Phytochemical Study, Antimicrobial Analysis and Partial Characterization of Medicinal plant: *Nyctanthes arbor- tristis*

A DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE AWARD OF DEGREE
OF

MASTER OF TECHNOLOGY

IN

INDUSTRIAL BIOTECHNOLOGY

Submitted By

SHEFALI ANJANKAR

(2K16/IBT/09)

Under the guidance of

Prof. Jai Gopal Sharma
Professor & Head

DEPARTMENT OF BIOTECHNOLOGY

Delhi Technological University

(Formerly Delhi College of Engineering)

Bawana Road, Delhi-110042

JUNE, 2018

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Bawana Road, Delhi-110042

CANDIDATE'S DECLARATION

I, SHEFALI ANJANKAR, Roll No. 2K16/IBT/09 of M. Tech. (Industrial Biotechnology), hereby declare that the project Dissertation title "Phytochemical Study, Antimicrobial Analysis and Partial Characterization of Medicinal plant: *Nyctanthes arbor-tristis*" which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Technology, is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any Degree, Diploma Associateship, Fellowship or other similar title or recognition.

Place, Delhi SHEFALI ANJANKAR

Date:

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Bawana Road, Delhi-110042

CERTIFICATE

I hereby certify that the Project Dissertation titled "Phytochemical Study, Antimicrobial Analysis and Partial Characterization of Medicinal plant: *Nyctanthes arbor-tristis*'' by SHEFALI ANJANKAR, Roll No. 2K16/IBT/09, Department of Biotechnology, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Technology, is a record of the project work carried out by the student under my supervision. To the best of my knowledge this work has not been submitted or full for any Degree or Diploma to this University or elsewhere.

Place, Delhi
Date:

Prof. Jai Gopal Sharma
Professor & Head
Department of Biotechnology
Delhi Technological University
Delhi, INDIA

ABSTRACT

Ayurveda is one of the oldest systems of medicine that uses plants and their extract for treatment and management of various diseased states. *Nyctanthes arbor-tristis* is well known Indian medicinal plant and the leaf extracts are used at a large extent in Indian traditional medicine. Present investigation deals with the qualitative analysis and quantitative estimation of phytochemicals in leaves of important medicinal *Nyctanthes arbor – tristis* plant. Qualitative analysis was carried out to identify the different classes of secondary metabolites in various chemical extracts such as water and methanol. Qualitative analysis of the extracts proved the presence of phytochemicals such as Flavonoids, Carbohydrate, Terpenoid, Phenols, Saponins, Tannins, Steroids and Glycosides. As per quantitative estimation we found that the Protein (19.22%) and Tannin (13.1%) are present higher than other compound in plant extracts. Phytochemicals like Flavonoid, Glycoside, Tannic acid, have been reported for significant hepatoprotective, antiviral, antifungal, antipyretic, antimalarial, antibacterial, anti-inflammatory and antioxidant activities. Herein we describe the synthesis of Fe and Cu nanoparticles using plant extract, which offers an efficient, inexpensive, and environmentally friendly method to produce nanoparticles and was characterized by Scanning electron microscope (SEM). The prepared nanoparticles were found to have better antibacterial and antifungal activity than the sample (water and methanol) leaf extract. *Nyctanthes* leaf extract were studied to determine the quantitative composition of amino acids using Amino Acid Analyzer. Also fatty acid quantitative composition was determined by Gas Chromatography (GC). The aqueous and methanol leaf extract were studied using TLC. Saponins were extracted from methanol leaf extract and were studied and quantified using HPLC.

ACKNOWLEDGEMENT

I would like to specially thank and show my gratitude towards my mentor, **Dr. Jai Gopal Sharma**, Professor and Head of Department of Biotechnology, for giving me this opportunity to undertake project training.

I would also like to thank **Mr. Mayank Raj Bhardwaj**, Founder and CEO of Rapture Biotech who gave me the opportunity to carry out the project. My project guide **Dr. Vinod Kumar Gupta,** Technical Head in Rapture Biotech is also to be thanked for giving me this project and helping with all the necessary resources and guidance.

