ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (25-33), Month: July - September 2018, Available at: www.researchpublish.com

Preliminary Phytochemical Screening and Quantitative Analysis of crude extracts of *Nyctanthes arbor-tristis* Indigenous to South Gujarat region

[1]Smita Parekh, [2]Apurva Patel, [3*]Anjali Soni, [4]Preeti Sharma

Department of Biotechnology, Veer Narmad South Gujarat University, Surat 395007 Gujarat, India

Abstract: ***Nyctanthes arbor-tristis*** **is commonly known as Night Jasmine, Harshinghar and Parijat is an important traditional medicinal plant. Present study deals with preliminary phytochemical screening and quantification estimation of bioactive constituents like phenolics, flavonoids, alkaloids, glycosides, cardiac glycosides, tannins, terpenoids and proteins using standard methods. These are the constituents which produces definite physiological actions on human body. Medicinal value of the plant lies in presence of these chemical substances commonly known as secondary metabolites. Synthesis of these active ingredients is organ/stage specific depending on physiological condition of the plant, different environmental parameters such as temperature and rainfall. Crude ethanolic extracts from leaf, stem, flower and fruits of** ***Nyctanthes arbor-tristis*** **plant were prepared using Soxhlet method. Presence of major phytochemicals like Phenolics, Flavonoids and Alkaloids had been detected in all the extracts whereas quinones, anthraquinones and carboxylic acids were found to be absent in all plant parts extracts. Quantitative analysis showed that Flower and Stem extracts possess higher phenolics (40.63 and 28.02 mg of GAE/g DW), Flavonoids (362.62 and 120.43 mg of QE/g DW) and Alkaloids (87.08 and 41.29 mg of AE/g DW) compared to Leaf and Fruit extracts. From this data, it can be concluded that both Flower and Stem extracts could be a good source of important bioactive constituents possessing valuable therapeutic compounds.**

Keywords: ***Nyctanthes arbor-tristis,*** **Phytochemical screening, Phenolics, Flavonoids, Alkaloids.**

I. INTRODUCTION

Nyctanthes arbor-tritis commonly known as 'Harshringar' or 'Night Jasmine' belonging to family Oleaceae found as hardy large shrub as well as small tree widely distributed in Indo-Pak subcontinent and South East Asia. It is categorized as one of important sacred medicinal plant in India. Whole plant parts are medicinally important having biological properties such as antibacterial, antifungal, anthelmintic, anti-inflammatory, hepatoprotective, anti-pyretic, antifilarial, anti-leishmanicidal, sedative, immunomodulatory etc. [1]. Medicinal plants have potential to treat different human diseases owing to the presence of organic compounds which are termed as phytochemicals. These chemicals are diverse in nature and have definite physiological action on human body. Widely known categories of such compounds are alkaloids, tannins, terpenoids, flavanoids, saponins and phenolics. Leaves of *N. arbor* contain Flavanol glycosides-Astragaline, Nicotiflorin, β-sitosterole, D-mannitol, Nyctanthic acid, tannic acid ascorbic acid, an amorphous glycoside, volatile oil, carotene, glucose, fructose, iridoid glycosides etc.[2, 3, 4] used for arthritis, sciatica, fevers and various painful conditions and as a laxative. Flower of this plant contain abundant mannitol also contain flavonoids, anthocyanins, diterpenoid nyctanthin, tannin, glucose, glycosides, essential oil similar to jasmine nyctanthin [5] and are investigated for their antileishmanial, antiviral, antifungal, Diuretic and antioxidant activities [6]. Seeds contain pale yellow brown oil, nyctoside A, Arbortristoside A & B, stearic, palmitic and myristic acids, D-glucose, D-mannose reported for their hepatoprotective, antileishmanial, anti-inflmmatory, antiallergic and immunostimulant activities [1, 7]. Bark contains

glycosides, alkaloids, phytosterols, flavonoids, fixed oil etc. and also reported for its tradition usage such as expectorant, liver disorder, blood disorder, anorexia, piles, skin diseases, fever, snake bite etc. [8]. Stem contains β-sitosterol and also revealed for new glycoside i.e. Naringenin-4'-0-e-glucopyranosyl-Z-xylopyranoside [9]. Study of these bioactive compounds is important because of their potential therapeutic usage and low toxicity. It is seen that climatic conditions have impact on the distribution and composition of these phytochemicals in these medicinal plants. Diversity within phytochemicals occurs due to changing temperatures and wind patterns in turn affecting precipitation levels [10]. To the best of our knowledge no systematic study is being conducted on the study of diversity of phytochemicals in this plant of any specific region. The aim of present work is to conduct qualitative and quantitative estimation of major phytochemicals in the crude organic extracts of different parts of the plant from South Gujarat region of Gujarat State in India.

