ASIAN JOURNAL OF PHARMACOGNOSY
Asian Journal of Pharmacognosy 5(3): 5-14
eISSN-0128-1119

Accepted August 9, 2021

Phytochemical and Biological study on leaves extract of *Nyctanthes arbor-tristis* L.

Bibek Sigdel[1]*, Dharma Prasad Khanal[2] , Bishwo Raj Dhungana[3,] Dikshita Lama[4]

[1,2,4]Manmohan Memorial Institute of Health Sciences, Soalteemode, Kathmandu.
[3]Janamatri Foundation, Hattiban, Lalitpur Nepal

*For correspondence: bibeksigdel94@gmail.com

Abstract: *Nyctanthes arbor-tristis* L is well known medicinal plant which is widely used in Ayurveda treatments due to the various pharmacological actions such as anti-arthritic, antispasmodic, antibacterial, anti-inflammatory, immunostimulant, antidiabetic, hepatoprotective, antioxidant, antimicrobial, antihelmintic, antileishmanial, antiviral, central nerve system (CNS) depressant activities. The objective of this study was to perform phytochemical screening, gas chromatography mass spectroscopy (GC-MS) analysis, total flavonoid and phenolic contents, *in-vitro* antioxidant activity and *in-vivo* hepatoprotective activity of ethanolic leaf extract obtained from *Nyctanthes arbor-tristis* L. The leaf extract was prepared by macerating dried leaves and crude extract was subjected for qualitative phytochemical analysis. The quantitative analysis was performed on GC-MS analysis of leaf extract, determination of total phenolic content (TPC) and the flavonoid content (TFC).. Antioxidant activity was determined by ferric reducing power assay (FRPA) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. *In-vivo* hepatoprotective activity was determined in paracetamol induced liver toxicity in Wistar Albino rats following standard protocol. Qualitative phytochemical analysis of extract showed the presence of alkaloids, glycosides, tannins, terpenoids, cardiac glycosides, saponins, carbohydrates, proteins, flavonoids and absence of anthraquinone glycosides. The results of GC-MS analysis of extract revealed the presence of palmitic acid, nonanoic acid, and phytol as main compounds. The phenolic content (TPC) and the flavonoid content (TFC) were found in indicative amount. In the reducing power assay, moieties present in extract with reducing power showed that they are electron donors and can reduce the oxidized intermediates. Maximum percentage inhibition of DPPH radicals by the extract was about 53% at 25 ug/ml and the IC50 value of extract was found to be 15.60 µg/mL. It showed decent hepatoprotective action against paracetamol induced hepatotoxic rats and was comparable with standard silymarin. The ethanolic extract of leaves are rich with many phytochemicals and showed hepatoprotective action and was comparable with standard. Therefore, it can be concluded that plant *Nyctanthes arbor-tristis* L may be a promising source for the search of hepatoprotective agent.

***Keywords*:** Hepatotoxicty, *Nyctanthes arbor-tristis*, paracetamol, hepatoprotective action

ASIAN JOURNAL OF PHARMACOGNOSY
Asian Journal of Pharmacognosy 5(3): 5-14
eISSN-0128-1119

INTRODUCTION

Hepatic ailment is a significant issue in developing countries and a cause of morbidity and mortality across the globe. Hepatitis A, B and C viruses, carbon tetrachloride, high doses of thioacetamide, paracetamol and some chemical agents are root causes of liver disease (Saleem et al., 2010). Liver is the fundamental organ for the metabolism and excretion of numerous endogenous and exogenous compounds, any trauma to it or deterioration of its function may see countless complication on one's health (Zhang et al., 2013). Successful treatment of hepatic disease is still a challenge to allopathic medicine. Corticosteroids and immunosuppressive agents are only accessible agents that are used for the management of hepatic disease which have considerable adverse drug reactions. This has guided to growing dependency on supportive and alternative medicine like plant-based medicine. Medicinal plants exert a crucial part in the treatment of liver ailment in the developing world for fundamental health care because of their easy availability in nature, lesser or no side effect (Sheetal & Singh, 2008).