I am highly obliged towards **Dr. Rajkumar Bidlan** and **Mr. Satish Kumar**, for guiding me and supporting me and giving never-ending inspiration and motivation throughout the project.

Lastly, I would like to thank my parents and friends for supporting me throughout.

SHEFALI ANJANKAR
M.Tech (IBT), DTU
Roll No. 2K16/IBT/09

CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

The term medicinal plants fuse distinctive kinds of plants which are used as a piece of herbalism and a portion of these plants have a therapeutic property. Medicinal plants are the "spine" of traditional medicine. The term herb has come from latin word ′herba′ and an old French word ′herbe′. Medicinal plant has been used for medicinal purpose since the ancient time. Nowadays, about 3.3 billion people in less developed countries used medicinal plant. There are around 17,000 sorts of higher plants, of which about 8,000 species, are seen as medicinally important and used by town gatherings, particularly tribal groups, or in traditional therapeutic frameworks, for instance, the Ayurveda [1-10]. The usage of conventional solution and therapeutic plants in most developing nations, as a reason for the upkeep of good wellbeing, has been generally seen by UNESCO, 1996 [11]. There is much more evidence in Unani Hakims, Indian Vadis and European culture. A great part of the therapeutic utilization of the plant is by all accounts created through perception of wild creatures, inborn individuals and experimentation technique. Medicinal plant consists of rich source of components that could be utilized in modern drug development.

Ascend in populace, lacking supply of medications, expanding expense of medicines, negative effects of several synthetic drugs and resistance development to currently used drugs for infectious diseases have prompted expanded utilization of plant materials as a wellspring of drugs for a wide assortment of human illnesses. As of late, it was evaluated by WHO (World Health Organization) that 80 percent of individuals overall depend on natural prescriptions for some part of their essential medicinal service needs. As per WHO, it is said that around 21,000 plant species have the potential for being utilized as therapeutic plants. It has been generally figured, that plant drugs constitute as much as 25% of the aggregate medications in developed nations, while 80% commitment in developing nations, for example, India and China. Accordingly, the monetary significance of therapeutic plants is more in nations like India than rest of the world.

Treatment with therapeutic plants is viewed as exceptionally safe on the grounds that there is no or less reactions and is likewise eco-accommodating and locally accessible. These cures are as a team with nature, which is one of the greatest preferred standpoint. The vital actuality is that the utilization of herbal medicines is independent of all ages and the genders. This is

the motivation behind why natural treatment is expanding its prominence everywhere throughout the globe.

Plants have hundreds of chemical compound for defense against fungi, insects, disease and herbivorous animals'. The restorative impacts of plants are because of the nearness of metabolites particularly secondary metabolites delivered by plant species. Plant metabolites include: essential metabolites and secondary metabolites. Phytotherapy is the utilization of plants or plant extracts for therapeutic purposes, particularly plants that are not portion of the daily diet. Phytochemistry is the investigation of phytochemicals created in plants, depicting the separation, cleaning, distinguishing identification, and structure of the expansive number of secondary metabolites found in plants. Chlorophyll, amino acids, protein, carbohydrates, nucleotides, fatty acids, steroids and lipids are the primary compounds. Secondary compounds are alkaloid, tannin, terpenoids, phenols etc. Terpenoids have different critical pharmacological activities, for example, anti-inflammatory, anticancer, against malarial, restraint of cholesterol union, anti-viral and against bacterial activities [12]. Terpenoids likewise assume an essential part in drawing in valuable parasites and devour the herbivorous insects [13]. Alkaloids are utilized as anesthetic agents and are found in therapeutic plants [14].

