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Phytochemical Evaluation and TLC Profile of Leaf and Bark in *Nyctanthes arbor–tristis* L.

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

This research work mainly deals with the collection of plants, the extraction of active compounds from the bark and leaves. The Introductory phytochemical screening of *Nyctanthes arbor–tristis* L. revealed that about 20 phytochemicals present out of the 21 phytochemicals were tested in the various solvent extract like Ethanol, Petroleum ether, Chloroform. In petroleum ether the 8 phytochemicals were found. The chloroform extract shows the presence of 11 phytochemicals and in ethanol 3 phytochemicals were found like carbohydrate, alkaloid and cardiac glycoside. All the solvent extract exhibits the occurrence of Carbohydrates, Alkaloids, Saponins while Emodin was totally absent in all kinds of extract. The compounds like alkaloids, glycosides, saponins, steroids, flavonoids have expectorant (cough out) action which is very useful in the management of upper respiratory tract inflammation. During performance of TLC, the extracted solvents were used in reducing sequence of polarity. In each one of them extract many of the solvents with their polarity the active secondary metabolites were observed. Coloured spots have been seen directly when

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performed mobile phase versus the stationary phase in TLC chamber. Apart from this the colourless secondary metabolites were visualized by spraying the TLC plates with specific reagent like Ammonia, Iodine regent and Dragandroff's reagent. The outcome of the performance of TLC profile showed that there are no similarities of Retention Factor (Rf) values among the plant extracts. On the basis of that finding all types of extract have different concentration of different kinds of phytochemicals as well as their rate of dissolution in different extracts.

Keywords: Phytochemicals; medicinal uses; bioactive compounds; TLC profile.

1. INTRODUCTION

The World Health Organization (WHO) reported by, greater than 80% of the total world population of expanding countries are depends on the traditional medicines that isolated from plant parts for the body. NAT plant normally grows in tropical and subtropical regions of the various countries in over all the world. The numerous phytochemicals are being discovered with proven biological functions. However, the consequences of taking a complete plant as medication are unknown because a single plant contains a large variety of phytochemicals. Furthermore, many plants with medicinal promise still lack comprehensive scientific research to determine their phytochemical components and pharmacological activities [1]. Recently, many researchers have explored the medicinal importance of bioactive phytochemical components of leaf, flower, fruit as well as seed of *Nyctanthes arbortristis* L. [2]. Antibacterial activity of extracts of *Nyctanthes arbortristis* L. prepared with various solvents like chloroform, petroleum ether, butanol, Water, and ethanol were evaluated by agar well diffusion method [3]. It has been attempted for various pharmacological actions such as anti-arthritic, antispasmodic, antibacterial, anti-inflammatory, immunostimulant, anti-diabetic, hepato-protective, antipyretic, anti-allergic and Central Nervous System depressant [4]. Today's, natural products are responsible with regards to partially sanctioned drugs that are now days available [5]. The WHO also supported the use of phytochemical plants for remedial used [6]. Just like that, phytomedicinal plants are far more fascinated in that drug revelation. The phytomedicinal importance of diverge plants sub-sits in its plant derived chemical constituents that build a well define physiological steps in human body [7]. In addition to overdose of minerals than their almost exact quantity daily dose, may generate toxicity in human body [8]. Perilous toxic Phyto metals like Al, As, Hg, Cr, Cd, and Pb can be available in phytomedicinal plants [9]. Today's, people have more alert about the uncertainty companion with the presence of dangerous metals in phytomedicinal plants and their entanglement [10]. The best way of supervision of Osteoarthritis is daily workout and managed diet in routine life. Approximately all body junctures in any way damaged by Osteoarthritis but knee junctures are highly pretentious, traced by the pelvic girdle joints [11]. Osteoarthritis of lower limbs decadence pliability of important organs and cause constraint [12]. The Global grade of Osteoarthritis detailed as the major extensive locomotor system disease in the middle of the globe [13]. It is a most prevalent speculation of joints affliction in approximately or more than 100 million people in the middle of globe having age more than 45 years [14]. and which is more or less than 15% of all locomotory system disorders by WHO centre [15]. The terpenoids presence in phytomedicinal plants were first time proclaimed [16]. It is indispensable by virtue of their correspondence with necessary compounds like vitamin A and could be an enormous medical demand [17].

1.1 Collection of Plant Material

Nyctanthese arbor–tristis L. leaves and stem bark were collected from the wild forests of Wardha and Nagpur District (Latitude 21.1153363 and Longitude 79.0618455). The plants were collected and was authenticated at the Department of Botany, Bajaj College of science, Wardha. (Latitude 20.740043° and Longitude 78.613932°).

List 1. Taxonomic classification: according to APG–IV (Angiosperm Phylogeny Group IV system) 2016

Kingdom	Plantae
Clade I	Tracheophytes
Clade II	Angiosperms
Clade III	Eudicots
Clade IV	Asterids
Order	Lamiales
Family	Oleaceae
Genus	*Nyctanthes*
Species	*Nyctanthes arbor–tristis* L.