Nyctanthes arbor-tristis belongs to the family Oleaceae and is precious medicinal plant that grows in tropical and subtropical areas. N. arbor-tristis often known as night jasmine, parijat & Harasinghar (Doughari & Ioryue, 2009; Meshram et al., 2012). A research study has exhibited diverse pharmacological actions of different parts of plant. Phytochemicals like flavanoids, alkaloids, proteins, carbohydrates, essential oils, tannic acid, friedeline, benzoic acid have been reported for remarkable hepatoprotective, anti-diabetic, anti-viral, anti-malarial, anti-oxidant, anti-bacterial activities (Shrivastava & Bharadwaj, 2018; Omkar et al., 2006). The work described in this research aims to study the chemical constituents of the *Nyctanthes arbor-tristis* Linn and to access their pharmacological activities. Hence, the purpose of this work is to investigate the likelihood of evolving new drug candidates from this plant for the treatment of hepatic diseases.

.

ASIAN JOURNAL OF PHARMACOGNOSY
Asian Journal of Pharmacognosy 5(3): 5-14
eISSN-0128-1119

Nyctanthes arbor-tristis L.

METHODS

Plant collection: The leaves of *Nyctanhes arbor-tristis L* were collected from Bhimad-6, Tanahun, on 20th March 2019 at 2:00 p.m.

Plant processing: The leaves were washed with water and they were shade dried for several months. After complete drying, and powder was prepared using an electric grinder. 100 gram of the powder was taken in conical flask and 500 ml of ethanol added and left 72 hours for maceration and then extracts were filtered through double folded muslin cloth at first then through whattmann filter paper No.1. Filtrate was evaporated in rotary evaporator. Dry extract was kept in desiccator for further analysis.

Chemicals used: All the chemicals were purchased and manufactured from Merck and Qualigens Fine Chemicals. Chemicals used were Gallic acid, Quercetin, sodium carbonate, Folin-ciocalteu reagent, Trichloroacetic acid, Aluminium trichloride, Sodium hydroxide, Sodium nitrate, Potassium ferricyanide, Ferric chloride, Paracetamol & Silymarin (Lomus Pharmaceuticals Pvt Ltd).

Instruments used: Computer set with spectrophotometer software (Sync master 5915, Samsung), UV visible spectrophotometer (EI-2372, India), Digital pH meter (Labotronics, India), GC-MS –QP2010(ultra gas chromatograph mass spectrometer by Shimadzu, Japan), Digital centrifuge (Remi-R-8C, India), Digital hot air oven (Accumax-UG37, India), Digital incubator (Memmert, Germany), Rotary vacuum evaporator (RC5100, Accumax india), Digital weighing balance (RADWAG- AS 220 R2, USA), and all the glassware were from Borosil, Religlas & Aarosil.

Phytochemical screening: Phytochemical screening was performed using standard protocols to detect the presence of glycosides, alkaloids, carbohydrates, proteins, flavonoids, saponins, phenols and tannins (Chetia et al., 2014).

GC-MS analysis: It was conducted in Department of Food Technology and Quality Control, Babarmahal, Kathmandu Nepal for the analysis of compounds present in the extract.

Total phenolic content: Folin-Ciocalteu assay published (reference) with slight modifications was performed for the determination of total phenolic content in the extract. Stanadard curve was prepared by dissolving gallic acid in methanol in order to prepare the concentration series of 10, 25, 50, 100 and 200 µg/ml. The plant extract (1mg/ml) was prepared in methanol and 0.5 ml of each sample was placed into test tubes and mixed with 2.5 ml of a 10 times dilute Folin- Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. All the tubes were kept stationary for 30 minutes at room temperature and absorbance was taken spectrometrically at 760 nm. All determinations were performed in triplicate and the results were expressed as mg/g gallic acid equivalents (GAE) (Sultana et al., 2012).

Total flavonoid content: Aluminum trichloride method was used for the determination of total flavonoid content in the extract. The plant extract (1.0 ml) was mixed with 4 ml of distilled water and then with 0.30 ml of a $NaNO_2$ solution (10%). After 5 min, 0.30 ml $AlCl_3$ solution (10%) was added followed by addition of 2.0 ml of NaOH solution (1%) to the mixture. The mixture was mixed and absorbance was taken at 510 nm spectrometrically versus the blank. Standard curve of quercetin was prepared by using the concentration series of 10, 25, 50, 100 and 200 µg/ml and expressed as mg quercetin equivalent (QE)/g of dried plant material. All the determinations were performed in triplicate (Sultana et al., 2012).

