International Journal of Early Childhood Special Education (INT-JECSE)
DOI:10.9756/INTJECSE/V14I5.8 ISSN: 1308-5581 Vol 14, Issue 05 2022

PHYTOCHEMICAL, IN-VITRO ANTIOXIDANT AND IN-VITRO CYTOTOXICITY ANALYSIS OF *NYCTANTHES ARBOR-TRISTIS* ETHYL ACETATE EXTRACT

Selvakumar S, Tina S. Biju, Nisha R, Malarvizhi R, Senthil J.

Department of Biotechnology, Bharath Institute of Higher Education and Research, Chennai, Tamil Nadu, India.

Department of Microbiology and Biotechnology, Bharath Institute of Higher Education and Research, Chennai, Tamil Nadu, India.

Corresponding author

Dr.S.Selvakumar,

Professor, Dept. of Biotechnology, Bharath Institute of Higher Education and Research, Chennai-600073, India

ORCID ID: https://orcid.org/ 0000-0001-7765-4801

ABSTRACT

Background: Phytomedicine can be defined as the herbal medicine with therapeutic and healing properties. They are the drugs purified and standardized fractions with defined minimum of more than four phytochemical compounds. **Objectives and Rationale:** A single herbal medicinal plant may consist of thousands of phytoconstituents or secondary metabolites and the possibilities of making new drug development and discoveries become evident.The aim of the present study is to explore the phytochemical profile, invitro radical scavenging and invitro cytotoxicity effect of ethyl acetate extract of *Nyctanthes arbor-tristis*. **Brief Methods:** Thepreliminary phytochemical screening was performed by qualitative methods and total content of phytoconstituents such as polyphenol, alkaloids, tannins etc in plant extract were quantified by spectrophotometric method. Total content of flavonoid was determined by aluminium chloride colorimetric assay. The concentration of alkaloids was analysed by spectrophotometrically. The antioxidant property of the extracts was determined by DPPH free radical scavenging analysis. Cytotoxicity of the extracts of *Nyctanthes arbor-tristis* was evaluated by MTT assay using LLCMK2 monkey kidney epithelial cells and

MCF-7. **Results and Conclusion:** The aim of the present study is to evaluate the presence of various phytochemicals i.e., alkaloids, flavonoids, tannins, polyphenols, etc., antioxidant potential and cytotoxicity of medicinal plant *Nyctanthes arbor-tristis*. Our results indicate that the plant possess antioxidant activity and cytotoxic nature of the plant extract due to presence of phytoconstituents, and their concentrations such as the total content of alkaloids, flavonoids, tannins and polyphenol compounds. The study demonstrated that the phytochemicals in a *Nyctanthes arbor-tristis* may contribute significantly to the different activities of this plant in further studies.

Keywords: Antioxidants; Cancer; Cytotoxicity; *Nyctanthes arbor-tristis* Phytomedicine; Phytoconstituents.

Introduction

Phytochemicals or phytocompounds are a class of non-nutritive phytochemicals which have either provide defence or disease protection to plants. Dietary consumption of these phytochemicals or plant-based products would provide benefit to health, protection for disorders and/or diseases i.e., cancer, heart and neurological disorders. These phytomolecules act independently and in combination have significant efficiency as therapeutics in different ailments. Phytomolecules of nutraceutical potential in diet are of tremendous benefit to human health [1]. *Nyctanthes arbor-tristis* has great therapeutic implication in Indian system of medicine (Siddha and Ayurveda) for various formulations to treat digestive disorders, piles, insect poison, anti-rheumatic. The flowers and fruit shell have potential to cure asthma, utensil [2]. They belong to the family Oleaceae and are commonly called as the night flowering jasmine which is native to South Asia. Thus, the in vitro preliminary phytochemical screening, quantification (alkaloids, flavonoids, tannins), antioxidant, cytotoxicity potential of the *Nyctanthes arbor-tristis* are investigated in this study. The results demonstrated that the chloroform extract contains various phytochemicals with strong antioxidant potential with non-cytotoxic properties. Antioxidants are important in the inhibition of oxidation and protect the cells from damage due to the scavenging of free radicals. These free radicals will attack healthy cells which then alters its DNA allowing tumour to grow. Thus in-vitro antioxidant analysis is performed by DPPH an antioxidant assay that produces a violent colour solution in ethanol which will turn colourless solution upon the presence of antioxidant molecules. Cytotoxicity evaluation is the most important

step in any *in-vitro* studies of drug formulations. It is important to understand the toxicity of the drug which can pose certain health risks to humans. Therefore, MTT assay is performed which is a colorimetric assay which can be used to analyse the cells for its metabolic activity. Here, MCF-7 human breast cancer cell lines which are positive for glucocorticoid, estrogen and progesterone receptor and LLC MK2 Rhesus monkey renal epithelial cells were used for analysis.

Materials and methods

Collection of samples

The plant *Nyctanthes arbor-tristis* Linn was used for the present investigation. Plant was collected nearby gardens, Chennai, India and authenticated by botanist. The aerial parts such as leaves, flowers and stem were used for extraction.

Preparation of extracts

1000 grams of finely ground plant material was charged into a separate round bottom flask for sample extraction using the solvent, namely ethyl acetate. Extract with 250 ml of solvent for 96 hours. At the end of the extraction, each solvent was concentrated under reduced pressure. The resulting extract was filtered to precipitate inorganic materials and debris, and then concentrated on a water bath (50°C) and vacuum dried. Now store the extracted experimental solution in the refrigerator for future use.

Chemicals and Reagents

Analytical grade chemicals were used in the experimental part.

Preliminary phytochemical analysis

Preliminary phytochemical analysis of the collected different solvents ethanol, chloroform, acetone, aqueous, ethyl acetate extracts of the plant was carried out. [3-8]

Quantification of Phytochemicals

Total phenolic, tannin, alkaloid, flavonoid content present in the extract were determined by standard protocols [9-15].