Image 1. Collection of plant material from Bajaj

Image 2. (Google location of plant material collection) college of science Wardha)

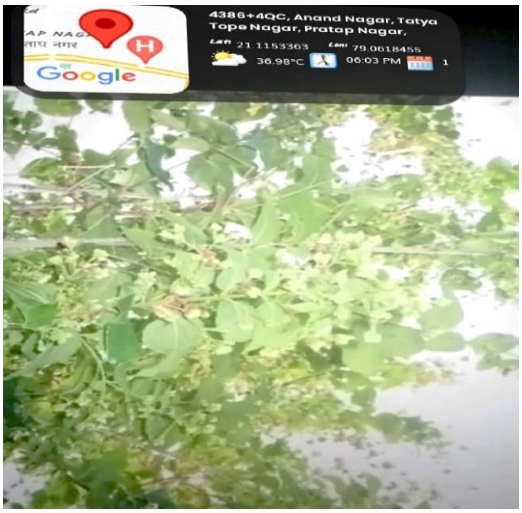

Image 3. Collection of plant material form Nagpur district

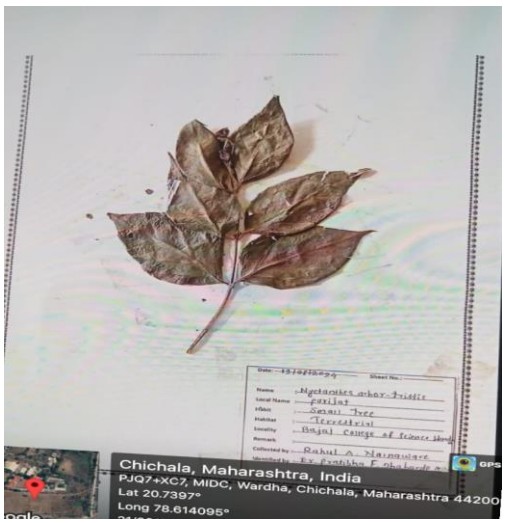

Image 4. Herbarium sheet of *Nyctanthes arbor-tristis* L.

Image 5. Tight container of refrigerator

1.2 Preparation of Plant Extraction

The plant material was processed to prevent the deterioration of secondary metabolites be available in the samples. The NAT plant collected materials washed and cleaned by distilled water, followed by peeling or striping leaves from the stem and desiccate at room (acceptable) temperature up to four weeks to remove moisture content. The dry sample was crushed into powder using mortar and pestle. The dry crushed sample was stored for further analysis. The powder sample of plant material (50/500ml) was extracted successively with ethanol, petroleum ether and chloroform. Allow the mixture to soak for 24 to 48 hours at room temperature, shaking occasionally to ensure through mixing. After soaking, filter the mixture using Whatman's filter paper. The extract was concentrated using a rotary evaporator to recollect the solvent. The extract can be further dried to obtained solid extract by evaporating the remaining solvent completely. Stored the concentrated extract in clean, air tights container in a refrigerator at 4°C. [18-19].

2. MATERIALS AND METHODS

Drying can be done by Artificial and Natural processes.

A) Artificial process: The common method was used in drying the plant material i.e. Hot Air Oven at temperature range in between 40°C to 70°C for 6 to 8 hours.

B) Natural Process: The plant material was dried under sun- drying.

C) Powdering: Powdering of plant material was made by mixer grinder.

D) Methods of Extraction

1. Soxhlet extraction: Soxhlet extraction method: Leaves of selected plants were collected locally. Leaves were washed; air dried under shade and powdered with the help of Grinder. Powdered leaves were weighed and packed in soxhlet. Solvent used for soxhletion was petroleum ether and ethanol. Extraction was continued at the temperature of 35°C till clear solvent was observed in thimble. Extract was concentrated in water bath at 40°C. Concentrated extract was concentrated at 40°C in hot air oven. Concentrated extract was packed in an air tight container. Qualitative Phytochemical screening: Nyctanthes arbor- tristis with petroleum ether extract were subjected to various qualitative tests for the identification of plant constituents present in this species [20].

2. Incubator Orbital shaker:

The incubator orbital shaker was used in the extraction of plant material. The shaker was set at temperature 30° C and humidity range from 20% to 80% with 120 rpm speed per second for 48 hours [21].

Image 6. Soxhlet extraction was used to extract the plant material

Image 7. Incubator orbital shaker

Phytochemical analysis

The phytochemical test for various phytochemicals presents in the extract was carried out using standard methods.

I. Carbohydrate Test

Molisch's test: 2ml of the extract taken with 2 drops of alcoholic solution of α-napthol was added and after mixture properly well shaken further, few drops of conc. H_2SO_4 was added at the edge of the sides of the test tube. The violet ring shows to indicates the sugar is present in the extract.

II. Protein Test

Biuret test: Up to 2ml of filtrate was taken to and 1 drop of 2% $CuSO_4$ solution was added after that 1ml of 95% CH_3CH_2OH was added. Then it was continuous by excess added of KOH. The pink colour appearance which indicates that the protein is confirm.

III. Amino acids Test:

Ninhydrin test: Take 0.5mg of extract with 2 drops of freshly prepared 0.2 Ninhydrin reagents were added and heated. The appearance of purple or pink colour which indicates that the presence of protein, and amino acid in the extract.