Reducing power assay: Mixture of 2.5 ml of phosphate buffer (200 mM, , pH 6.6) and 2.5 ml of 1% potassium ferricyanide was mixed with different concentrations of extracts (10, 20, 30, 40, 50 µg/ml). The mixtures were incubated for 20 min at 50 ^{0}C. Trichloroacetic acid (10%) was mixed into the above mixture and was centrifuged at 650 rpm for 10 min. The upper layer (5ml) of the mixture was mixed with 5 ml of distilled water and 1ml of 0.1% ferric chloride and the absorbance of the final solution was measured at 700 nm. The activity of the extract was compared with that of control. Ascorbic acid was used as standard (Azarmehr et al., 2019).

DPPH radical scavenging assay: DPPH (0.1 mM) was prepared using methanol and 3.0 ml of this solution was added to 1.0 ml of extract solution in methanol at different

concentrations (10, 20,30, 40, 50 µg/ml). The absorbance was taken spectrometrically at 517 nm after thirty minutes. A blank solution was prepared without adding extract. Different concentration of ascorbic acid (10, 20, 30, 40, 50 µg/ml) was used as standard (Saeed et al., 2012).

$$\text{DPPH scavenging activity}(\%) = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

In-vivo hepatoprotective action against paracetamol induced hepatotoxicity: Acute toxicity study was performed in Natural plant resources, Thapathali, Kathmandu following method – OECD TG 425 Up and Down procedure, 2001. Animals: Female albino rats weighing 180-200 gram were purchased from Department of plant resources, Thapathali. Animals were housed in the animal house of MMHIS under 12 hours light and 12-hour dark cycle after the approval from MMIHS IRB. Animals had free access to food and water and were acclimatized prior to experiments. Selection of extract dose was based on acute toxicity study.Experimental design: Animals were grouped as follows: Group I: Negative control (Animals had free access to water and food *ad libitum*). Group II: Positive control (Liver injury was induced by Paracetamol at dose 2000 mg/kg body weight). Group III: Standard control (Silymarin at dose 100 mg/kg body weight). Group IV: Low dose (250 mg/kg body weight) of tested extract. Group V: High dose (500 mg/kg body weight) of tested extract. The method described by Nahid Azeamehr et al was used (Azarmehr et al., 2019). Group I received distilled water for seven days. Group III pretreated by silymarin at dose 100 mg/kg for seven days. Group IV and V pretreated by NALE at dose 250 and 500 mg/kg respectively for seven days. To induce acute hepatotoxicity in group III, IV, V rats post-treated by orally intoxicated with single dose of paracetamol at dose 2000 mg/kg on the sixth day. Blood samples were taken from animals by cardiac puncture, collected and centrifuged 3000 rpm for 10 minutes. Plasma was separated and tested for determination of enzyme like GOT, GPT, ALP. Percentage changes in enzyme level are calculated using following formula (10).

$$\%\text{ change in enzyme level} = \frac{\text{initial value} - \text{final value}}{\text{initial value}} \times 100$$

Statistical analysis: The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Tukey's test for comparing SGOT, SGPT, ALP values. The values were expressed as mean ± SEM and $p<0.05$ was considered significant. Group II was compared with Group I; Group III, Group IV and Group V were compared to Group II.

RESULTS

Phytochemical screening: Phytochemical analysis of this study showed the presence of alkaloids, glycosides, tannins, terpenoids, cardiac glycosides, saponins, carbohydrates, proteins, flavonoids and absence of anthraquinone glycosides. Similar study carried out by pushpendra kumar Jain et.al showed similar result (Jain & Pandey, 2016).

GC-MS analysis: GC-MS analysis of extract showed 13 compounds which are given in Table 1.

Table 1: Top five Compound with their area percentage and reported activity

Name	Area percentage	Reported Biological activity
Gamma – stearolactone	19.80	Flavoring agent
Arachic alcohol	18.42	Used as an emollient in cosmetics
Palmitic acid	15.37	Anti inflammatory, anti oxidant, hypocholestrolemic, hemolytic
7-hexadecenal	9.42	It is a derivative of essential oils with potential antibacterial activities.
Nonanoic acid	6.61	Antibacterial

Total phenol content: The greater amount signifies the presence of different constituents having phenolic moiety in their structures. The phenolic content with respect to gallic acid was found to be 158±0.25 mg/g GAE of extract with reference to the standard curve shown in Fig 1. Similar study carried out by sumita kachwaha et.al found value as 94.78 mg/g GAE (Vyas et al., 2014).