Determination of Antioxidant activity (DPPH free radical scavenging activity)

Based on the DPPH free radical activity the antioxidant potential of the collected extracts was determined by DPPH radical scavenging activity.The antioxidant activity of the plant extracts was examined on the basis of the scavengingeffect on the stable DPPH free radical activity [16]. Ethanolic solution of DPPH (0.05 mM)as added to 40: l of extract solution with different concentrations (0.02-2 mg/mL).DPPH solution was freshly prepared and kept in the dark at 4°C. Ethanol 96% (2.7 mL) wasadded and the mixture was shaken vigorously. The mixture was left to stand for 5 min andthe absorbance was measured spectrophotometrically at 517 nm. Ethanol was used to set theabsorbance zero. A blank sample containing the same amount of ethanol and DPPH was alsoprepared. All determinations were performed in triplicate. The radical scavenging activitiesof the test samples, expressed as percentage of inhibition were calculated according to thefollowing equation.

Percent (%) inhibition of DPPH activity = [(AB – AA) / AB] x 100

Where AA and AB are the absorbance values of the test and of the blank sample,respectively.A percent inhibition versus concentration curve was plotted and theconcentration of samplerequired for 50% inhibition was determined and represented as IC_{50} value for each of the test solutions [17].

Determination of In vitro cytotoxicity

Preparation of stock solutions

The stock solution is prepared by dissolving 4 mg of crude extract in 100 µL of dimethyl sulfoxide (DMSO), and then diluting it to 400 µg/mL with RPMI-1640 cell culture medium. All solutions are sterilized through a 0.22 µm syringe adapter filter and stored at -20°C until use

Cytotoxic activity

In vitro cytotoxicity activity of the collected extract on LLCMK2 monkey kidney epithelial cells and MCF-7 breast cancer cells are used for MTT assay [18]. The cytotoxicity of the crude extract was evaluated on LLCMK2 monkey kidney epithelial cells. The cells were grown in RPMI-1640 medium containing L-glutamine and 25 mM HEPES. The medium was supplemented with 2 mg/mL NaHCO3, 10 µg/ml hypoxanthine , 11.1 mM glucose, 10% FBS (BioWhittaker®, Verviers, Belgium) and 5 µg/mL gentamicin. Before being used for cytotoxicity determination, the cells were incubated in a humidified incubator at 37°C in 5% O_2, 5% CO_2, and 90% N2 until confluence. Trypsin-treated cells are distributed in a 96-well

microtiter plate at a density of 10,000 cells, 100 µL per well, incubated for 48 hours to allow attachment, and then the extract is added. After 48 hours, completely remove the medium from each well, and then add 100 µl of fresh medium. Thereafter, 100 µl of crude extract (400 µg/ml) was added to row H, and then serially diluted to row B to provide a concentration range of 200 – 3.125 µg/ml. The cells in row A serve as a control without drug (100% growth). The cells with or without extracts were incubated at 37°C for 72 h before determining their viability. Each concentration level was tested in triplicate [19, 20].

MTT Assay

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used to determine cell viability [18]. After 72 hours of incubation, the medium with or without extracts in each well is completely removed from the assay plate and replaced with 100 µl of fresh medium. Then add 10 µl of 5 mg/mL thiazolyl blue tetrazolium bromide, MTT (Sigma) to each well to reach a final concentration of 0.45 mg/ml, and then incubate at 37°C for 3 hours. After 3 hours, carefully remove the medium containing MTT, then add 100 µl dimethylsulfoxide to dissolve the formazan crystals, then incubate for 1 hour, and then record the optical density at 595 nm (Emax-Molecular Devices Corporation, California, USA).

Data analysis

The percentage viability and percentage mortality were calculated from the OD values using Microsoft Excel 2010. The mean results of the percentage mortality were plotted against the logarithms of concentrations using the Fig P computer program Ver 4.189/07 (BiosoftInc, USA). Regression equations obtained from the graphs were used to calculate the fifty percent cytotoxic concentration (CC 50), which is the concentration killing fifty percent of the cells. An extract with CC50 > 25 µg/ml is considered non-toxic [21].

Results and Discussion

The phytochemical constituents of prepared ethanol, chloroform, ethyl acetate, acetone and aqueous extracts of *Nyctanthes arbor-tristis* were presented in Table 1. The obtained results demonstrated that the presence of flavonoids, alkaloids, saponins, triple sugar, phenol, tannins, steroids, anthraquinones, and cardiac glycosides. Alkaloids were present in chloroform, ethyl acetate and acetone extracts whereas, the ethanol and aqueous extracts