IV. Alkaloid Test:

1. Mayer's test: Take few ml of the filtrates of extract and drop of Mayers reagent ware added at the edge of the test tube. A creamy or white precipitate that means test is positive.

2. The Wagner's test (Iodine-potassium iodine reagent) Up to 2ml of extract with few drops of Wagner's reagent the formation of Reddish-brown precipitate which indicates that the alkaloids are present.

V. Glycosides Test:

Borntrager's test: Take 2ml of filtrate and add 3 ml of chloroform with well shake for few minutes. Then chloroform layer had separated after that 10% ammonium solution were added into it. Pink colour indicates that the glycosides present.

VI. Cardiac glycosides

Test (Keller Killani test): Up to 5ml of extract mixed to 2ml of glacial acetic acid with a drop of ferric chloride sol^n. were added continuously by the add^n. of 1ml of concentrated H_2SO_4. The brown ring in the interface indicates that the presence of deoxy sugars of cardenoloides in the compound. The violet ring appeared below the brown ring whilst acetic acid layer a (green ring) form just side by side approach the layer in test tube.

VII. Phenol Test

Gelatine test: Up to 5ml extract, 2ml of 1% solution of gelatine containing 10% of NaCl was added. Appearance of white precipitate which indicates that phenol is present.

VIII. Tannins Test:

Ferric chloride test: The tannin present in the extract were done by taking 5ml of extract in a test tube side by side added a drop of 0.1% Ferric chloride solution. A bluish black colour / brownish green precipitation which indicates that tannin is present.

IX. Flavonoids Test

This Methods was used to determine the flavonoids present in the plant sample or extract [22]. 5 ml diluted ammonium sol^{n}. were added to a portion of the aqueous filtrate of each plant extract continuously by add^{n}. of concentrated H2SO4. A yellow colouration observed in sample of each extract which indicated that the flavonoids are present. After few minutes yellow colouration was disappeared at standing position.

X. Steroids Test:

Salkowski test: Up to 10 ml of chloroform were added in 1ml of each sample extract in the test tube. After that 10ml concentrated sulphuric acid was dissolved in this test tube. 2 layers were formed. The lowering layer shows yellow colour as supports with green colour fluorescence even as uppermost layer showing reddish colour. The formation of these two layers which indicates that the steroids were present.

B. Thin layer chromatography:

Chromatographical representations were performed on Thin Layer Chromatography Silica Gel 60F254, Aluminium Sheets of Size 6.5 cm x 5 cm (Merck, Germany). The Aqueous and Methanol Extracts of *Nyctanthes arbor–tristis* L. Were Resuspended in Respective Solvents at a Concentration of 100 mg/ml and used for TLC Analysis. The Extracts of 10 µL were Manually Applied to the Plate as Spot Using the Hamilton 50 µL Syringe, Positioned 1cm from the Bottom and 1.5 cm from Side of the Plate, On Each Plate with Four Applications. The Space Between Two Spot was 1.5 cm. The Spotted TLC Plates were Subjected to Development in the TLC Developing Glass Chamber Pre-saturated with Different Solvent as Mobile phase. The Developing Distance was 80mm and the developed plate was removed from the chamber and dried over the hot plate for the Evaporation of Solvents used as Mobile Phase. The TLC Plates were Transferred into the Mobile Phase Consisting of Numerous Blending of Solvent Systems of Different Polarity such as $CHCl+_{3,}$ $C_6H_{14,}$ C_2H_5OH (4:2:4), $CHCl_{3,}$ $C_6H_{14,}$ H_2O, PE, $C_4H_8O_2$ (4:2:2:4), PE, $CHCl_{3,}$ C_6H_{14} (4:3:3) and permit to move on the receptive adsorbent silica gel. The consequent spots were observed under UV visible light and spraying by iodine reagent stain. The compute of the distance a compound travelled is considered as the retention factor (R_f), which was evaluated by using the following TLC formula: The Different Spots were Developed in each Solvent System which was Identified by means of Post-development Derivatisation with Different Spraying Agents like Iodine, Ammonia and Dragendorff's Reagents. The appearing a coloured spots by using this reagent with calculations of different Rf values on the TLC plates in different extracts indicates the desired phytochemicals was found in the extract of plants. The particular phytochemical of the plants shows the specific colour, on the basis of that coloured spots place on the TLC plates the Rf value was calculated and the specific phytochemical shows a particular Rf values which indicates that the plant contains such type of phytochemicals. On the basis of this investigations, we confirmed that the specific type of phytochemicals found in NAT plant.

Retention factor (R_f) values was determined by following formula:

$$R_f = \frac{Distance\ travelled\ by\ solute\ (cm)}{Distance\ travelled\ by\ solvent\ (cm)}$$

3. RESULTS AND DISCUSSION

The following Table 1 are the results obtained.

