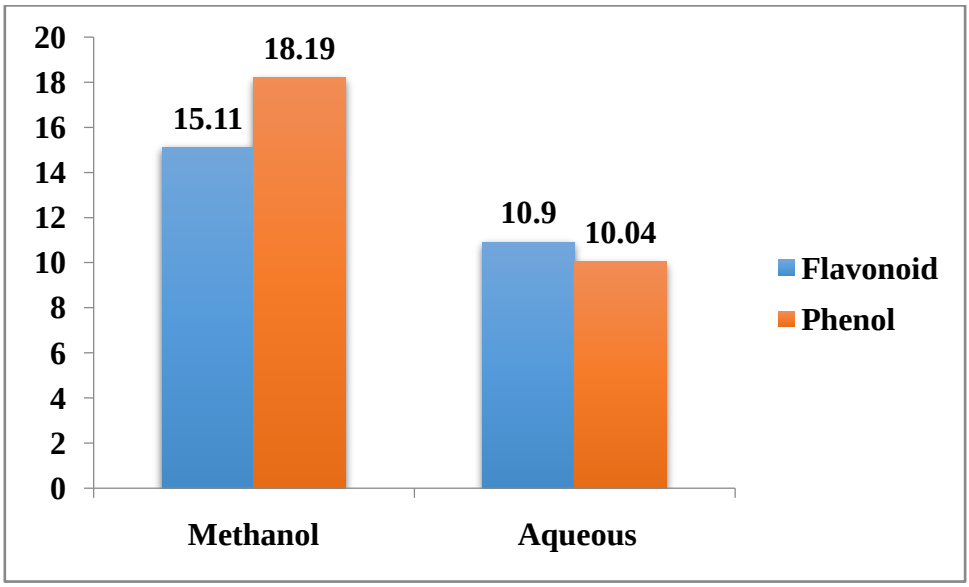

Fig.2 Quantitative phytochemical analysis of N. *arbor-tristis* flower extracts

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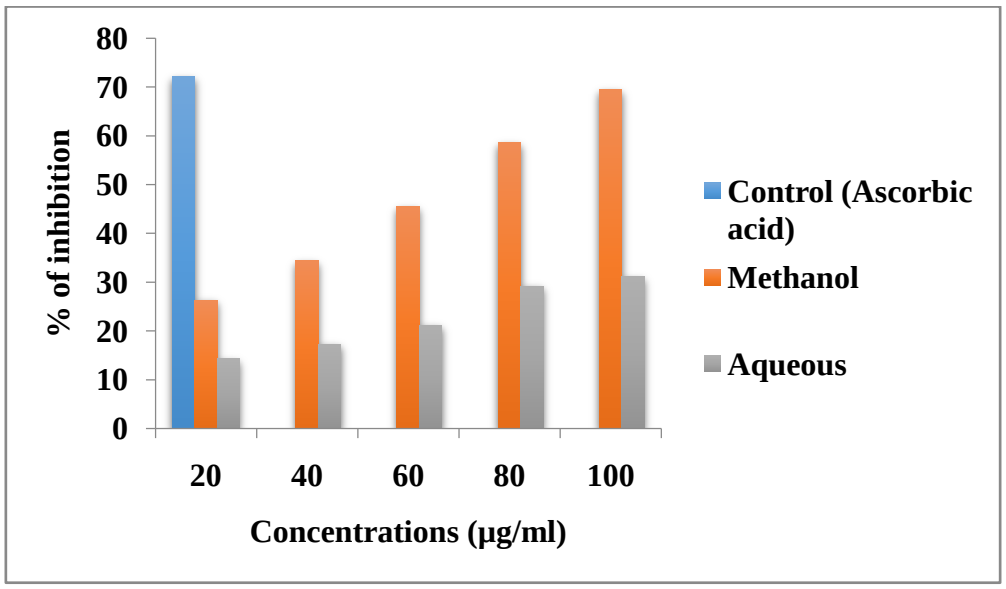