shows absence. Flavonoids, saponins, tannins, anthraquinones and steroids was present in both ethyl acetate, acetone and chloroform extract respectively. The acetone extracts show the presence of phenol and anthroquinones whereas chloroform extracts show negative result. In the case of terpenoids, glycosides, proteins and amino acids which shows negative result in ethyl acetate, chloroform and acetone extract. These phytochemicals represent the pharmacological activities of *Nyctanthes arbor-tristis* (Figure 1-8).The total phenolic compound ranged from 5.69 to 14.25 µg/ml. Total phenolic contents in the ethanol, chloroform, ethyl acetate, acetone and aqueous extract were expressed as Gallic acid equivalent (mg of GAE/g of extract) (y=0.004x-0.024, R^2=0.965). The ethanol extract of *Nyctanthes arbor-tristis* shows y=14.25x+66.91, R^2=0.430; chloroform extract y=11.40x+ 53.54, R^2=0.430; ethyl acetate extract of *Nyctanthes arbor-tristis* y=8.552x+40.13, R^2=0.430; acetone extract y=2.843x+13.38, R^2=0.429; aqueous extract y=5.643x+26.73, R^2=0.429 [22]. Alkaloid contents in the extracts of *Nyctanthes arbor-tristis* was expressed as atropine equivalent (y= 0.005x+0.274, R^2=0.982). The ethanol extract of *Nyctanthes arbor-tristis* shows the total concentration of alkaloids were y=11.35x+53.48, R2 = 0.428; chloroform extract y=14.20x+66.87. R2=0.428; the ethyl acetate extract y=8.528x+40.13, R2=0.428; acetone extract y=5.654x+26.74, R2= 0.427; aqueous extract was shown y=2.812x+13.4, R2 =0.426. The total alkaloid content ranges from 2.81-14.20 µg/ml [23]. Total tannin contents in the extract were expressed as Gallic acid equivalent (y=0.002x+0.073, R^2=0.973). Total content of tannins in ethanol y=11.39x+53.53, R2=0.430 chloroform y=14.25x+66.91, R2=0.430, ethyl acetate y=8.533x+40.14, R2=0.429 acetone y=5.678x+26.76, R2=0.428 and aqueous extract y=2.831x+13.38, R2=0.428 of *Nyctanthes arbor-tristis*. The total tannin content ranges from 2.83-14.25 µg/ml. Total flavonoid content expressed in terms of atropine equivalent (y=0.007×0.82, R^2 = 0.981). The total content of flavonoids was y=14.12x+67.04, R2=0.428; y=11.27x+53.69, R2=0.428; y=8.429x+40.34, R^2=0.428; y=5.594x+27.00, R2=0.429; y=2.751x+13.63, R2=0.421, ethanol, chloroform, ethyl acetate, acetone, and aqueous extracts of *Nyctanthes arbor-tristis* respectively. The total concentration of flavanoids ranges from 2.75-14.25 µg/ml [24].

The antioxidant effects of ethanol, chloroform, ethyl acetate, acetone, and aqueous extracts of *Nyctanthes arbor-tristis* was shown in Figure 9. The present study focuses the percentage of antioxidant activity in different concentrations such as 5, 10, 25, 50, 100, 125, 250, and 500 µg/ml. Our results revealed that the chloroform extract of *Nyctanthes arbor-tristis* shows significant activities as compared to ethanol, ethyl acetate, aqueous, and acetone extracts.

The plant-based formulations used today provides safety and also compatibility with human physiology. Dietary sources provide a significant quantity of antioxidants to humans. Phytomolecules are considered to be an important antioxidant since it interacts with the free radical neutralization, thus providing protection from damage. The exogenous plant-based antioxidants like fruits, vegetables, and grains are known as dietary antioxidants [25]. Most of the health ailments are associated with free radicals i.e., aging, cancer cells, heart diseases, and gastrointestinal disorders etc. The antioxidants act as protective agent by free radical neutralization and leads to normal metabolism [26]. Many research results demonstrated that the foods may supply many antioxidant substances to humans and considered as a richest source of antioxidant molecules [27].

The cytotoxic activity of the extracts of *Nyctanthes arbor-tristis* were shown in Figure 10. Based on our result the cytotoxicity analysis was carried out in the chloroform extract of plant extract on MCF-7 cells through MTT. The results showed the dose dependent response in the cell viability and proliferation inhibition. The IC_{50} value was determined as 28.6 µg/ml. The chloroform extract of *Nyctanthes arbor-tristis* contains potent phytochemicals like alkaloids, phenols, flavonoids, terpenoids, glycosides, saponins, steroids, and tannins and supports their antioxidant and anti-proliferative activity as reported in other plants [21, 28].

Figure 1: Shows the total phenolic compound standard calibration curve.