II. MATERIAL AND METHODS

A. Collection of plant material:

Various parts like leaves, stem, flowers and fruit from healthy *Nyctanthes arbor-tristis* plant were collected from Jolva village of Gujarat state, India (Lat-21.1719896; Long-73.0003919). Flowers were collected in the month of July-August. Fruits/seeds collected in the month of October. The plant was botanically authenticated by botanist Dr. Meenu Parabia (Rtd), Proffessor & Head, Department of Biosciences, Veer Narmad South Gujarat University, Surat, Gujarat, India. All freshly collected parts were washed thoroughly under tap water; shade dried at room temperature, powdered and kept in airtight containers for further use.

B. Preparation of plant extract:

To prepare plant extract 5 gm powder of each part was taken in 150 ml of ethanol and extracted using Soxhlet apparatus for 7-8 hours. All four extracts were filtered using Whatman no. 1 filter paper in order to remove all unextractable particles. The filtrates obtained were concentrated by evaporating at room temperature, weighed and redissolved in ethanol to have final concentration 1mg/ml for each extract. These extracts were kept in air tight glass bottles and stored at 4^{o}C till further use. The yield obtained for each extracts were shown in TABLE I.

C. Preliminary Qualitative Phytochemical Screening:

The extracts were assessed for preliminary phytochemical screening using the following standard methods [11-13].

Test for Flavonoids

a. Ammonia Test: To small amount of sample extract 1% NH_3 (diluted ammonia) solution was added followed by the addition of conc. H_2SO_4 or HCL and observe for the yellow coloration.

b. Lead acetate test: To small volume of sample extract few drops of $PbCl_2$ added and observe for the yellow precipitates.

Phenolic compounds

a. Folin's test: To small amount of sample few drops of Folin's reagent added and observe for the appearance of violet or brown color.

b. Ferric chloride test: To small volume of sample 3-4 drops of 5% $FeCl_3$ added and observe for the bluish black coloration.

c. Lead acetate test: To small volume of sample few drops of 10% lead acetate added and observe for the bulky white precipitates.

Alkaloids

a. Dragendorff's test: To small amount of sample few drops of dragendorff's reagent added and observe for the prominent yellow precipitates.

b. Mayer's Test: To small amount of sample few drops of 1%HCl added followed by addition of few drops of Mayer's reagent (potassium mercuric chloride solution). Observe for the formation of cream white precipitation or else turbid extract is observed.

c. Wagner's test: To small amount of sample Wagner's reagent (iodine in potassium iodide) was added and observed for the formation of reddish brown precipitates.

d. Hager's test: To small amount of sample small amount of saturated Aq. Solution of picric acid was added and observe for the Yellow color.

Glycosides

To small volume of sample extract few drops of conc. HCl added and was boiled for 4-5 hours. To this 1 ml of distilled water added followed by the addition of 10% NaOH. Observe for the formation of yellow color.

Cardiac glycosides

a. Keller Killani test: To small volume of sample extract conc. H_2SO_4 or Glacial acetic acid added followed by 1 drop of 5% $FeCl_3$ solution. Observe for the formation of various colored rings i.e. brown ring at the interface or bluish green ring just above the brown ring.

Saponins

a. Foam test: To small volume of sample 5 ml of distilled water added and mixed vigorously for 15 sec. and observe for persistent froth appearance.

Tannins

a. Ferric chloride test: To small amount of sample few drops of 5% ferric chloride solution was added and see for blue-black or blue-green color formation.

Terpenoids

Small volume of sample treated with chloroform and filtered. Filtrates were treated with conc. H_2SO_4, shaken vigorously and allowed to stand. Observed for the formation of reddish brown color at the lower layer which indicates presence of sterols and yellow color at the lower layer indicates the presence of terpenoids.

Carbohydrates

a. Molisch's test: To small amount of sample few drops of alpha napthol solution was added followed by the addition of conc. HCl or H_2SO_4 slowly. Observe the purple or violet ring.

Reducing Sugars

a. Benedict's test: To 1 ml of sample, 5 ml of Benedict's reagent added and boiled for 2 minutes and let it cool. Observe the formation of brick red precipitates.