The Successful evaluation of botanical phytocompounds from plant sample was largely dependent on the type of solvent were used in the extraction procedure. Today's phytochemical study on the plant of Nyctanthes arbor-tristis using different solvent containing extracts betray the alkaloids, glycosides, flavonoids, phenols, saponins, steroids, amino acids, tannins, terpenoids, quinones, anthraquinones and coumarin was present. Next to ethanol and showed the presence of rich variety of secondary metabolites. The results revealed that *Nyctanthes arbor–tristis* L. leaves and Bark as a rich source of bioactive compounds. These findings suggested that *Nyctanthes arbor–tristis* L. leaves and Bark have a potential source of natural, antioxidant which have great importance as therapeutic agent for many chronic diseases.

Table 1. Presence (+Ve) or Absence (–Ve) of Primary and Secondary metabolites with Different solvents in the Extract of *Nyctanthes arbor –tristis* L.

Sr. No.	Name of sample	Ethanol	Petroleum Ether	Chloroform
I	Carbohydrate	+ve	+ve	+ve
II	Amino acid	–ve	+ve	–ve
III	Protein	–ve	+ve	–ve
IV	Steroids	–ve	+ve	–ve
V	Alkaloids	+ve	+ve	+ve
VI	Flavonoids	–ve	+ve	–ve
VII	Glycosides	–ve	–ve	+ve
VIII	Cardiac glycosides	+ve	–ve	+ve
IX	Tannins	–ve	+ve	+ve
X	Phenols	–ve	+ve	+ve

Table 2. Phytochemical evaluation and TLC Profile Perform by using chromatographic Rf values of different solvent extracts of leaves and Bark in *Nyctanthes arbor–tristis* L.

Extract	Solvent System	Normal slide Number of spots	R_f values	UV light Number of spots	R_f values	Iodine and Dragendorff" s Reagent, Ammonia Number of spots	R_f values
NATCE	$CHCL_3$, C_6H_{14}, C_2H_5OH	1 green, 1 yellow	0.231, 0.136	2 light green, 1 dark green, 1 yellow green	0.316, 0.366, 0.533, 0.716	1 light green, 1 dark yellow, 1 brown, 1dark green	0.316, 0.312, 0.716, 0.366
NATWE	(4:2:4)	1 yellow	0.312	1 dark yellow, 1 brown	0.483, 0.716	1 light green, 1 light yellow	0.316, 0.76
NATPEE		2 green, 2 yellow	0.423, 0.321, 0.521, 0.211	2 dark green, 3 dark yellow, 1 light yellow	0.266, 0.35, 0.483, 0.65, 0.716, 0.75	1 dark green, 2 brown, 1 light yellow, 1 dark yellow	0.312, 0.713, 0.716, 0.76, 0.483
NATEAE		1 brown, 2 yellow	0.412, 0.421, 0.321, 0.134	2 dark yellow, 2 dark green, 1 brown	0.216, 0.516, 0.55, 0.683, 0.716	1 dark green, 1 light green, 1 dark yellow	0.316, 0.366, 0.438
NATEE		1 brown, 1 yellow	0.416, 0.612	1 dark green, 1 dark yellow, 1 light yellow, 1 brown	0.2, 0.35, 0.566, 0.783	1 light green, 1 dark green, 1 orange and brown	0.345, 0.232, 0.266
NATCE	$CHCL_3$, C_6H_{14}, H_2O, PE,	1 green, 1 yellow, 1 brown	0.352, 0.132, 0.326	2 dark yellow, 1 dark yellow, 2 brown	0.15, 0.266, 0.4, 0.566, 0.7	1 dark blue green, 1 dark yellow, 1 light green, 1 light blue	0.766, 0.483, 0.283, 0.416
NATWE	$C_4H_8O_2$ (4:2:2:4)	1 brown	0.423	1 dark yellow, 1 light yellow, 1 light green	0.283, 0.483, 0.766	1 dark blue, 1 light green, 1 dark yellow	0.25, 0.7, 0.483
NATPEE		2 yellow, 1 brown, 1 green	0.521, 0.412, 0.324, 0.231	2 dark green, 1 light green, 1 dark green, 1 brown	0.266, 0.416, 0.616, 0.8, 0.816	1 dark green, 1 dark yellow, 1 light yellow, 1 light green	0.083, 0.116, 0.683, 0.516
NATEAE		2 yellow, 1 brown, 2 green	0.116, 0.45, 0.321, 0.126	2 dark green, 1 dark green, 1 light green, 2 brown	0.083, 0.25, 0.383, 0.55, 0.633, 0.683	1 blue, 1 dark yellow, 1 light yellow, 1 light green	0.683, 0.283, 0.15, 0.65
NATEE		1 green, 1 yellow, 1 brown	0.241, 0.512, 0.116	1 dark green, 1 light yellow, 2 light green, 1 brown	0.283, 0.483, 0.6, 0.7	1 dark green, 1 brown, 1 dark yellow	0.433, 0.733