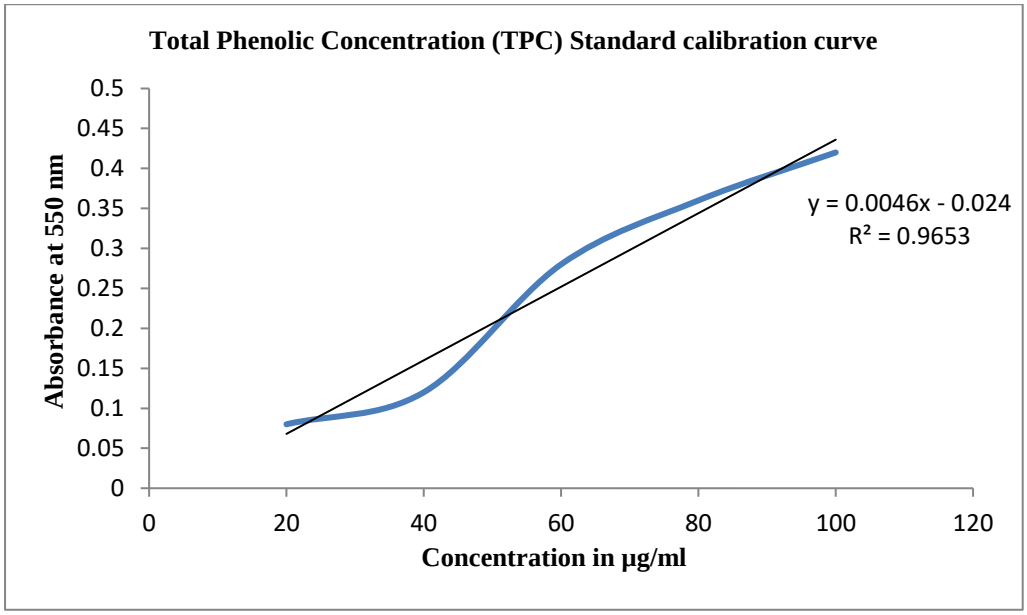

Figure 2: Shows the Total alkaloid content standard calibration curve

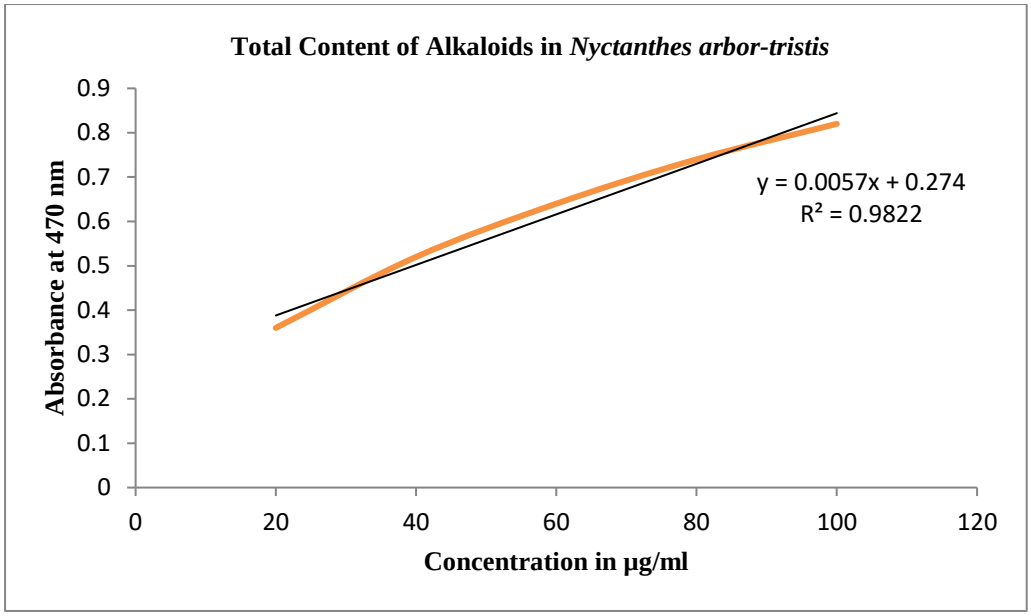

Figure 3: Standard calibration curve of total content of tannins.

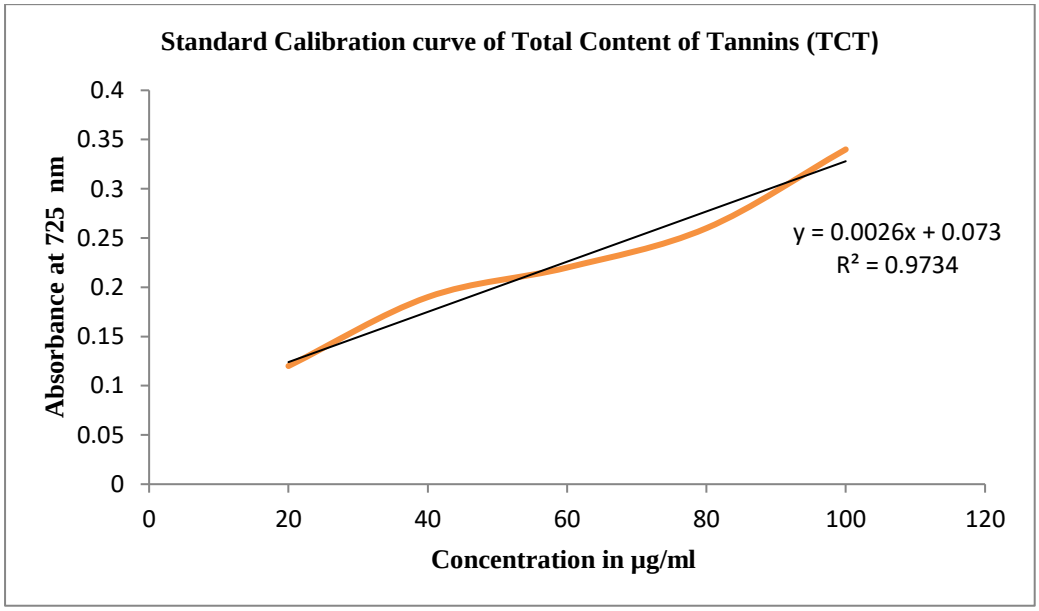

Figure 4: Total Flavonoid content standard calibration curve.

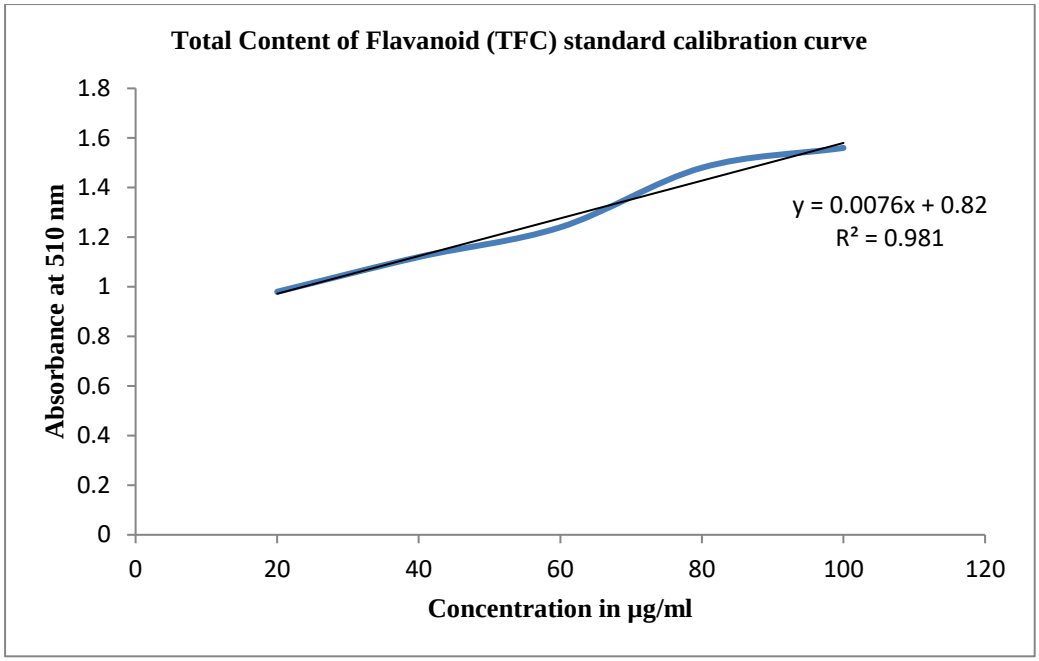

Figure 5:Shows the total content of polyphenols in ethanol, chloroform, ethyl acetate, acetone, and aqueous extracts of *Nyctanthes arbor-tristis*