Proteins or Amino acids

a. Biuret test: To small amount of sample, 4% NaOH added followed by the addition of 1% $CuSO_4$ and observe the violet or pink coloration.

b. Ninhydrin test: To small amount of sample few drops of Nynhydrin reagent added and boiled. Observe the blue or purple coloration.

Quinone Test

Small amounts of sample treated with conc. HCl and observe for the yellow colored precipitates or yellow coloration.

Anthraquinone test

a. Brontrager's test: Few ml of chloroform added to the few ml of sample extract. This was heated in water bath for 5 min. and filtered and allowed to cool. The filtrate was added with equal volume of 10% ammonia solution. This was shaken and the upper aqueous layer was observed for bright pink coloration.

Carboxylic acid test

To small amount of sample sodium hydrogen carbonate ($NaHCO_3$) was added to produce CO_2 gas which can be observed by the effervescence.

D. Quantitative Phytochemical Screening:

Total Phenolics Content

The total phenolics content was determined by the Folin Ciocalteu colorimetric method based on oxidation-reduction reaction described by Singleton et al., (1965) with slight modifications [14]. The calibration curve was plotted for 10, 20, 30, 40, 50 µg/ml Gallic acid solution (1mg/ml) prepared in methanol and 1 ml of sample extracts (1mg/ml) in test tube. 1ml of FC reagent added to each test tube. After 5 min incubation in dark condition 1 ml 10% Na_2CO_3 added and allowed to stand for 30 min at room temperature against blank (i.e. without sample extract). Absorbance was measure at 550 nm using spectrophotometer. The total phenolic content in all the extracts were calculated using following formula:

C = c V/M

Where, C= total phenolic content mg GAE/g dry extract, c = Concentration of gallic acid obtained from calibration curve in mg/ml (This unit would be depend on the standard assay performed), V= Volume of extract taken for experiment in ml, M= Dry weight of the extract in gram. Hence, final result can be expressed as mg of Gallic acid equivalents per gram of dry extract weight.

Total Flavonoid content

The determination of total flavonoid content was done by Aluminium trichloride method using Quercetin as standard compound using Zhishen *et al*., method with few modifications [15]. The calibration curve was plotted for 10, 20, 30, 40, 50 µg/ml Quercetin solution (1mg/ml) prepared and 1 ml of sample extracts (1mg/ml) in test tube. 0.1 ml of 1M potassium acetate added to each test tube. This mixture was allowed to stand for 6 min and 0.1 ml 10% $AlCl_3$ added in each test tube. Mix it well. After 5 min, 1 ml 1 M Sodium hydroxide added and finally 1.8 ml distilled water added to each test tube and mix it well. It was allowed to incubate the mixture for 30 min and absorption was taken at 415 nm against blank (i.e. without sample extract). Total flavonoid contents in all the extracts were calculated using following formula:

C = c V/M

Where, C= total flavonoid content mg QE/g dry extract, c = Concentration of quercetin obtained from calibration curve in mg/ml (This unit would be depend on the standard assay performed), V= Volume of extract taken for experiment in ml, M= Dry weight of the extract in gram. Hence, final result can be expressed as mg of quercetin equivalents per gram of dry extract weight.

Total Alkaloid Content

The estimation of total alkaloid content was done using method described by *Gonzales et al*. (2014) [16].The plant extract (50mg/ml) was dissolved in methanol in which 1 ml 2N HCl added. This mixture was transferred to a separating funnel. 5 ml bromocresol green (BCG) solution and 5 ml of phosphate buffer (pH 4.7) were added. This mixture was shaken with 2- 2 ml batch of chloroform, mixed well and allowed to stand for a while and separated chloroform layer was collected. This step was repeated till the clear chloroform layer has obtained. The calibration curve of standard solution of Atropine (2 – 10mg/ml) aliquots was prepared and same procedure followed as for plant extracts. Alkaloids dissolved in chloroform layer were detected taking O.D at 460 nm with UV/Visible spectrophotometer. The total alkaloid contents in all the extracts were calculated using following formula:

C = c V/M

Where, C= total alkaloid content mg AE (Atropine Equivalents)/g dry extract, c = Concentration of quercetin obtained from calibration curve in mg/ml (This unit would be depend on the standard assay performed), V= Volume of extract taken for experiment in ml, M= Dry weight of the extract in gram. Hence, final result can be expressed as mg of Atropine equivalents per gram of dry extract weight (12, 13).

III. RESULTS

In the present study plant parts were collected, dried, powdered. The dried powdered of plant parts were extracted with Soxhlet using ethanol and then subjected to qualitative and quantitative phytochemical screening.