Extract	Solvent System	Normal slide Number of spots	R_f values	UV light Number of spots	R_f values	Iodine and Dragendorff" s Reagent, Ammonia Number of spots	R_f values
NATCE	PE, $CHCL_3$, C_6H_{14} (4:3:3)	1 yellow, 1 brown	0.321, 0.514	1 light green, 1 dark yellow, 1 brown	0.116, 0.516, 0.683	1 blue, 1 light green, 1 dark green, 2 dark yellow	0.516, 0.866, 0.733, 0.65, 0.35
NATWE		1 brown	0.341	1 dark green, 1 dark yellow	0.283, 0.483	1 brown, 1 dark green, 1 dark yellow	0.55, 0.65, 0.383
NATPEE		1 yellow, 1 green	0.534, 0.162	1 light green, 1 dark green, 2 dark yellow	0.15, 0.35, 0.483, 0.65	1 light blue, 1 light green, 1 dark green, 2 dark yellow, 1 brown	0.25, 0.266, 0.283, 0.6, 0.7, 0.15
NATEAE		2 yellow, 1 green, 1 brown	0.465, 0.112, 0.116, 0.341	2 dark green, 1 light green, 1 dark yellow, 2 brown	0.266, 0.35, 0.433, 0.516, 0.733, 0.866	1 dark green, 1 brown, 2 dark yellow	0.283, 0.416, 0.8, 0.633
NATEE		1 green, 1 yellow, 1 brown	0.172, 0.342, 0.521	2 dark yellow, 1 dark green, 1 light green, 1 brown	0.25, 0.35, 0.383, 0.483, 0.65	1 brown, 1 dark yellow, 1 dark green	0.483, 0.616, 0.55

In addition, the Ethyl Acetate, Petroleum Ether, Chloroform as well as ethanol extracts for *Nyctanthes arbor–tristis* L. leaves and Bark contain a higher content of bioactive compounds, which will be used for future research on this plant.

The Introductory phytochemical screening of *Nyctanthes arbor–tristis* L. revealed that about 20 phytochemicals present over the 21 phytochemicals were tested in the various solvent extract (Ethanol, Petroleum ether, Chloroform), with Emodins was totally absent in all kinds of extract which shows the occurrence of 8 phytochemicals which includes Carbohydrate, Amino acid, Protein, Steroid, Alkaloid, Saponin, Tannin, Terpenoids, Triterpenes, Fatty acids, Resins, Quinones. The chloroform extract shows the presence of 11 phytochemicals which includes, carbohydrate, Alkaloid, Glycoside, Cardiac glycoside, Saponins, Tannin, Fatty acid, Coumarins, Phenol, Quinone, Resin. All the solvent extract exhibits the occurrence of Carbohydrates, Alkaloids, Saponins while Emodins was totally absent in all kinds of extract (Table 1). TLC is used in separating various phytochemicals based on their polarity and interaction with the stationary or the standing phase and the moving or mobile phase. Different classes of compounds in *Nyctanthes arbor–tristis* L. such as Alkaloid, Flavonoids, Tannins, Saponins, Terpenoids and Phenolic compounds which exhibit different R_f values. The R_f values are used to characterize the different phytochemical compounds which are present in the extracts. The phytochemical compounds will be obtained by different Rf values, due to their polarity. The polar compound will have strong interaction with stationary phase on TLC and travel shorter path. Meanwhile, the non-polar compounds have weaker interaction with stationary phase and travel longer path. The plates below show the developed chromatogram which resulted from the various solvent extracts and the R_f values of the constituents separated (Table 2).

The indispensable class of compounds were clearly identified on chromatogram. The different coloured spots in CHCL3, C6H14, C2H5OH (4:2:4) solvent system 2 light green, 1 dark green, 1 yellow green with 0.316, 0.366, 0.533, 0.716 Rf values in NATCE, 1 dark yellow, 1 brown with 0.483, 0.716 Rf values in NATWE, 2 dark green, 3 dark yellow, 1 light yellow with 0.266, 0.35, 0.483, 0.65, 0.716, 0.75 Rf values in NATPEE, 2 dark yellow, 2 dark green, 1 brown with 0.216, 0.516, 0.55, 0.683, 0.716 Rf values in NATEAE, 1 dark green, 1 dark yellow, 1 light yellow, 1 brown with 0.2, 0.35, 0.566, 0.783 Rf values in NATEE. In CHCL3, C6H14, H2O, PE, C4H8O2 (4:2:2:4) solvent system 2 dark yellow, 1 dark yellow, 2 brown with 0.15, 0.266, 0.4, 0.566, 0.7 Rf values in NATCE, 1 dark yellow, 1 light yellow, 1 light green with 0.283, 0.483, 0.766 Rf values in NATWE, 2 dark green, 1 light green, 1 dark green, 1 brown with 0.266, 0.416, 0.616, 0.8, 0.816 Rf values in NATPEE, 2 dark green, 1 dark green, 1 light green, 2 brown with 0.083, 0.25, 0.383, 0.55, 0.633, 0.683 Rf values in NATEAE, 1 dark green, 1 light yellow, 2 light green, 1 brown with 0.283, 0.483, 0.6, 0.7 Rf values in NATEE. In PE, CHCL3, C6H14 (4:3:3) solvent system 1 light green, 1 dark yellow, 1 brown with 0.116, 0.516, 0.683 Rf values in NATCE, 1 dark green, 1 dark yellow with 0.283, 0.483 Rf values in NATWE, 1 light green, 1 dark green, 2 dark yellow with 0.15, 0.35, 0.483, 0.65 Rf value in NATPEE, 2 dark green, 1 light green, 1 dark yellow, 2 brown with 0.266, 0.35, 0.433, 0.516, 0.733, 0.866 Rf value in NATEAE, 2 dark yellow, 1 dark green, 1 light green, 1 brown with 0.25, 0.35, 0.383, 0.483, 0.65 Rf values in NATEE which was observed by using UV-light confirmed that the desired phytochemicals present in the plant extract.