Total flavonoids content: The greater amount signifies the presence of more flavonoids moieties in the constituents. The flavonoid content was found to be as 175±0.05 mg/g QE of extract with reference to the standard curve shown in Fig 2. Similar study carried out by Harsha lad et.al found 162.13 ± 1.52 mg/g QE (hydroethanolic extract) (Lad & Bhatnagar, 2017).

Antioxidant effect: In the reducing power assay, the more antioxidant composites change the ferric (Fe^{+3}) ions to ferrous (Fe^{+2}) ions. The reducing activity of the extract and standard ascorbic acid at various concentrations were shown Fig 3. Moieties with reducing power shows that they are electron donors and can reduce the oxidized intermediates which can act as antioxidants.

DPPH scavenging assay: Maximum percentage inhibition of DPPH radicals by the extract was about 53% at 25 ug/ml while that of ascorbic acid was 97% at the same concentration (Fig 4). The IC50 value of Standard was found to be 7.581 µg/mL and the IC50 value of extract was found to be 15.60 µg/mL. Similar research carried out by Manjulatha Khanapur et.al found value as 32.71 ±1.32 µg/mL (flower extract). (Khanapur et al., 2014).

ASIAN JOURNAL OF PHARMACOGNOSY
Asian Journal of Pharmacognosy 5(3): 5-14
eISSN-0128-1119

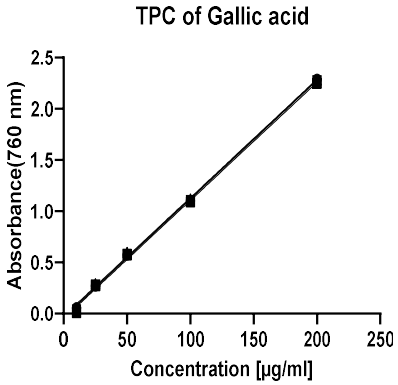

Fig 1: Standard plot of Gallic acid

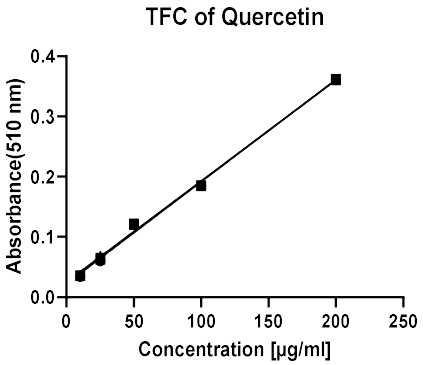

Fig 2: Standard plot of Quercetin

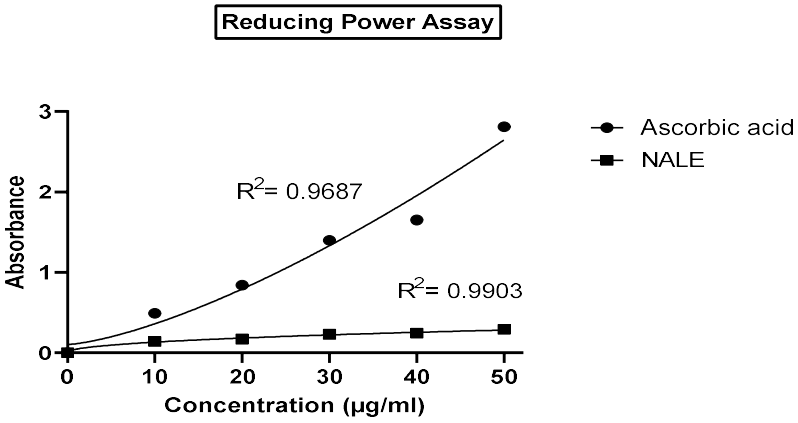

Fig. 3: Reducing power assay of tested extract

ASIAN JOURNAL OF PHARMACOGNOSY
Asian Journal of Pharmacognosy 5(3): 5-14
eISSN-0128-1119