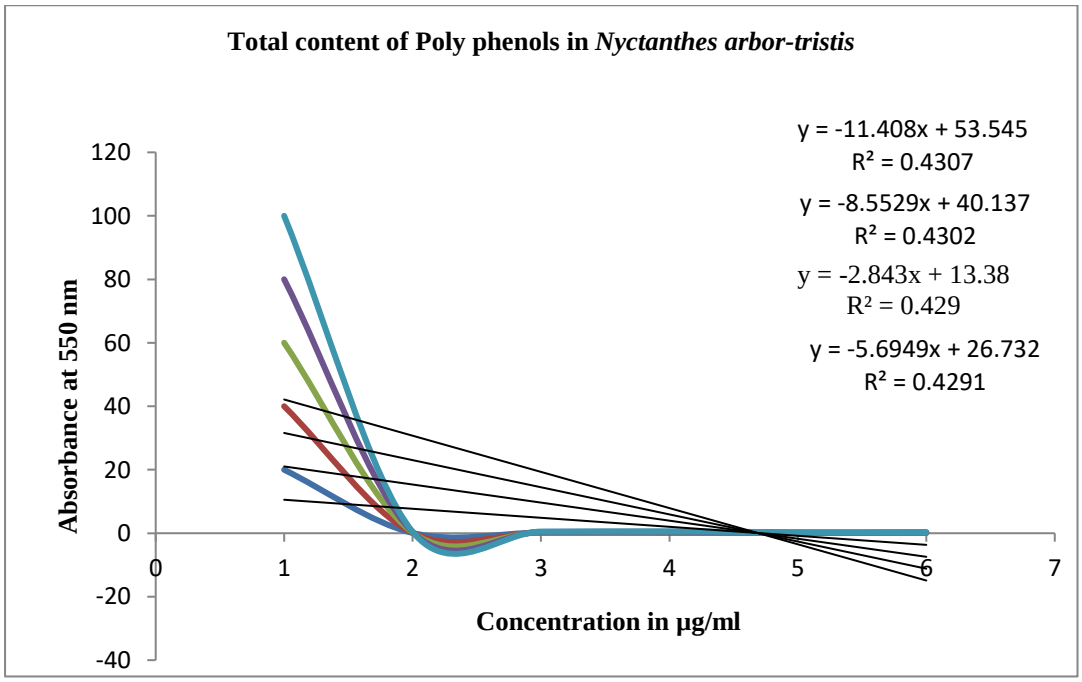

Figure 6: Shows the total content of Alkaloids in ethanol, ethyl acetate,chloroform, acetone, and aqueous extracts of*Nyctanthes arbor-tristis*

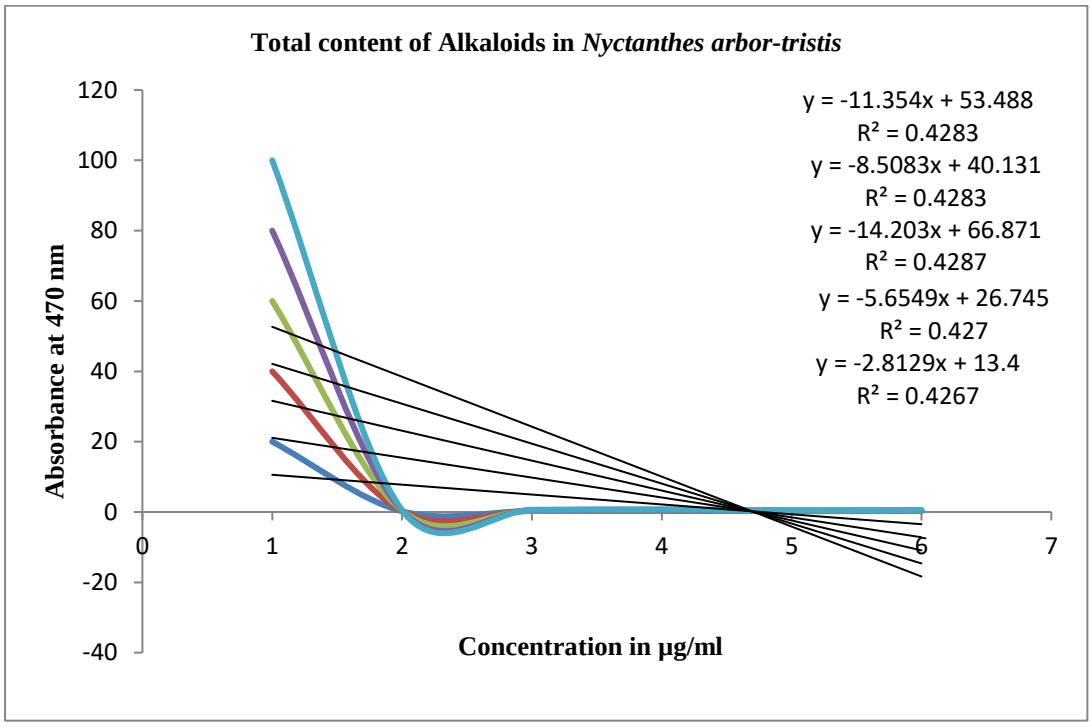

Figure 7: Shows the total content of tannins in various extracts of *Nyctanthes arbor-tristis*