In CHCL3, C6H14, C2H5OH (4:2:4) solvent system, 1 light green, 1 dark yellow, 1 brown, 1dark green with 0.316, 0.312, 0.716, 0.366 Rf values in NATCE, 1 light green, 1 light yellow with 0.316, 0.76 Rf values in NATWE, 1 dark green, 2 brown, 1 light yellow, 1 dark yellow with 0.312, 0.713, 0.716, 0.76, 0.483 Rf values in NATPEE, 1 dark green, 1 light green, 1 dark yellow with 0.316, 0.366, 0.438 Rf values in NATEAE, 1 light green, 1 dark green, 1 orange and brown with 0.345, 0.232, 0.266 Rf values in NATEE.

In CHCL3, C6H14, H2O, PE, C4H8O2 (4:2:2:4) solvent system 1 dark blue green, 1 dark yellow, 1 light green, 1 light blue with 0.766, 0.483, 0.283, 0.416 Rf values in NATCE, 1 dark blue, 1 light green, 1 dark yellow with 0.25, 0.7, 0.483 Rf values in NATWE, 1 dark green, 1 dark yellow, 1 light yellow, 1 light green with 0.083, 0.116, 0.683, 0.516 Rf values in NATPEE, 1 blue, 1 dark yellow, 1 light yellow, 1 light green with 0.683, 0.283, 0.15, 0.65 Rf values in NATEAE, 1 dark green, 1 brown, 1 dark yellow with 0.433, 0.733 Rf values in NATEE.

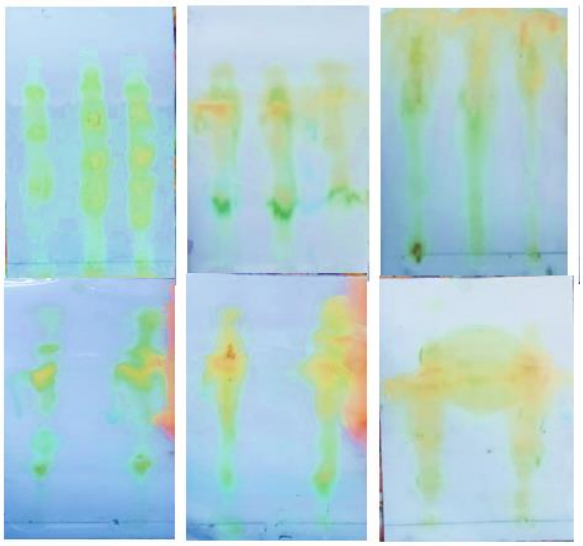

Image 8. TLC profile of *Nyctanthes arbor–tristis* L. Different solvent systems examined under UV light to find phytochemicals found in the extracts. (A) $CHCL_3$, C_6H_{14}, C_2H_5OH (4:2:4) B) $CHCL_3$, C_6H_{14}, H_2O, PE, $C_4H_8O_2$ (4:2:2:4) (C) PE, $CHCL_3$, C_6H_{14} (4:3:3) NATCE: *Nyctanthes arbor–tristis* L. Chloroform Extract, NATWE: *Nyctanthes arbor–tristis* L. Water Extract, NATPEE: *Nyctanthes arbor–tristis* L. Petroleum Ether Extract, NATEAE: *Nyctanthes arbor–tristis* L. Ethyl Acetate Extract, NATEE: *Nyctanthes arbor–tristis* L. Ethanol Extrac.

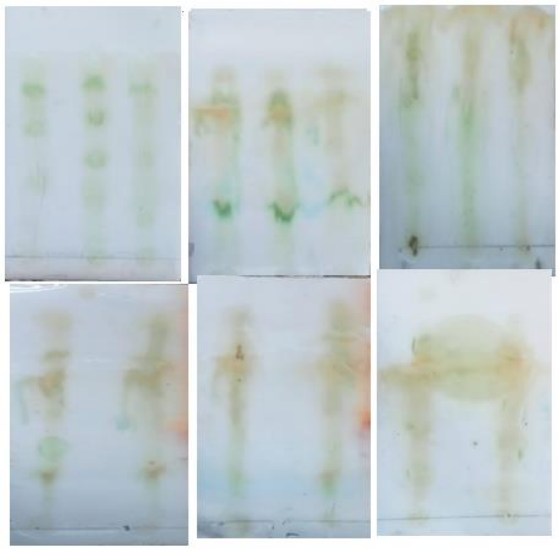

Image 9. TLC profile of *Nyctanthes arbor–tristis* L. Different solvent systems shows visualised spots to find phytochemicals found in the extracts. (A) $CHCL_3$, C_6H_{14}, C_2H_5OH (4:2:4) B) $CHCL_3$, C_6H_{14}, H_2O, PE, $C_4H_8O_2$ (4:2:2:4) (C) PE, $CHCL_3$, C_6H_{14} (4:3:3) NATCE: *Nyctanthes arbor–tristis* L. Chloroform Extract, NATWE: *Nyctanthes arbor–tristis* L. Water Extract, NATPEE: *Nyctanthes arbor–tristis* L. Petroleum Ether Extract, NATEAE: *Nyctanthes arbor–tristis* L. Ethyl Acetate Extract, NATEE: *Nyctanthes arbor–tristis* L. Ethanol Extract