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (25-33), Month: July - September 2018, Available at: www.researchpublish.com

A. Preliminary qualitative phytochemical analysis

The data given in TABLE II shows phytochemical screening of ethanolic extracts of different parts of *N. arbor*. These tests reveal the presence of medicinally important secondary metabolites. All the parts viz. leaf, stem, flower and fruit of *Nyctanthes arbor-tristis* contain phenolic compounds, flavonoids, alkaloids, glycosides, cardiac glycoside, tannin, proteins. Leaf and stem observed for carbohydrates and saponins presence whereas flower and fruit lacks for the same compounds. Reducing sugars only present in leaf whereas quinones, Anthraquinones and carboxylic acids completely gave negative test for all four parts. Steroids are absent in fruit extract whereas present in remaining all three extracts (TABLE II).

TABLE I: Yield of crude ethanolic extracts of *N. arbor*

Plant part extract	Yield ($mg \cdot ml^{-1}$)
Leaves	358
Stem	210
Flower	362
Fruit	250

TABLE II: Qualitative phytochemical analysis of *Nyctanthes arbor-tristis* Leaf, Stem, Flower and Fruit

Phytochemicals	Leaf	Stem	Flower	Fruit
Phenolic compounds	+	+	+	+
Flavonoids	+	+	+	+
Alkaloids	+	+	+	+
Glycosides	+	+	+	+
Cardiac Glycosides	+	+	+	+
Saponins	+	+	-	-
Tannins	+	+	+	+
Terpenoids	+	+	+	-
Carbohydrates	+	+	-	-
Reducing Sugars	+	-	-	-
Proteins or Amino acids	+	+	+	+
Quinones	-	-	-	-
Anthraquinones	-	-	-	-
Carboxylic acid	-	-	-	-

(+) Sign denotes presence and (–) sign denotes absence of phytochemicals.

B. Quantitative determination of the major secondary metabolites:

Total phenolics content

Total phenolic content of ethanolic extract of various part of *N. arbor* was ranging from 0.38±0.03 to 40.63±1.69 mg GAE/g extract (TABLE III). The calibration curve showed the linearity of Gallic acid in the range of 10 - 50$\mu g \cdot ml^{-1}$ with a correlation coefficient (R^2) of 0.9991. Flower and Stem extracts showed higher total phenolic content (40.63±1.69 and 28.02±2.82 mg GAE/g extracts respectively) than the other extracts.

Total flavonoids content

The calibration curve showed the linearity of Quercetin in the range of 20 - 100$\mu g \cdot ml^{-1}$ with a correlation coefficient (R^2) of 0.9922. The total flavonoid content of ethanolic extracts of flower and stem (362.62±2.36 and 120.43±2.76 mg QE/g extracts respectively) was higher whereas leaf and fruit of the plant reported for lower flavonoid content (102.62±1.95 and 28.87±2.66 mg QE/g extracts respectively) (TABLE III).

Total alkaloids content

The total alkaloid content of the various parts of *N. arbor* was determined to be ranged between 2.06±0.10 to 87.08±5.26 mg AE/g extracts (TABLE III). The calibration curve showed the linearity of Atropine in the range of 2 – 10 $mg \cdot ml^{-1}$ with a correlation coefficient (R^2) of 0.9932. Among the ethanolic extracts of various parts the stem and flower extracts were registered for higher alkaloids content 41.29±2.58 and 87.08±5.26 mg AE/g extracts respectively.

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (25-33), Month: July - September 2018, Available at: www.researchpublish.com

TABLE III: A comparative data of Total phenolic, Flavonoids and Alkaloid contents in *Nyctanthes arbor-tristis* different plant parts

Plant Part	Total Phenolic content $mg \cdot g^{-1}$ (GAE)	Total Flavonoids Content $mg \cdot g^{-1}$ (QE)	Total Alkaloid Content $mg \cdot g^{-1}$ (AE)
Leaf ethanolic Extract	16.73±0.83	102.62±1.95	2.06±0.10
Stem ethanolic Extract	28.02±2.82	120.43±2.76	41.29±2.58
Flower ethanolic Extract	40.63±1.69	362.62±2.36	87.08±5.26
Fruit ethanolic Extract	0.38±0.03	28.87±2.66	14.98±2.35

Data of total phenolic contents and total flavonoids contents and total alkaloid contents are expressed as mg of GAE/g of dry weight and QE/g of dry weight, mg of AE/g dry weight of the extracts respectively. All values expressed as mean of triplicates ± Standard Deviation (SD).