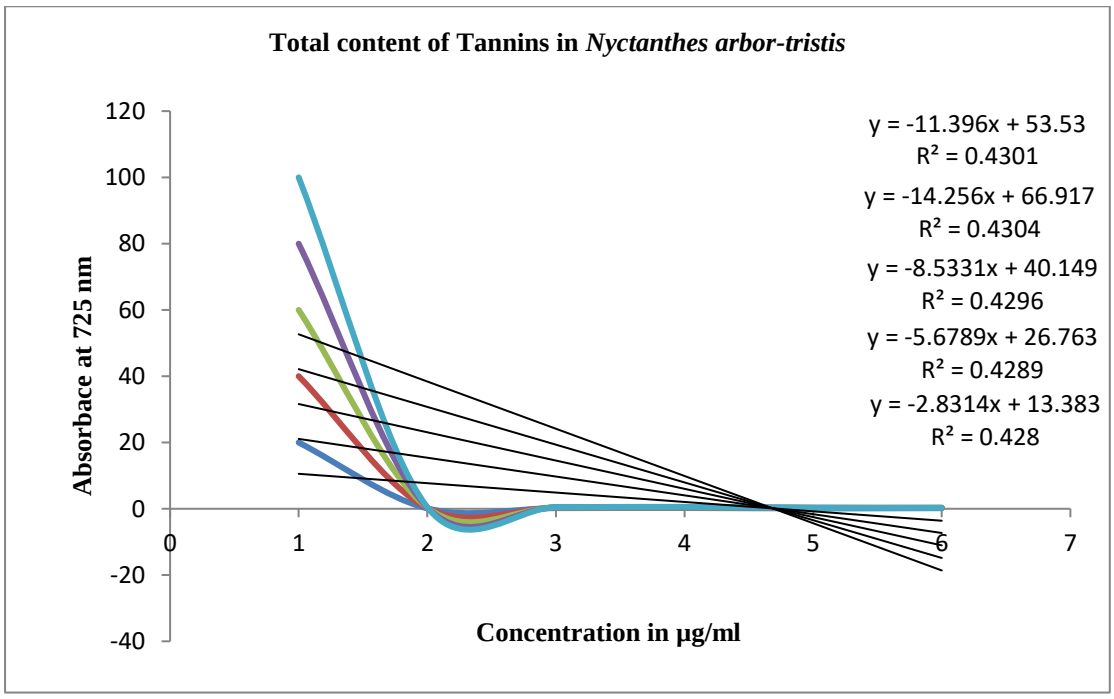

Figure 8: Shows the total content of flavonoids in ethanol, ethyl acetate, chloroform, acetone and aqueous extracts of *Nyctanthes arbor-tristis*

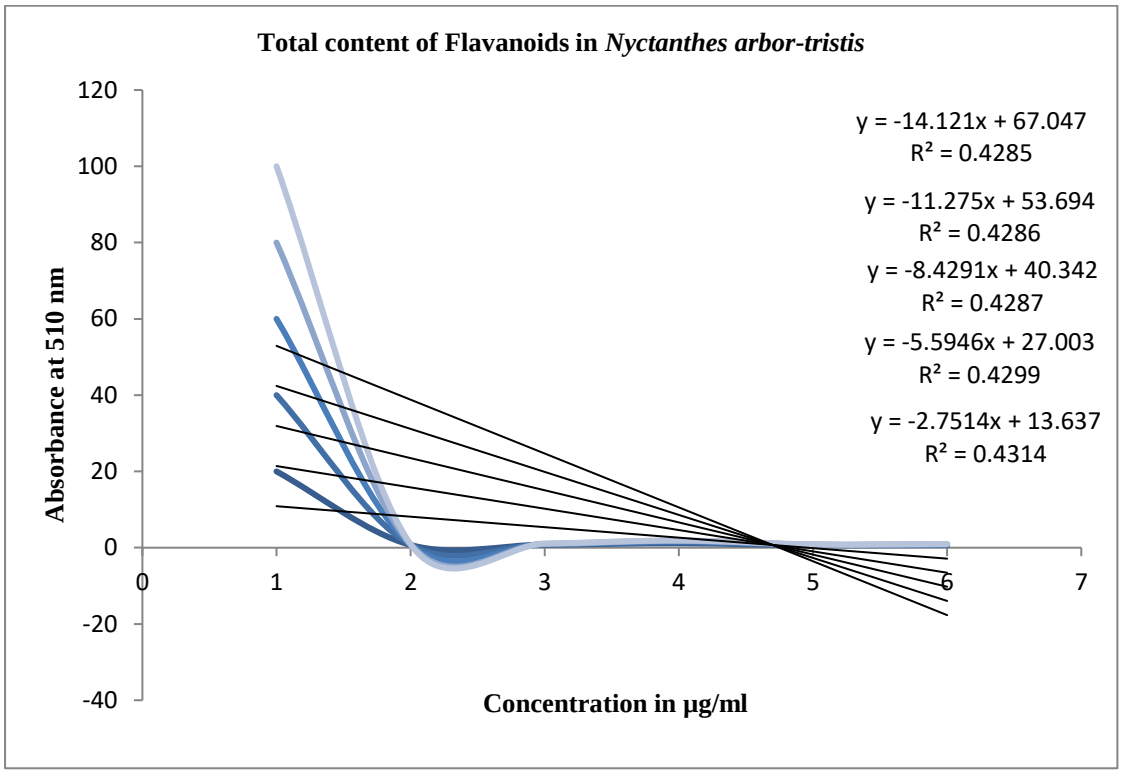

Figure 9: Shows the antioxidant effects of ethanol, chloroform, ethyl acetate, acetone, and aqueous extracts of *Nyctanthes arbor-tristis*