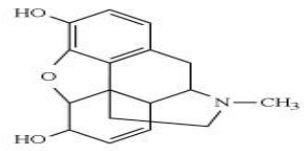
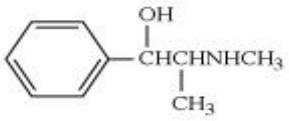
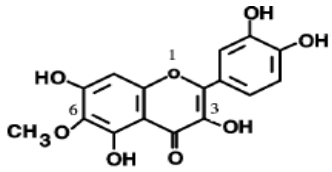
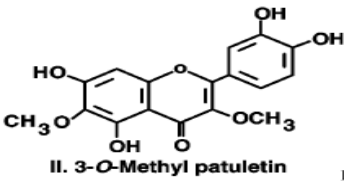
Phytochemicals are the biologically active chemical compounds which provides health benefit to humans. Around 4000 phytochemicals have been recorded and characterized by functions, chemical and physical property. These secondary metabolites have several biological properties such as antimicrobial, antioxidant, modulation of detoxification of enzyme and stimulation of immune system. These medicinal properties are due to the phytochemicals that produces certain definite physiological actions on the human body.

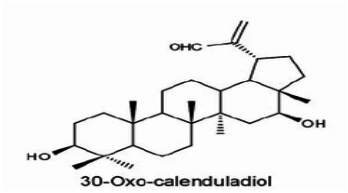
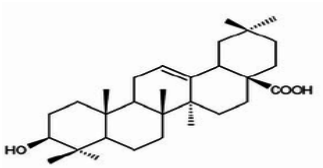
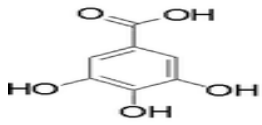
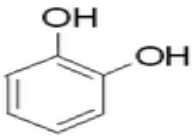
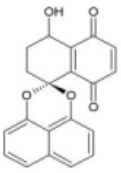
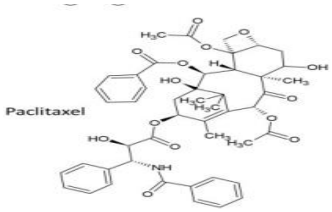
The phytochemical examination of the plants is critical industrially and have wide enthusiasm for pharmaceutical organizations for the generation and improvement of the new medications for curing of different diseases. Phytochemicals have different macronutrients and micronutrients which shield plants from ailment and harm yet in addition add to the plant's color, fragrance and flavor. By and large, a few synthetic concoctions exhibit in plant protect plant cells from natural hazards, for example, contamination, stress, dry season, UV exposure and pathogenic assault are referred to be considered as phytochemicals [15,16].

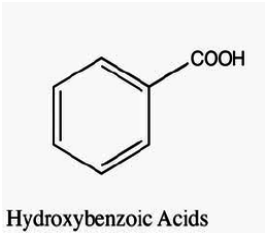
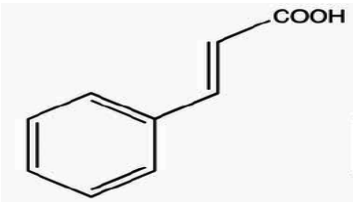
Phytochemicals are insignificant supplements and are consequently not required by the human body for maintaining life, however have a few vital properties to avert or to battle against some basic ailments.

Phytochemicals can kill free radicals and can repress catalysts that would actuate cancer-causing agents, and furthermore enact compounds that detoxify cancer-causing agents.

Table 1: - List of some phytochemicals and their medicinal value

Sr.No	Phytochemical	Medicinal value	Example
1.	Alkaloid	Antimalarial, Antiasthma, anticancer, antibacterial, analgesic.	 morphine  ephedrine
2.	Flavonoid	Antioxidant, anti-inflammatory, cardiovascular disease, antibacterial.	 I. Patuletin  II. 3-*O*-Methyl patuletin
3.	Saponin	Antitumor genic, anti-inflammatory,	

		hypocholestrolemic, immune stimulating.	 30-Oxo-calenduladiol  Oleanolic acid
4.	Tannin	Antioxidant, antimicrobial.	 Gallic acid  Pyrocatechol
5.	Terpenoid	Anti-inflammatory, expectorant, bronchodilator, local antiseptic	 Trichodermin 

6.	Phenols	Infection and degenerative disease, antimicrobial.	 Hydroxybenzoic Acids  Hydroxycinnamic Acids

Phenolic mixes are a considerable and complex group of manufactured constituents found in plants as secondary metabolites, and have a basic part as resistance compounds. Phenolics show a couple of properties which are essential to individuals and its cell reinforcement properties are fundamental in choosing their part as guaranteeing authorities against free radical - mediated illness forms.