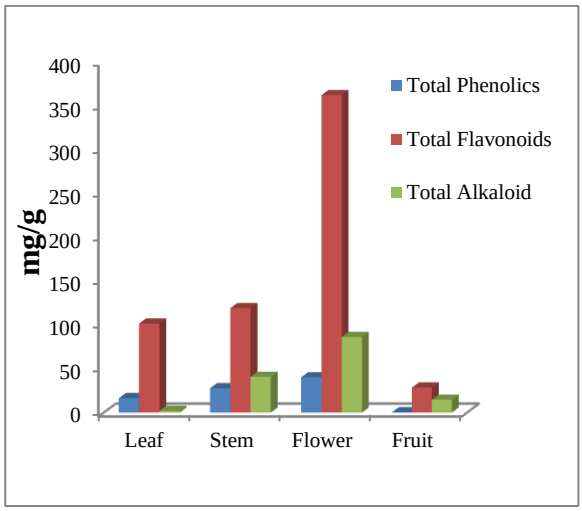

Fig 1: Comparative analysis of Total phenolics, Flavonoids, Alkaloids in different plant parts crude extract.

IV. DISCUSSION

Plants possess several medicinal properties like hypoglycemic, antidiabetic, antioxidant, antimicrobial, antiinflammatory, anticarcinogenic etc. due to the presence of different secondary metabolites. In the present study ethanolic extracts were prepared from four parts i.e. leaf, stem, flower and fruit by Soxhlet extraction method. Final yield was calculated and found to be highest in flower and lowest in stem extracts. Preliminary phytochemical screening of all four extracts was performed to know the presence of chemical constituents. Phytoconstituents and bioactivity of the plant are correlated to each other and presence and absence of these components may determine their application for different ailments. In all four extracts Phenolics, Flavonoids, Alkaloids, Glycosides especially Cardiac glycosides and Tannins were found to be present as obtained by other research group [17] while quinones, anthraquinones and carboxylic acid found to be absent in our study. Tannins have the ability to heal wounds and inflammatory mucous membrane, Flavonoids act as antioxidant as well as anticancer agents. Saponins were found to be present in leaf and stem extracts while Terpenoids (Phytosterols) show their presence in leaf, stem and flower extracts. Steroids and Saponins helps in reducing cholesterol and regulating immune responses and terpenoids known to possess several important activities like antimicrobial, antifungal, antiparasitic, antiviral etc [18]. Phenolics, glycosides and alkaloids have major therapeutic potential and found in almost every medicinal plant and *Nyctanthes* is no exception. Since the plant is rich in iridoid glycosides, a detailed study on iridoid glycoside of this plant was reported [19]. As the plant is lacking in quinones, anthraquinones and carboxylic acid class of phytochemicals, absence of therapeutic potential of these phytoconstituents is suggested. The results were in compliance with the work reported previously [20, 21]. Literature studies have revealed that presence or absence of specific phytochemical depends on environmental conditions of the plant. In our studies *Nyctanthes* growing in South Gujarat region was taken for present study. Different researcher groups from different states of India have studied *N. arbor* for its phytochemicals. Leaves of *N. arbor* were collected from Hubli, Karnataka state and screened for presence and absence of various phytochemicals such as alkaloids, steroids, carbohydrates, flavonoids, glycoside and proteins [21]. The leaves and stem parts of *N. arbor* observed for the presence of phenolics, alkaloids, saponins, flavonoids, reducing

sugars, tannins and Terpenoids from Dhapakhel region of Nepal [22]. Leaves of *N. arbor* from Madurai city of Tamilnadu has shown the absence of was Saponins and Terpenoids whereas our region plant possesses these phytochemicals [23]. Leaves, Flowers and fruits of *N. arbor* were studied by Priya and Deepak (2007) collected from Vallore district of Tamilnadu. They reported the absence of alkaloids in leaves of *N. arbor*, one of the major secondary metabolite but fruit of this plant possess phytosterols which is absent in our region plant [17]. Leaves and Stem parts of *N. arbor* procured from Jabalpur city of Madhya Pradesh and different extracts prepared in different solvent systems. All the extracts were found to be devoid of Saponins which are present in leaves and stem of our region plant [11] and similar absence of saponins in leaves of Guntur district of Andhra Pradesh state of India obtained with the research work of Yadav *et al.*,(2013) [13]. Fresh leaves of *N. arbor* screened for phytochemicals showed the absence of major secondary metabolites such as Phenols, Tannins and Glycosides, a study from Warangal region of Telangana state of India which is not the case with our regional study [19].