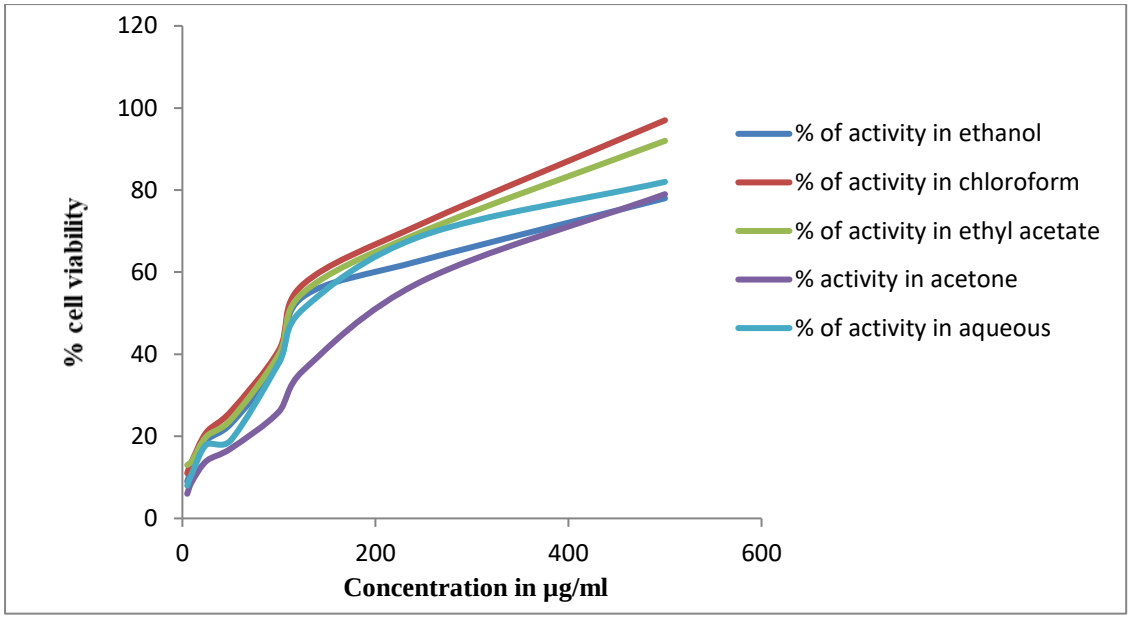

Figure 10: The percentage of cell viability of *Nyctanthes arbor-tristis*on control and experimental cell lines

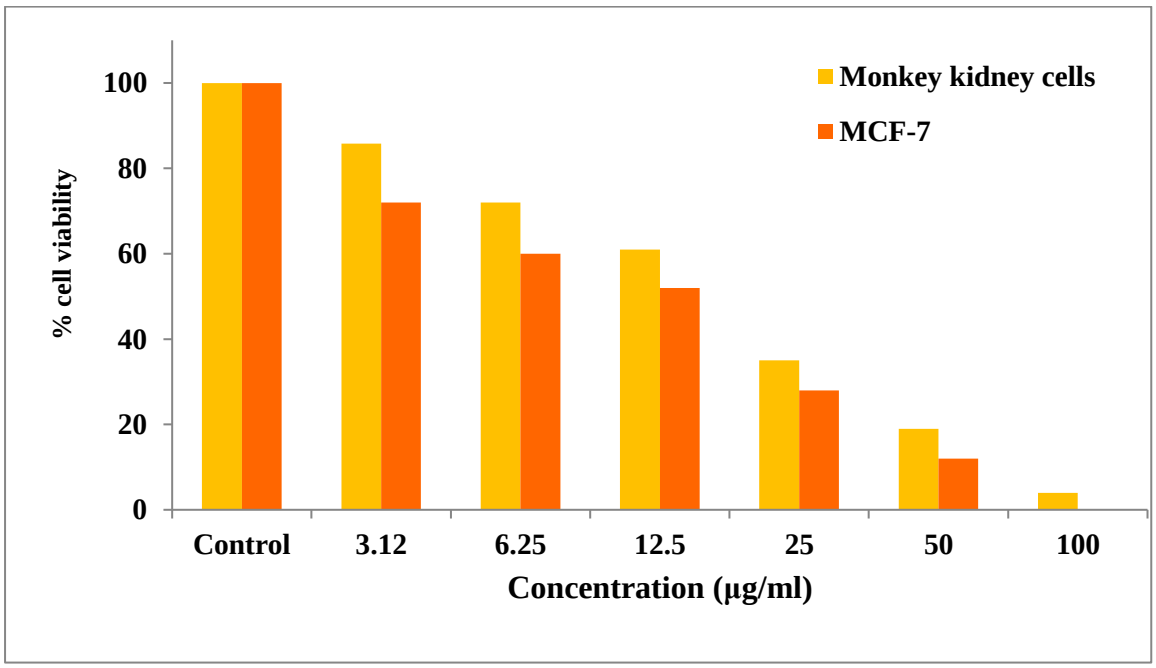

Table 1: The preliminary phytochemical analysis of *Nyctanthes arbor-tristis*

Sl. no	Phytochemical Constituents	Ethanol Extract	Chloroform Extract	Ethyl acetate Extract	Acetone Extract	Aqueous Extract

1	Flavonoids	-	+	+	+	+
2	Alkaloids	-	+	+	-	-
3	Triterpenoids	-	-	-	-	-
4	Proteins	-	-	-	-	-
5	Triple sugars	+	+	-	+	-
6	Saponins	+	+	+	+	+
7	Tannins	-	+	+	+	+
8	Amino acids	-	+	+	-	-
9	Anthraquinones	+	-	-	+	+
10	Steroids	+	-	+	+	+
11	Cardiac glycosides	-	+	+	-	-
12	Phenolic compounds	-	-	-	-	-

'+' indicates Positive and '-' indicates Negative

References

1. Prakash D and Gupta C(2011) Role of Phytoestrogens as nutraceuticals in human health. A review Biotechnology: An Indian J Vol 5 No 3 Pages 1-8.
2. Selvakumar S and Gangatharan S (2017) HPTLC Fingerprinting and antifungal efficacy of ethyl acetate extract *Couroupitaguianensis* on Selected fungal pathogens. Int J Pharmaceutical Sci and Nanotechnology Vol 10 No1 Pages 3594-3599.
3. Siddiqui AA, Ali M (1997) Practical pharmaceutical chemistry. Ist edition. New Delhi. CBS Publishers and Distributors Pages 126-131
4. Segelman AB, Fransworth NR, Quimbi MD (1969) False negative saponins test results induced by the presence of tannins. Lloydia Vol 32 Pages 52-58.
5. Sofowora A (1982) Medicinal Plants and Traditional Medicine in West Arica. New York. John Wily and Sons P. 256
6. Trease GE and Evans W. Pharmacognosy 11th Edn. BrailliarTiridacanb Macmillian Publishers. 1989.