Flavonoids are the greatest gathering of plant phenols and the most thought secondary metabolite [17,18]. Flavonoids have various natural properties including antimicrobial, cytotoxicity, protein obstruction, oestrogenic development, hostile to hypersensitive action, cell reinforcement action, vascular activity, antitumor activity and likewise moderating yet the best-property of relatively every gathering of flavonoids is their capacity to go about as extraordinary tumor aversion operators which can shield the human body from free radicals and open oxygen species. The farthest point of flavonoids to go about as tumor avoidance agents depends on their molecular structure [19].

Plants which contain tannins can be utilized as astringents, against looseness of the bowels, as diuretics, against stomach and duodenal tumors and furthermore as mitigating, germ-free, cell reinforcement and haemostatic pharmaceuticals [20,21]. Tannins can be utilized as a part of the dyestuff business as caustics for cationic colors (tannin colors), and furthermore in the generation of inks (iron gallate ink). In nourishment industry tannins can be utilized as a part

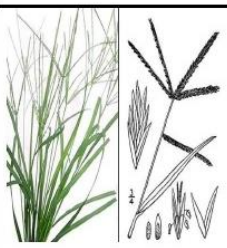
of clearing up wine, brew, and organic product juices. Other industrial uses of tannins include textile dyes, and also added as antioxidants in the fruit juice, beer, and wine industries, and furthermore utilized as coagulants in elastic generation [22].

Alkaloids play an essential part for security and survival of plants as they guarantee their survival against small scale living beings (antibacterial and antifungal exercises), insects and herbivores and furthermore against different plants by methods for allelopathically dynamic synthetic substances [23].

India has 2.4% of world's region with 8% of worldwide bio-diversity. India is one of the 12 mega – diversity hot-spot region of the world. India has 4 biological hotspot I) The western Himalaya, II) The Eastern Himalaya, III) Indo-Burma, IV) Sunderland. There are around 17.000 types of higher plants of which 8000 types of plant are thought to be medicinal. Just around 10% of the known therapeutic plants of India are confined to the non-forest environment.

Table 2: - List of medicinal plants and their action

Plant Name	Ayurvedic name	Family	Action	Plant
1) *Abelomoschus esculentus (Linn) Moench.*	Bhaandi, Bhendaa.	Malvaceae	Immature pods(decoction) – emollient, demulcent and diuretic (in catarrhal affections, ardor urine, dysuria, dysentery). Seeds-antispasmodic. [3,4,7]	
2) *Abies pindrow Royle*	Taalish (related sp.)	Pinaceae	Expectorant, bronchial sedative, antiseptic, carminative.[3,4,7]	
3) *Beta vulgaris Linn.subsp.c*	Palanki	Chenopodiaceae	Leaf- used for burns and bruises, also for disease of spleen and liver. [3,4,7]	

icla(L.) Moq.				
4) *Betula alnoides Buch.-Ham. Ex D.DON.*	Bhojpatra	Betulaceae	Used in supportive therapy of rheumatic ailments.[3,4,7]	
5) *Caesalpinia pulcherrima Sw*	Padangam, Krishnachuda.	Caesalpiniaceae	Leaves- laxative, antipyretic, Flowers- anthelmintic.[3,4,7]	
6) Caesalpinia sappan Linn	Pattanga, Pattraanga, suranga	Casealpiniaceae	Wood decoction- emmenagogue, antidiarrhoeal and skin disease.[3,4,7]	
7) Dactyloctenium aegyptium Beauy,	Takraa,takraahvaa.	Poaceae.	Astringent,bitter tonic, anthelmic.([3,4,7]	