Quantification of major class of phytochemicals i.e. Phenolics, Flavonoids and alkaloids was performed using standard procedures. The total phenolic content in the all four extracts was determined by Folin-Ciocalteu method and expressed as mg of GAE/g of dry extract weight. It is well-known that polyphenols have ideal structural chemistry for free radicals scavenging activities and more potent antioxidants *in vitro* compared to vitamins E and C [24]. As revealed by the data, total phenolics content of Flower and Stem (40.63 and 28.02 mg of GAE/g DW extract) was significantly higher than the Leaf and Fruit (16.73 and 0.38 GAE/g DW extract) (TABLE III). Overall, the order of the phenolic contents of the test extracts was Flower (FE)>Stem (SE)>Leaf (LE)> Fruit (FrE) (Fig. 1). Fresh leaves of *N. arbor* collected from panchgini (Maharashtra), India was found to possess strong reducing power, high phenolics (330 mg GAE/g) and flavonoids (368.7 mg ECE/g) contents responsible for its antioxidant activity [25].Total phenolic content of leaf methanolic extract (78.48 ± 4.26 mg TAE/g) was reported by Michael et al. (2013) [26]. The leaves of *N. arbor* collected from Tirunelveli district of Tamilnadu. They studied antioxidant activities of leaves methanolic extract and quantified its phenolic content (78.48±4.2 mg TAE/g) [22]. In our case phenolics content was found to be highest in flowers whereas study of other groups has found high phenolics content in their leaves.

Flavonoids show antioxidant potential and have importance in human nutrition and health. These health benefits of flavonoids are because of their scavenging or chelating mechanism of action [27]. In the present study, total flavonoids content of Flower and Stem (362.62 and 120.43 mg of QE/g DW extract) were quite higher than Leaf and Fruit (102.62 and 28.87 mg of QE/g dry weight of extract) determined by Aluminium trichloride method. The order of the total flavonoid contents of the plant extracts was FE>SE>LE>FrE (Fig. 1). Previous study of Nagavani *et al.*, (2010) reported the total phenolic content of *N. arbor* flowers ranged from 4.725±0.88 to 0.5±0.012 mg GAE/gm and total flavonoid content ranged from 0.75±0.24 to 0.05±0.01% catechin Eq/gm which was comparatively demonstrated for lower phenolics and flavonoids contents than our studies observations [28]. *N. arbor* leaf ethyl acetate extract further subfractioned with acetone showing highest amount of phenolics (~ 330 mg GAE/g and flavonoids (368.7 ± 0.5 mg ECE/g) reported by Rathee *et al.* (2007) [25] which may account for its antioxidant potential. Recently Mishra *et al.* (2016) has studied flower for its antioxidant potential and quantified major secondary metabolites. They collected flowers from Varanasi region of India. Total phenolic content was higher in aqueous extract was 177±0.17 $\mu g \cdot mg^{-1}$ GAE and total flavonoid content was higher in ethanolic flower extract was 29.25±0.13 $\mu g \cdot mg^{-1}$ rutin equivalent. They correlate these phenolic and flavonoid contents with the obtained antioxidant activities from ethanolic extracts of flowers [29]. *N. arbor* of our region was found to possess highest flavonoid content in all the parts compared to other region India. One more observation from our study was to have higher flavonoids content than the phenolics content in each extracts.

Alkaloids represent the large group of secondary metabolites [30]. They are well known for their anticancer, antimalarial, antibacterial and analgesic properties [31]. Total alkaloid content of Flower and Stem extracts (87.08 and 41.29 mg of AE/g dry weight of extract) was higher than Leaf and Fruit (2.06 and 14.98 mg of AE/g dry weight of extract) (Fig. 1). The order of the total alkaloid content of the different plant extracts was found to be FE>SE>FrE>LE. To our knowledge, no study was found on total alkaloids estimation of N. arbor of any region.All these data for phytochemical investigations and quantification of major secondary phytochemicals suggest that phytochemicals compositions differ as the environmental conditions changes. Overall, from quantitative analysis it can be said that the order of total phenolic, flavonoid and alkaloid contents of the all four samples was Flower > Stem > Leaf > Fruit (Fig. 1). These three secondary metabolites are the important classes of all the known such metabolites. Phenolics, Flavonoids and Alkaloids were already known for their antioxidant and anticancer properties [32-34].