7. Adetuyi AO. Popoola AV (2001) Extraction and Dye ability potential studies of colourantin zanthoxylumzanthoxyloides Plant on cotton fabric J SciEng Tech 8 (2):
8. Salehi-Surmaghi MH, Aynehchi Y, Amin GH, Mahmoodi Z (1992) Survey of Iranian plants for saponins, alkaloids, flavonoids and tannins IV, DARU Vol 2 Pages 1-11.
9. Singleton VL, Orthofer R, Lamuela-Raventos RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent Methods Enzymol Vol 299 Pages 152-178.
10. Ali Ghasemzadeh, Hawa, Hawa ZE, Jaafar, Asmah Rahmat 2010) Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia Young Ginger (*Zingiber officinale Roscoe*) Mol Vol 15 Pages 4324-4333.
11. Nasir Rasool, Komal Rizwan, Muhammad Zubair, Kaleem Ur Rahman Naveed, Imran, Viqar Uddin Ahmed (2011) Antioxidant potential of different extracts and fractions of *Catharanthus roseus* shoots Int J Phytomed Vol 1 No 3 Pages 108-114.
12. Milan S. Stankovic (2011) Total phenolic content, flavonoid concentration and antioxidant activity of *Marru biumperegrinum L,* extracts Kragujevac J Sci Vol 33 Pages 63-72.
13. Marinova, D, Ribarova, F, Atanassova M (2005) Total Phenolics and Total Flavonoids in Bulgarian Fruits and Vegetables. J University Chem Technol Metallurgy Vol 40 No 3 Pages 255-260.
14. Rajeev Singh, Pawan Kumar Verma, Gagandeep Singh(2012) Total phenolic, flavonoids and tannin contents in different extracts of *Artemisia absinthium* J Intercult Ethnopharmacol Vol 1 No 2 Pages 101-104.
15. AfifyAel-M, El-Beltagi, HS, El-Salam, SM, Omran AA (2012) Biochemical changes in phenols, flavonoids, tannins, vitamin E, β-carotene and antioxidant activity during soaking of three white sorghum varieties Asian Pac J Trop Biomed Vol 2 No 3 Pages 203-9.
16. Braca A, Sortino C (2002) Politi M. Antioxidant activity of flavonoids from Licanialicaniae flora. J. Ethnopharmacol Vol 79 Pages 379-381.
17. Yen GC, Duh PD (1994) Scavenging effect of methanolic extracts of peanut hulls on free-radical and active oxygen species J Agric Food Chem Vol 42 Pages 629-632.
18. Niles, AL, Moravec, RA, Riss TL (2008) Update on in vitro cytotoxicity assays for drug development Expert Opin Drug Discov Vol 3 No 6 Pages 655-69.

19. Merlin, NJ, Parthasarathy, V, Manavala R and Kumaravel S (2009) Chemical investigation of aerial parts of *Gmelina asiatica Linn* by GC-MS Pharmacog. Res Vol 1No 3 Pages 152-156.
20. Niles, AL, Moravec, RA, Riss TL (2009) In vitro viability and cytotoxicity testing and same-well multi-parametric combinations for high throughput screening Curr Chem Genomics Vol 3 Pages 33-41.
21. Fadeyi, SA, Fadeyi, OO, Adejumos, AA, Okoro C, Myles EL (2013) In vitro anticancer screening of 24 locally used Nigerian medicinal plants BMC Compl and Altern Med Vol 13 Pages 7952.
22. Milan S Stankovic (2011) Total phenolic content, flavonoid concentration and antioxidant activity of *Marru biumperegrinum L.* Extracts Kragujevac J Sci Vol 33: 63-72.
23. Afify Ael, M, El Beltagi, HS, El-Salam, SM, Omran AA(2012) Biochemical changes in phenols, flavonoids, tannins, vitamin E, β-carotene and antioxidant activity during soaking of three white sorghum varieties Asian Pac J Trop Biomed Vol 2 No3 Pages 203-209.
24. S. Selvakumar, S, Madhan Kumar, U, Prashanth, A, Barnali Sarkar and Sindhuja J (2017) Quantitative Analysis of Phytoconstituents of Chloroform Extract of Poly Herbal Formulation Int J Pharm Sci Rev Res Vol 46 No 2 Pages 157-160.
25. Bouayed J, Bohn T (2010) Exogenous antioxidants double-edged swords in cellular redox state: health beneficial effects at physiologic doses versus deleterious effects at high doses Oxidative Medicine and Cellular Longevity Vol 3 No 4 Pages 228-237.
26. Mahantesh, SP, Gangawane AK and Patil CS (2012) Free radicals, antioxidants, diseases and phytomedicines in human health: future prospects World Res J Medicinal & Aromatic Plants Vol 1 No1 Pages 6-10.
27. Selvakumar S, Sindhuja J (2016) Quantitative Analysis of Phytoconstituents of Various Extracts of Poly Herbal Formulation Nallamarunthu Int J Pharm Sci Rev Res Vol 47 No 1 Pages 13-16.
28. Selvakumar S and Barnali Sarkar (2017) In vitro Cytotoxicity analysis of chloroform extract of novel poly herbal formulation Int J Pharmacog and Phytochem Res Vol 9 No 2 Pages 193-196.