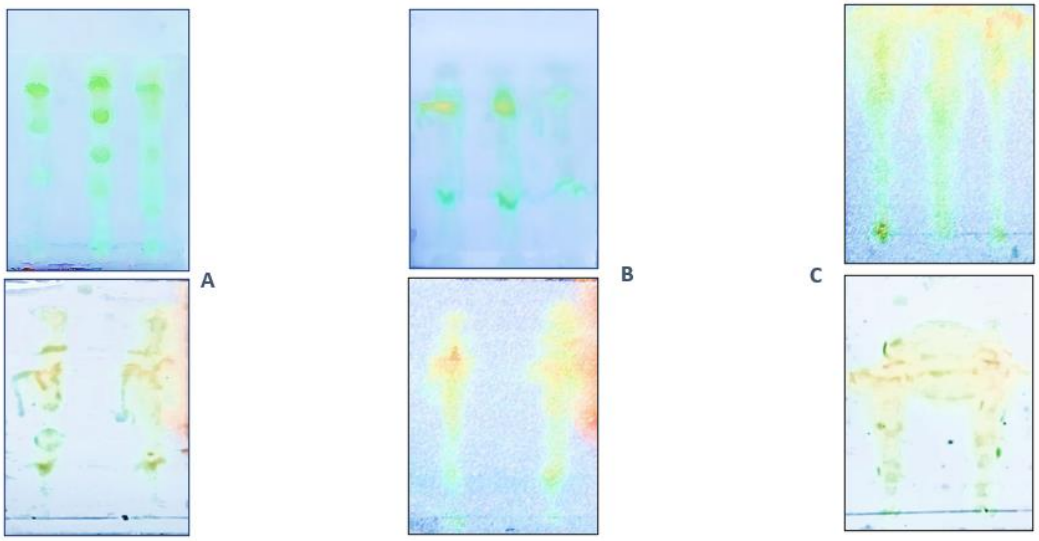

Image 10. TLC profile of *Nyctanthes arbor–tristis* L. Different solvent systems examined by spraying Iodine reagent to find phytochemicals present in the extracts. (A) $CHCL_3$, C_6H_{14}, C_2H_5OH (4:2:4) (B) $CHCL_3$, C_6H_{14}, H_2O, PE, $C_4H_8O_2$ (4:2:2:4) (C) PE, $CHCL_3$, C_6H_{14} (4:3:3) NATCE: *Nyctanthes arbor–tristis* L. Chloroform Extract, NATWE: *Nyctanthes arbor–tristis* L. Water Extract, NATPEE: *Nyctanthes arbor–tristis* L. Petroleum Ether Extract, NATEAE: *Nyctanthes arbor–tristis* L. Ethyl Acetate Extract, NATEE: *Nyctanthes arbor–tristis* L. Ethanol Extract

In PE, CHCL3, C6H14 (4:3:3) solvent system 1 blue, 1 light green, 1 dark green, 2 dark yellow with 0.516, 0.866, 0.733, 0.65, 0.35 Rf values in NATCE, 1 brown, 1 dark green, 1 dark yellow with 0.55, 0.65, 0.383 Rf values in NATWE, 1 light blue, 1 light green, 1 dark green, 2 dark yellow, 1 brown with 0.25, 0.266, 0.283, 0.6, 0.7, 0.15 Rf values in NATPEE, 1 dark green, 1 brown, 2 dark yellow with 0.283, 0.416, 0.8, 0.633 Rf values in NATEAE, 1 brown, 1 dark yellow, 1 dark green with 0.483, 0.616, 0.55 Rf values in NATEE, by using a specific reagents like ammonia, dragendorff's reagent and iodine reagent which indicates that the presence desired phytochemicals like tannins (dark green colour), flavonoid (dark yellow colour), alkaloid (brown colour), light yellow green colour indicates the occurrence of steroids, blue green colour signify the occurrence of phytosterols (Image 9), this clearly indicates that the most necessary secondary metabolites or phytochemicals are found in the bark and leaves extract in *Nyctanthes arbor–tristis* L. The compounds like alkaloids, glycosides, saponins, steroids, flavonoids Saponins, a special class of glycosides (Images 10 and 4), have expectorant action which is very useful in the management of upper respiratory tract inflammation. The extracted solvents used are reducing sequence of polarity there in each one of them extract many of the solvents with their polarity depend on the active secondary metabolites containing in the plant. Coloured compounds have been seen directly when the stationary phase absorbed specific reagent even as colourless categories were encountered by spraying the plate with specific reagent which is produced colour-full areas at the spots, which are they absorbed [23]. The following spraying systems were used. The Alkaloid present in the sample were detected by spraying the freshly prepared Dragendorff" s reagent, Ammonia, and Iodine on TLC plates. The above Rf values of specific phytochemicals tally with the earlier researches to confined the accurate prediction of particular phytochemical.