8) Enhydra fluctuans Lour.	Hil-mochikaa	Asteraceae	Leaf-antibilious,laxative,demulcent.[3,4,7]	
9) Fagus sylvatica Linn	-	Fagaceae	Seeds and fatty oil- used externally in skin disease. Rheumatism and gout.[3,4,7]	
10) Gallum aparine Linn.	-	Rubiaceae	Choleretic, stomachic, refrigerant,lymphatic,antiscorbutic.[3,4,7]	
11) Habenaria intermedia D. Don	Riddhi, vriddhi.	Orchidaceae	Nervine and cardiac tonic. ([3,4,7]	

1.1 Scientific classification of plant

Botanical Name: - *Nyctanthes arbor-tritis*

Kingdom: - Planta

Division: - Magnoliophyta

Class: - Magnoliopsida

Order: - Lamiales

Family: - Oleaceae

Genus: - *Nyctanthes*

Species: -*N. arbor-tritis*

Fig1: - *Nyctanthes arbor-tristis* plant

Nyctanthes arbor-tritis plant is once in a while called as the "tree of sorrow", in light of the fact that the blooms lose their brilliance amid day-time. The logical name arbor-tristis itself signifies "sad tree". The blooms are the official flower of the province of West Bengal, India, which is otherwise called Parijat in nearby West Bengal locale in India. *Nyctanthes arbor-tristis* is normally called as night-blooming jasmine or coral- jasmine.

1.2 Plant Morphology

Growth form – Large shrub or little tree up to 9m tall.

Trunk – Grey coloured, rough and flaky bark.

Foilage – Hairy and rough green leaves, intersected and simple, margin entire, measuring about 6 to 12 cm long and 2 to 6.5 cm wide.

Flowers – Fragrant white flowers, 5 to 8 lobed corolla having an orange red centre, usually in group of 2 to 7. Flowers bloom profusely at night and lose their fragrance and colour as day approaches, and dropping off in the morning.

Fruits – Flat, brown, heart-shaped fruit with 2 sections each containing a single seed.

Habitat**:** Usually found on rocky ground in dry slopes and as undergrowth in dry deciduous woodlands.

1.3 Ethnobotanical uses

Medicinal (In Ayurveda, this plant is used to treat sciatica (leg pain caused by lower back problems) and arthritis. This use was provided by Saxena et al. (1984) who found that the leaf extract has anti-inflammatory activity. Puri et al. (1994) demonstrated that flowers, leaves and especially seeds have compounds that stimulate the immune system. Other studies showed that it has antiviral, anti-fungal and anti-protozoan activity, as well as a protective effect on liver cells. Flower and leaf extracts contain compounds with mosquito larvicidal activity (Mathew et al. 2009).

LEAVES

Antibacterial, Antifungal, Anti-inflammatory, Antipyretic, Antioxidant - Sciatica, Rheumatism and various painful conditions, Ringworm.

SEEDS

Anti-bacterial, Anti-fungal. Antileishmanial - Piles, Baldness, Scurvy and Hair tonic.

FLOWER

Contains essential oil - The blooms can be utilized as a wellspring of yellow color for dress.

STEM

Antipyretic, Antioxidant - Relieves headache, relieves swelling of lungs.

1.4 Objective

Investigations were carried out with respect to the following objectives:

1) To perform phytochemical and antimicrobial analysis of *Nyctanthes* leaf extract.
2) Synthesis of Fe and Cu nanoparticle from leaf extract of *Nyctanthes.*
3) Study of secondary metabolites (Saponins) from leaf extract of *Nyctanthes* by using TLC and HPLC.

2. REVIEW OF LITERATURE

Nyctanthes arbor tristis (NAT) is outstanding and most valuable therapeutic plant. It is commonly called Night jasmine, as its flowers give an exceptionally very strong and lovely scent amid entire night. The flowers begin falling after 12 pm and continuously break, the plant seems dull. The generic name *Nyctanthes* has been invented from two Greek words Nykhta (night) and Anthos (flowers). Actually particular name *arbor tristis* implies that it deprived of its brightness amid day-time. NAT is a vast bush or a little tree generally developed in tropical and sub-tropical locales everywhere throughout the world. Leaves, natural products, flowers, stem and barks demonstrate different pharmacological action.