Fig.3 DPPH assay of *N. arbor-tristis* flower extracts

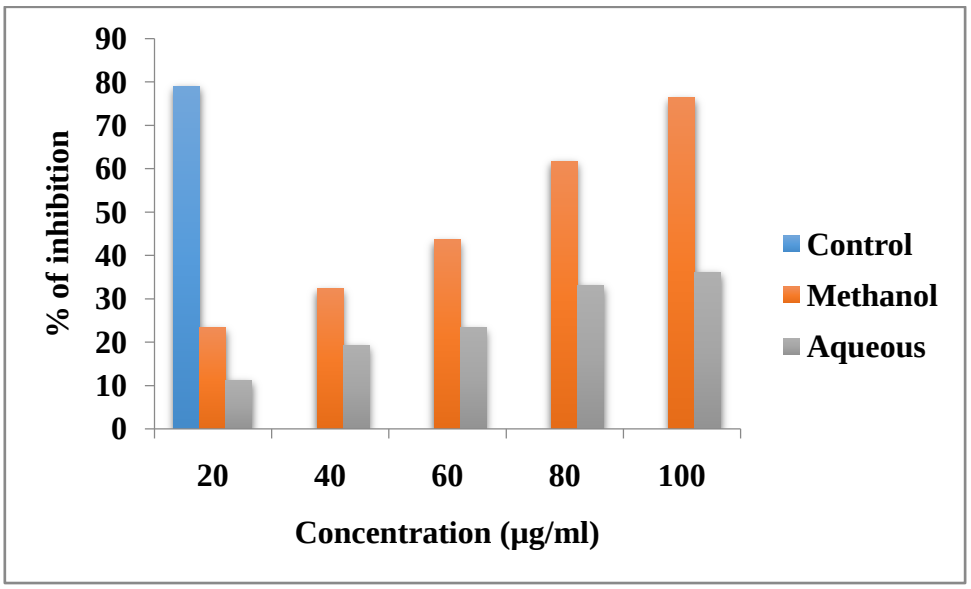

Fig.4 ABTS assay of *N. arbor-tristis* flower extracts

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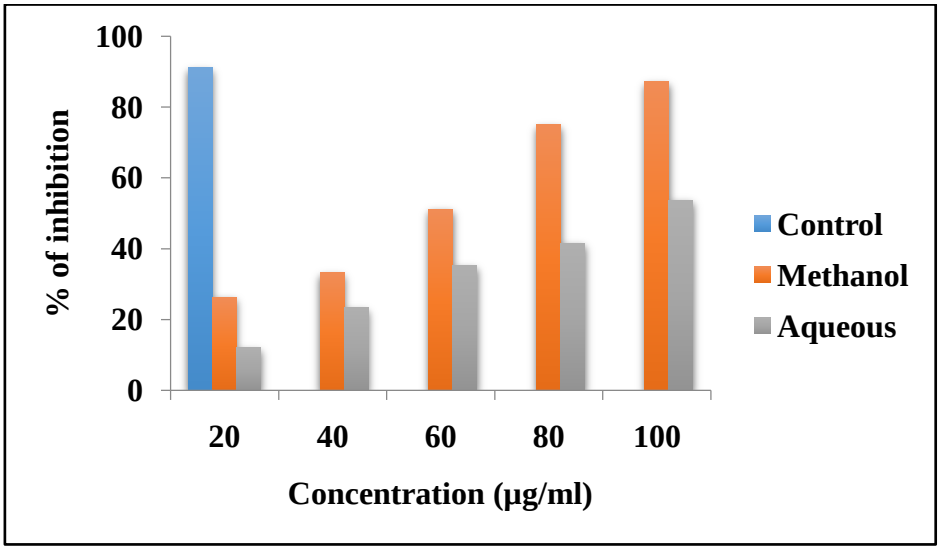