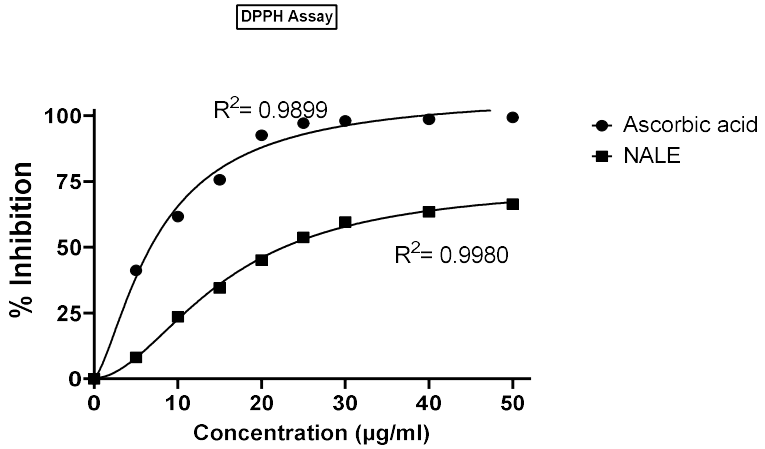

Fig. 4: DPPH assay of of tested extract

Hepatoprotective action In-vivo hepatoprotective activity of the extract was determined in Paracetamol (PCM) induced hepatotoxic model in rats. Results are shown in Table 2 and Table 3.

Table 2: Values of serum enzymes in different groups

Group	SGOT(IU/L) [mean±SEM]	SGPT(IU/L) [mean±SEM]	ALP(IU/L) [mean±SEM]
I(Normal)	158.5±0.885	38.83±0.401	125.2±1.327
II(Disease Induced)	380±0.980	210.8±1.302	381.2±0.703
III(Silymarin 100mg/kg)	164.2±0.600	53.83±1.376	134.2±0.872
IV(Low dose extract 250 mg/kg)	281.5±0.562	152±1.125	294.2±0.600
V(High dose extract 500 mg/kg)	162.5±0.846	64.5±0.957	151.5±0.619
Values are expressed as mean ± SEM (n = 6)			

Table 3: Change in enzyme percentage

Group	SGOT(%+or-)	SGPT(%+or-)	ALP(%+or-)
I(Normal)	-	-	-
II(Disease Induced)	+140%	+443%	+204%
III(Silymarin 100mg/kg)	-57%	-74%	-65%
IV(low dose extract 250mg/kg)	-26%	-28%	-23%
V(high dose extract 500mg/kg)	-57%	-69%	-60%

ASIAN JOURNAL OF PHARMACOGNOSY
Asian Journal of Pharmacognosy 5(3): 5-14
eISSN-0128-1119

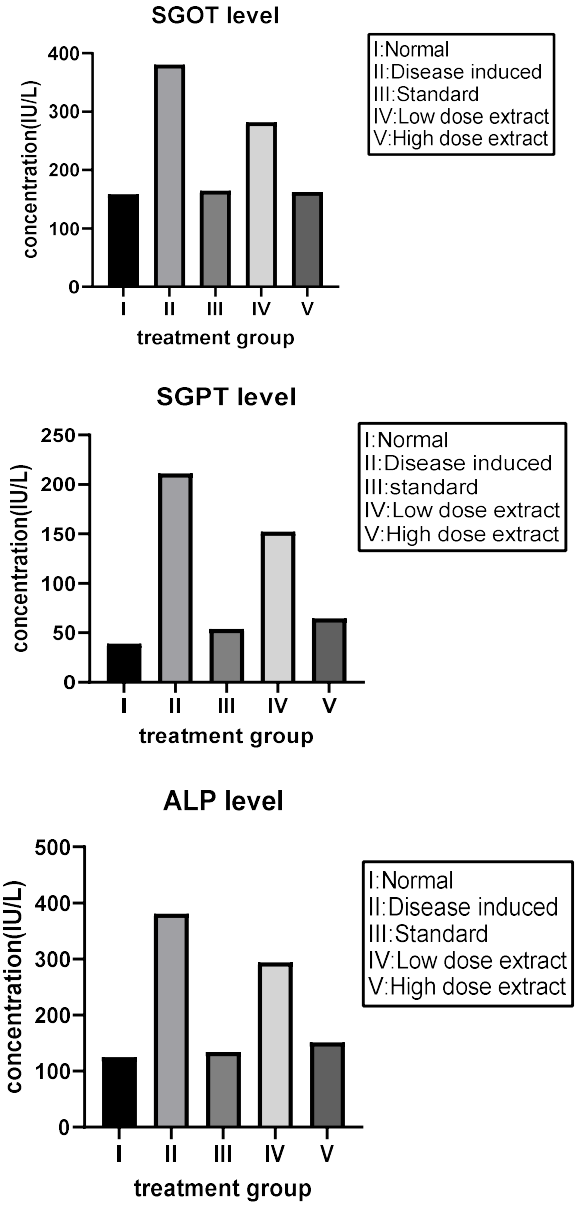

Fig 5: Effects of extracts on Serum SGOT, SGPT, ALP

CONCLUSION

The present study demonstrated that the plant possessed significant phenolic and flavonoid content and exhibited strong antioxidant and hepatoprotective activity which were comparable with standard. Further, it can be concluded that the plant can be considered as

good source for novel hepatoprotective drug. Further research is recommended for the exploration of new drugs with hepatoprotective action from taking samples from different geographical locations of Nepal.