V. CONCLUSION

From the result of present studies, it can be conclude that all four parts of *Nyctanthes arbor-tristis* were showing presence of secondary metabolites. The qualitative test of phytochemicals revealed the presence of major secondary metabolites viz. Phenolics, Flavonoids, Alkaloids, Glycosides, Tannin, Terpenois and Proteins. Quantification of important phytochemicals like phenolics, flavonoids and alkaloids was performed on different extracts of plant parts. The amount of Phenolics, Flavonoids and Alkaloids were higher in Flower and Stem extracts. Hence, it was found that this study would be useful in deciding of which plant extract would be suitable for further planned antioxidant and cytotoxicity studies.

REFERENCES

[1] Sah, A. K., & Verma, V. K. (2012). Phytochemicals and pharmacological potential of *Nyctanthes arbor-tristis*: A comprehensive review. *Int J Res Pharm Biomed Sci*, *3*(1), 420-427.

[2] Talakal, T. S., Dwivedi, S. K., & Shamra, S. R. (2000). In vitro and in vivo antitrypanosomal potential of *Nyctanthes arbor-tristis* leaves. *Pharmaceutical biology*, *38*(5), 326-329.

[3] Paul, B. N., & Saxena, A. K. (1997). Depletion of tumor necrosis factor-α in mice by *Nyctanthes arbor-tristis*. *Journal of ethnopharmacology*, *56*(2), 153-158.

[4] Saxena, R. S., Gupta, B., & Lata, S. (2002). Tranquilizing, antihistaminic and purgative activity of *Nyctanthes arbor tristis* leaf extract. *Journal of ethnopharmacology*, *81*(3), 321-325.

[5] Khatune, N. A., Islam, M. E., Rahman, M. A. A., Mosaddik, M. A., & Haque, M. E. (2003). In-vivo cytotoxic evaluation of new benzofuran derivative isolated from *Nyctanthes arbor-tristis* L. on Ehrlich Ascite Carcinoma cells (EAC) in mice. *J Med Sci*, *3*(2), 169-173.

[6] Ratnasooriya, W. D., Jayakody, J. R. A. C., Hettiarachchi, A. D. I., & Dharmasiri, M. G. (2005). Sedative Effects of Hot Flower Infusion of *Nyctanthes arbo-tristis*. on Rats. *Pharmaceutical biology*, *43*(2), 140-146.

[7] Agrawal, J., & Pal, A. (2013). *Nyctanthes arbor-tristis* Linn—A critical ethnopharmacological review. *Journal of ethnopharmacology*, *146*(3), 645-658.

[8] Suresh, V., & Arunachalam, G. (2012). Pharmacognostical and preliminary phytochemical studies of bark of *Nyctanthes arbor–tristis* linn. *Int J Pharm Sci*, *4*(Suppl 1), 356-363.

[9] Rani, C., Chawla, S., Mangal, M., Mangal, A. K., Kajla, S., & Dhawan, A. K. (2012). *Nyctanthes arbor-tristis* Linn.(Night Jasmine): A sacred ornamental plant with immense medicinal potentials.

[10] Kumar, S., Yadav, A., Yadav, M., & Yadav, J. P. (2017). Effect of climate change on phytochemical diversity, total phenolic content and in vitro antioxidant activity of Aloe vera (L.) Burm. f. *BMC research notes*, *10*(1), 60.

[11] Bansal, S., Bharati, A. J., & Bansal, Y. K. (2013). In vitro Callogenesis and Phytochemical Screening of Harsingar (*Nyctanthes arbor-tristis*) a Multipotent Medicinal Tree. *Int. J. PharmTech Res*, *5*(4), 1786-1793.

[12] Paikara, D., Singh, S., & Pandey, B. (2015). Phytochemical Analysis of leave extract of *Nyctanthes arbortristis*. *J Environmental Science, Toxicology and Food Tech*, *1*(3), 39-42.

[13] Yadav, C. S. P. D., Bharadwaj, N. S. P., Yedukondalu, M., Methushala, C. H., & Kumar, R. (2013). Phytochemical evaluation of *Nyctanthes arbor tristis*, *Nerium oleander* and *Catharathnus roseus*. *Indian Journal of Research in Pharmacy and Biotechnology*, *1*(3), 333.

[14] Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture*, *16*(3), 144-158.

[15] Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food chemistry*, *64*(4), 555-559.

[16] Gonzales, M. V. M., & Tolentino, A. G. (2014). Extraction And Isolation Of The Alkaloids From The *Samanea Saman* (Acacia) Bark: Its Antiseptic Potential. *International Journal of Scientific and Technology Research*, *3*(1).