Fig.5 Anti-inflammatory activity of *N. arbor-tristis* flower extracts

Fig.6
Klebsiella pneumoniae

Fig.7
Pseudomonas

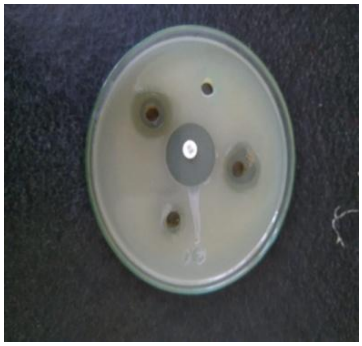

Fig.8
E. coli

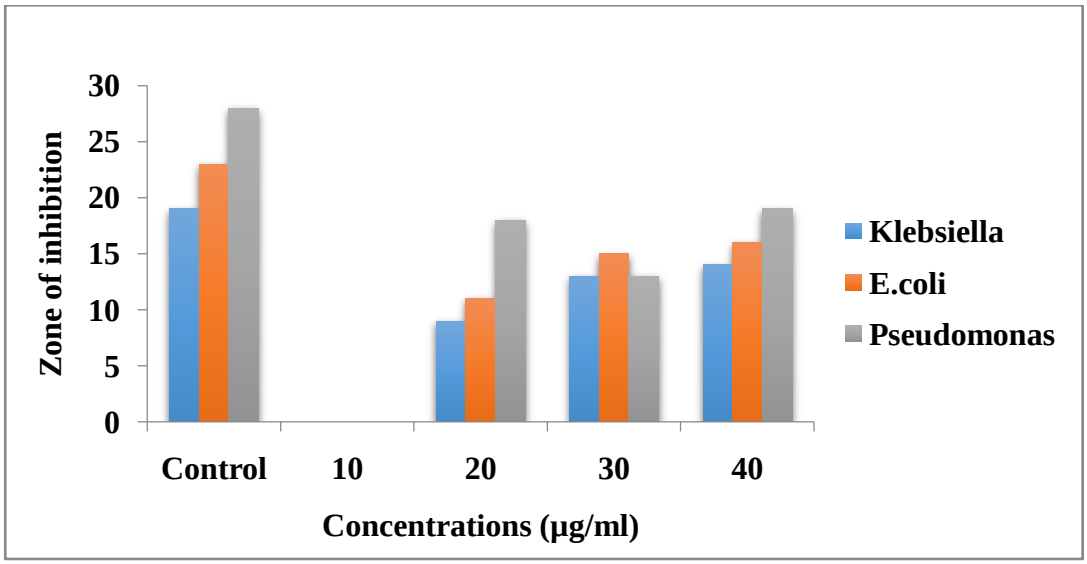

Fig.9 Antibacterial activity of *N. arbor-tristis* flower methanolic extract

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The plants are considered as natural resources because of innumerable pharmacological properties. In fact the pharmacological properties (**Patra, 2013)** can be attributed to the presence of several phytochemicals such as alkaloids, terpenoids, phenols, tannins etc. Carbohydrates, fats, proteins, minerals are the primary metabolites; the phytoconstituents (**Kumar and Pandey, 2013)** such as phenols, flavonoids are secondary metabolites that are derived from the primary metabolites. Literature studies have associated the presence of secondary metabolites to the biological potential of the plants

Screening for the presence of phytochemicals in methanol and aqueous extracts of *N. arbor-tristis* revealed the phytocompounds **(Fig.1)** such as phenols, terpenoids, and tannins alone in the methanolic extract **(Table 1)**. Whereas, alkaloids and steroids were present in both the extracts obtained through cold percolation technique. The quantification of flavonoids and phenols in the methanol and aqueous extracts were studied. The results of the present study revealed that methanolic extract possess high phenolic and flavonoid content **(Fig.2)**. The TFC in the methanolic extract was 15.11 ±0.13, QE /100gm while the TFC in the aqueous extract was 10.9±0.04 QE /100gm. The TPC of *N. arbor-tristis* in methanolic extract was found to be 18.19±0.13 GAE/100gm and 10.04±0.03 GAE/100gm in the aqueous extract. From the quantitative analysis of *N. arbor-tristis* TPC was higher when compared with the TFC.

The total yield of flavonoid and phenol content vary due to the different extraction technique and the solvent used for extraction (**Kannaian *et al.*, 2020).** A number of free radicals are produced during the metabolic process in the biological system. This will enable the radicals to cause various diseases **(Phaniendri *et al.*, 2015).** Therefore, antioxidant mechanism is necessary to protect the biological system from free radicals such as reactive oxygen species. Diseases like diabetes, cancer are directly or indirectly associated with reactive oxygen species **(Kibiti and Afolayan, 2015).** The results of the antioxidant potential revealed the inhibition in a dose dependant manner. The DPPH and ABTS assay showed maximum inhibition of free radicals in the methanolic extract whereas the aqueous extract showed very less inhibition.

In DPPH assay the methanolic extract exhibited the inhibition percent 69.42 at the concentration of 100µg/ml while aqueous extract showed only 31.14 percent inhibitions at the same concentration **(Fig.3)**. The ABTS assay of methanolic extract reported higher inhibition 76.31 at 100µg/ml concentration whereas aqueous extract reported only 36.14 percent inhibition **(Fig.4)**. In the present study the free radicals were predominantly scavenged by methanolic extracts. Previous studies also suggested the use of methanol extracts **(Sharma and Bhat, 2009)** enabled free radical scavenging ability of plants.

Anti-inflammatory activity

The *in vitro* anti-inflammatory assay of methanol and aqueous extract of *N. arbor-tristis* flower was carried out using egg albumin denaturation assay the results revealed that the activity was in a dose dependent manner **(Fig.5)**. The activity was determined based on the IC_{50} value. The IC_{50} value is the measure of extract required for 50% inhibition. When the IC_{50} value is lesser it denotes higher anti-inflammatory activity. The methanolic extract showed maximum anti-inflammatory activity than the aqueous extract. Inflammation is one of the focused area in the era of scientific research, as the synthetic anti-inflammatory drugs **(Gowrie *et al.*,**

2017) pose several adverse reactions. The results of anti-inflammatory provide a clear evidence for the use of *N. arbor-tristis* as a natural remedy for inflammation

Antibacterial activity

The methanolic extract of *N. arbor-tristis* flower was selected to study the antibacterial activity. The results of the antibacterial activity are presented in **(Fig.6 –Fig .8).** Three bacterial strains namely *Klebsiella pneumoniae*, *Pseudomonas* and *E. coli* were studied for antibacterial activity utilizing the methanolic extract of *N. arbor-tristis* flower. The results revealed that the methanolic extract of *N. arbor-tristis* were potentially effective in suppressing the growth of bacteria. Among the three bacterial strains the highest zone of inhibition was observed in *Pseudomonas* **(Fig.9)** followed by *E. coli* and *Klebsiella pneumoniae*.

Conclusion

The results of this study show that the methanolic extract of *N. arbor-tristis* flower contain secondary metabolites that are biologically active. The antioxidant activity of the extracts also clearly reveal that it has potential antioxidants. The anti-inflammatory activity showed that the methanolic extract was capable of inhibiting the process of denaturation of proteins than the aqueous extract. The antibacterial activity also exhibited significant zone of inhibition. Therefore, this study has revealed the potential benefits of the *N. arbor-tristis* flower.

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