ACKNOWLEDGMENTS

The author is grateful for management of Manmohan Memorial Institute of Health sciences for providing the necessary laboratory facilities throughout the research. Authors are thankful to the Director of "Food Laboratory, Babarmahal, Kathmandu." Under the Ministry of Food and Agriculture, Government of Nepal, for the GC-MS analysis of the extract.

DECLARATION OF CONFLICT OF INTEREST

No conflict of interest to declare.

REFERENCES

Saleem TS, Chetty SM, Ramkanth S, Rajan VST, Kumar KM, Gauthaman K (2010) A review on Hepatoprotective herbs. Int J Res Pharma Sci. 1:1-5.

Zhang A, Sun H, Wang X (2013) Recent Advances in Natural products from plants for the treatment of Liver diseases. Eur J Med Chem. 63:570-577.

Sheetal V, Singh SP (2008) Current and Future status of Herbal Medicine. Vet world. 1:347-350

Doughari JH, Ioryue AS (2009) Antimicrobial activity of stem bark extracts of Ceiba pentandra. Pharmacologyonline. 1(1):1333-1340

Meshram MM, Rangari SB, Kshirsagar SB, Gajbhiye S, Trivedi MR, Sahane RS (2012) Nyctanthes arbor-tristis a Herbal Panacea. International J of Pharmaceutical science and Research. 3(8):2432-2440

Shrivastava R, Bharadwaj AK (2018) Nyctanthes arbor-tristis an Important Medicinal Plant of Madhya Pradesh State. UK journal of Pharmaceutical Biosciences. 6(6):10.

Omkar A, jeeja T, Chhaya G (2006) Evaluation of Anti-inflammatory activity of Nyctanthes arbor-tristis and Onosma echioides. Pharmacognosy Magazine. 2(8):258-260.

Chetia J, Upadhyaya S, Bora DK (2014) Screening of Phytochemicals, Antioxidant and Antimicrobial activity of some tea garden weeds. Int J Pharm Sci Rev Res. 26(1):193-196.

Sultana M, Verma PK, Raina R, Prawez S (2012) Quantitative analysis of total phenolic, flavonoids and tannin contents in acetone and n-hexane extracts of Ageratum conyzoides. Int J ChemTech Res. 4(3):996-999

Azarmehr N, Afsar P, Moradi M, Sadeghi H, Alipoor B, Doustimotlagh AH (2019) Hepatoprotective and Antioxidant activity of watercress extract on Acetaminophen-induced hepatotoxicty in rats. Heliyon. 5(7).

Saeed N, Khan MR, Shabbir M (2012) Antioxidant activity, Total phenolic and Total flavonoid contents of whole plant extracts Toris leptophylla L. BMC Complement Altern Med. 12(1):1-12

Jain PK, Pandey A (2016) The wonder of Ayurvedic medicine-Nyctanthes arbor-tristis. Int J Herb Med. 9(4):9-17

Vyas S, Kachhwaha S, Kothari SL (2014) Comparative analysis of the in vitro Antioxidant activity and polyphenolic content of successive extracts of Nyctanthes arbor-tristis L. Int J of Pharmacy and Pharmaceutical science. 6(11):373-376.

Lad H, Bhatnagar D (2017) Modulation of oxidative stress mediators in the liver adjuvant induced arthritis rats by Nyctanthes arbor-tristis. Clinical Phytoscience. 3(1):1-8

Khanapur M, Avadhanula RK, Setty OH (2014) In vitro Antioxidant, antiproliferative, and Phytochemical study in different extracts of Nyctanthes arbor-tristis flowers. Biomed Res Int.

Chaudhary S, Gupta RK, Kumar A, Tarazi H (2018) Hepatoprotective and antioxidant potential of Nyctanthes arbor-tristis L. leaves against Antitubercular drugs induced hepatotoxicity. Journal of Pharmacy and Pharmacognosy Research. 6(3):205-215