Nyctanthes prefers semi-shady place to grow. Flowering usually occurs from July to October. NAT is found on ground in dry slopes and as undergrowth in dry deciduous woodlands. Soil required for the development of NAT ought to be loamy and pH from 5.6 to 7.5. Plant really acquire conditions which differs from full daylight to incomplete shade and they should be watered routinely, yet doesn't require overwatering.

S. Bansal. et al. done in Vitro Regeneration of a Medicinal Plant *Nyctanthes arbor-tristis*. It was effectively established from axillary bud explants on MS media. Greatest number of numerous shoots was acquired on MS containing benzylaminopurine. Half quality of MS supplemented with Napthaleneacetic corrosive (NAA) gave the greatest recurrence of root commencement. The plantlets were effectively hardened further [27].

Prabodh Satyal. et al. extracted fundamental oil from the leaf and bark of *Nyctanthes arbor-tristis* and then gathered and afterward hydrodistilled and dissected by GC-MS. An aggregate of 26 compounds were recognized in the leaf oil while a sum of 20 compounds were distinguished in the bark oil. Both the leaf and bark oil had comparative amounts of hexadecanoic acid and octadecanoic acid. In any case, the leaf oil likewise comprised of linalool, (E)- phytol and (3 Z)- hexenyl benzoate, which were missing in the bark oil. Other than unsaturated fats, the bark oil displayed essentially unique arrangement with mostly β-eudesmol and other eudesmol isomers. The oil was screened for antimicrobial activity and indicated negligible action against *Bacillus cereus* and *Aspergillus niger* [28].

Sonu Sharma, et al. considered antibacterial action of the different concentrates of bark of *Nyctanthes arbor - tristis* Linn. was assessed by the filter paper disc method. This strategy

depends on the diffusion. The diffusion of an antibiotic from a filter paper disc through the set culture media of a petridish was utilized for contemplate. Development of inoculated micro-organism is restrained altogether in a roundabout territory called as "Zone", around the filter paper disc containing a solution of the anti-biotic and the plant extract [29].

Repon Kumer Saha, et al. explored antibacterial examine by disk diffusion technique. Cell surface receptor restricting test was performed by hemagglutination hindrance measure and hemolysis examine. Methanolic concentrate of the leaves of *Nyctanthes arbor tristis* contains flavanoids and other naturally dynamic compounds. The concentrate demonstrated cancer prevention agent, peroxide searching and add up to diminishing movement and furthermore indicated antibacterial activities against a few strains of microbes. They additionally demonstrated hemaglutination restraint activities and hydrogen peroxide actuated hemolysis hindrance movement in human platelets [30].

K. Priya, Deepak Ganjewala studied the antibacterial action of dried leaf, fruits and seed separate of *Nyctanthes arbor-tristis*. The extract was prepared using chloroform and ethyl acetate using gram positive and gram-negative bacteria. In case of zone of inhibition, bacterial growth was evaluated. The concentrate showed significant antibacterial action against gram negative (*E. coli, Klebsiella pneumonia and Pseudomonas aeruginosa*). They also studied synergetic effect of combined extract and found less activity against individual extract. These activities were due to various secondary compounds i.e phenolics and glycosides which was measured using spectrophotometrically [31].

P. Kannan, D. Ganjewala reached conclusion that the IR range of fruits and seed melanins contrast from each other. The melanins was then described and tried for their stability after incubating at various temperatures and in presence of oxidants ($KMnO_4$ and $K_2Cr_2O_7$) and metal particles (Mg^{++} and Zn^{++}) for a predetermined period. The stability was then checked by estimating changes in the absorbance at 285 nm. The outcomes revealed that the fruit melanin was steadier at 50 ºC while the seed melanin was very steady at 25 ºC. Consequently, their stability of melanin was influenced by oxidants and metal particles [32].

M. Sundrarajan, S. Gowri prepared titanium dioxide NPs from titanium isopropoxide solution by green synthesis method. These NPs were developed using *Nyctanthes* leaf extract. Further NPs were characterized using SEM and XRD and also used particle size analyzer

(PSA). Shape and size of NPs were studied using SEM and XRD result showed crystallinity and purity of NPs. NPs size was found in the range of 100 to 150 nm. These NPs found to have various applications in nanotech and biomedical without having any side effects [33].