[17] Priya, K., & Ganjewala, D. (2007). Antibacterial activities and phytochemical analysis of different plant parts of *Nyctanthes arbor-tristis* (Linn.). *Research Journal of Phytochemistry*, *1*(2), 61-67.

[18] Manisha, V., Neha, S., & Satish, S. (2009). Antimicrobial activity of stem bark extracts of *Nyctanthes arbortristis* Linn.(Oleaceae). *International Journal of Pharmacognosy and Phytochemical Research*, *1*(1), 12-14.

[19] Kumari, T. S., & Charya, M. S. (2017). PHYTOCHEMISTRY, ANTI-CANCER AND ANTI-INFLAMMATORY ACTIVITIES OF SOLVENT LEAF EXTRACTS OF *NYCTANTHES ARBOR-TRISTIS*. *International Journal of Pharmaceutical Sciences and Research*, *8*(4), 1654.

[20] Paikara, D., Singh, S., & Pandey, B. (2015). Phytochemical Analysis of leave extract of *Nyctanthes arbortristis*. *J Environmental Science, Toxicology and Food Tech*, *1*(3), 39-42.

[21] Akki, K. S., Krishnamurthy, G., & Bhojanaik, H. S. (2009). Phytochemical investigations and in vitro evaluation of *Nyctanthes arbor-tristis* leaf extracts for antioxidant property. *Journal of Pharmacy Research Vol*, *2*(4).

[22] Chidi, B. B., Pandeya, S., Gharti, K. P., & Bharati, L. (1993). PHYTOCHEMICAL SCREENING AND CYTOTOXIC ACTIVITY OF *NYCTANTHES ARBOR-TRISTIS*.

[23] Sathiya, M., Parimala, P., & Muthuchelian, K. (2008). Preliminary Phytochemical Screening and Antibacterial Studies on the Ethanolic Leaf Extract of *Nyctanthes arbortristis* Linn. *Ethnobotanical Leaflets*, *2008*(1), 40.

[24] Rice-Evans, C., Miller, N., & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in plant science*, *2*(4), 152-159.

[25] Rathee, J. S., Hassarajani, S. A., & Chattopadhyay, S. (2007). Antioxidant activity of *Nyctanthes arbor-tristis* leaf extract. *Food Chemistry*, *103*(4), 1350-1357.

[26] Michael, J. S., Kalirajan, A., Padmalatha, C., & Singh, A. R. (2013). In vitro antioxidant evaluation and total phenolics of methanolic leaf extracts of *Nyctanthes arbor-tristis* L. *Chinese journal of natural medicines*, *11*(5), 484-487.

[27] Pourmorad, F., Hosseinimehr, S. J., & Shahabimajd, N. (2006). Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *African journal of biotechnology*, *5*(11).

[28] Nagavani, V., Raghava, R. K. V., Ravi, K. C., & Raghava, R. T. (2010). In vitro screening of *Nyctanthes arbortristis* flowers for antioxidant activity and identification of polyphenols by RP-HPLC. *Pharmacologyonline*, *2*, 57-78.

[29] Mishra, A. K., Upadhyay, R., Chaurasia, J. K., & Tiwari, K. N. (2016). Comparative antioxidant study in different flower extracts of *Nyctanthes arbor-tristis* (L.)(Oleaceae): an important medicinal plant. *Brazilian Journal of Botany*, *39*(3), 813-820.

[30] Ibraheem, O., & Maimako, R. F. (2014). Evaluation of alkaloids and cardiac glycosides contents of *Ricinus communis* Linn.(Castor) whole plant parts and determination of their biological properties. *Int. J. Toxicol. Pharmacol. Res*, *6*(3), 34-42.

[31] Ezeonu, C. S., & Ejikeme, C. M. (2016). Qualitative and Quantitative Determination of Phytochemical Contents of Indigenous Nigerian Softwoods. *New Journal of Science*, *2016*.

[32] Carocho, M., & CFR Ferreira, I. (2013). The role of phenolic compounds in the fight against cancer–a review. *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents)*, *13*(8), 1236-1258.

[33] Galati, G., & O'brien, P. J. (2004). Potential toxicity of flavonoids and other dietary phenolics: significance for their chemopreventive and anticancer properties. *Free Radical Biology and Medicine*, *37*(3), 287-303.

[34] Habli, Z., Toumieh, G., Fatfat, M., Rahal, O. N., & Gali-Muhtasib, H. (2017). Emerging cytotoxic alkaloids in the battle against cancer: overview of molecular mechanisms. *Molecules*, *22*(2), 250.