A positive reaction in the chromatogram (orange and brown) was confirmatory evidence that the alkaloid was present in the extract [24]. The presence of flavonoid was detected by the absorption of colour on the plate a positive reaction was showed of yellow colour spot by using ammonia [25]. On the basis of this analysis the Alkaloid, Tannins and Saponins contents are responsible for its antibacterial activity [26]. The occurrence of Phenolic group in the plants keep safe them from Microbial attack, Insect and Herbivores damages [27]. The number of active chemical compounds also contain other functional characteristic features like Anti-inflammatory, Antimutagenic, Hypocholestemic and Antiplatelet Aggregation Properties [28]. Such phytochemical necessary compounds carried out their specific activity by Combining with Protein, Lipids or any other Components of the Bacterial Cell Membrane which are associated to many indispensable physiological functions by there, disrupting the characters as well as functional behaviour of the cell membrane [29]. Cardiac Glycosides is also Occurs to useful in treatment of Heart failure and Supraventricular Arrhythmias [30].

The necessary class of Phytoconstituents known as Cardiac Glycosides have very important role in Medicine because their proper actions on Heart and used in Cardiac Insufficiency [31]. It is a particular action helps in the treatment of Congestive Heart Failure [32]. Moreover, Glycosides, Flavonoids and Tannins have Hypoglycaemic Activities [33]. Saponins is a best class of compound which is an Active Constituents with a marked Hormonal Activity, support in the Absorption of Nutrients [34]. In accordance with previous phytochemical studies, phytoconstituents like steroids, flavonoids, alkaloids, terpenoids and tannins have been shown to possess anti-inflammatory and analgesic activity [35,36]. The phytochemical analysis of N. arbor-tristis and A. scholaris extracts confirmed the presence of steroids, flavonoids, tannins, glycosides, alkaloids and terpenes.

4. CONCLUSION

This study of Phytochemical screening of Petroleum ether and chloroform extracts of *Nyctanthes arbortristis* L. revealed more concentrated phytochemicals in Petroleum ether and chloroform extracts when compared to that of ethanol extracts of the plant during which was formed during various phytochemical tests by using standard protocol. When the actual demonstration was done of different phytochemicals in different solvents by using Thin Layer Chromatography which revealed that every phytochemical showed different absorption rate in different solvent to formed different R_f values and based on the Rf values calculations was found that the phytochemicals is present in plants which was shown above. It has been analysed and conclude that the plant containing phytochemicals present in different

concentrations in plants based on their rate of absorption on TLC plates by using different reagents to forms a cleared coloured spots at different solvent system concentration. This Study Confirmed that the use of *Nyctanthes arbortristis* L. Plant Material Supply for the pharmaceutical industry and demonstrated a suitable control of many biochemical and physiological activities like anti-bacterial, anti-arthritic, anti-malarial as well as anti-cancerous. Current study validated the traditional use of N. arbortristis in arthritis, rheumatism, and inflammatory disorders. The data showed that NAT extracts possessed antiarthritic property which was evident by inhibition of arthritic development during the course of treatment. These, inferences were further validated by suppression of paw edema, infiltration of inflammatory cells, bone erosion, and pannus formation found in this study. Treatment with NAT nearly normalized hematological parameters and was found safe in terms of hepatotoxicity and nephrotoxicity. Ethyl acetate extract showed the highest inhibition of paw edema among all extracts. Terpenes, terpenoids, fatty acids, and iridoid glycosides were majorly identified constituents in ethyl acetate extract. The antiarthritic activity might be attributed to the presence of these phytochemical constituents; however, further studies are required for isolation and confirmation of pharmacological activities. Combinational effect of various herbal plants along with NAT can be examined which could provide best alternatives for various ailments as was examined in case of wound healing activity of ethanolic extract of *Nyctanthes arbor-tristis*. High potential of plant in management of various ailments, easy availability and requirement of no special condition for its collection and cultivation make it a plant of clinical interest which requires more attention and clinical trials for manufacturing therapeutic preparations that can treat human ailments. Various synthetic chemicals are being widely used for treatment of different diseases but encompass adverse side effects. Due to these side effects of the drugs, various alternatives are being explored by the researchers and for the same plants are being studied. One such plant is *Nyctanthes arbor-tristis* (NAT). The broad-spectrum medicinal use of NAT is the matter of interest for the researchers. The anti-arthritic, antispasmodic, antibacterial, anti-inflammatory, immunostimulant, antidiabetic, hepatoprotective, antioxidant, antimicrobial, anthelminthic, antileishmanial, anti-pyretic, anti-allergic, antiviral and CNS depressant activities of the plant show its great value in the field of medicine. Considering NAT for treatment of various ailments can provide effective and efficient alternative against chemical drugs, which have no side effects and are cost-effective. Further attention and research are required for identification and characterisation of bioactive compound(s) responsible for the biological activity of plant and the elucidation of the mechanism of action in many cases. The toxicity of the various extracts should be considered, as human studies for safety and efficacy of the extracts for long term administration are needed to be proved.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declared that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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