The examination was done to halfway describe the unknown alkaloids of stem bark from methanol concentrate of *Nyctanthes arbor-tristis* by Bhavya Bandi, K. Venkatesan, Ujwala Mannarapu, M. Keerthi. At first utilizing dichloromethane extraction crude alkaloids were removed from the stem bark. At that point, TLC characterization was completed utilizing crude alkaloid extract and the presence of alkaloids was then distinguished by Dragendroff's reagent. Further characterization of alkaloids was finished by HPTLC and NMR. In HPTLC five peaks were got having diverse Rf esteems with various percentage of area representing as unknown compounds, so it is further mostly portrayed by 2D-NMR, which demonstrated the presence of new methyl hydroxyl group of alkaloids [34].

R.S Bhadouria et.al. isolated and described phytoconstituents from the ethanolic concentrate of *Nyctanthes arbor-tristis* leaves. Two alkaloid compounds were fractioned and isolated with the assistance of column chromatography from the ethanolic concentrate of the leaves and further structures of this compound were resolved based on spectral and chemical studies [35].

S. Meghashri. et.al. studied biochemical characterization of radical scavenging polyphenols from *Nyctanthes arbor-tristis.* With the help of various established in-vitro system antioxidant activity of the polyphenols of leaves were investigated. Active constituents of polyphenols which is responsible for antioxidant activity were investigated by HPLC [36].

3. MATERIALS AND METHODS

3.1 Collection of plant material: - Green fresh leaves of *Nyctanthes arbor-tristis* was collected from Noida Sector-10, Uttar Pradesh, India during the winter season in the month of January 2018. This leaf sample was washed thoroughly with tap water, some leaves were shade dried and grinded finely in to the powder form and some leaves were taken fresh which was then used for various form of extract preparation.

3.2 Preparation of Extract: - 10g per 100ml of each solvent such as methanol and water extracts of leaf of *Nyctanthes arbor- tristis* was prepared. Leaves were crushed using mortar and pestle. After extraction the extracts were filtered through Whatman No. 42 filter paper. The concentrated concentrates were subjected to qualitative test for the distinguishing proof of different phytochemical constituents according to standard fundamental phytochemical techniques given by Harbone, 1998; Kokate et al. 2006 [37,38].

Fig 2: *Nyctanthes* leaves

Fig 3: Powdered sample

3.3 Phytochemical Tests

A. Qualitative

Phytochemical analysis was done by following standard methods

a) Test for Saponin-To 1ml of plant extract,5ml of Dist. Water was added and the solution was then shaken well for 10-15 sec. Appearance of froth in the upper layer of the solution which remains stable for 2 min, indicates that the Saponins are present.

b) Test for Tannin-To 1ml of plant extract, 5% 1ml ferric chloride was added and mixed. Appearance of dark black or green color indicates that the Tannins are present in the solution.

c) Test for Flavonoid- To 2ml of dilute NaOH, 50µl of sample was added. Appearance of yellowish color of the solution indicates flavonoids present in it.

d) Test for Protein- To 2ml of plant extract, 2ml of copper sulphate and 2ml of NaOH solution was mixed. The color changes to pink which indicates the presence of protein.

e) Test for Steroid- To 1 ml of plant extract, 2ml chloroform was included and 1ml of conc. Hydrochloric acid was additionally included by sides of the test tube. The color of upper- layer changes to reddish & the hydrochloric acid part showed yellow color with green fluorescence. This appearance indicates that the steroids are present in plant sample.

f) Test for Terpenoid- 500µl of sample was mixed with 2ml of methanol and 1ml of chloroform. Then 1ml of H_2SO_4 solution was then added slowly through the wall of test tube. The solution appears reddish brown in color which indicates the presence terpenoids in sample.

g) Test for Quinine- To 500µl plant sample, 2ml of NaOH solution was added slowly. Formation of blue, green or red color specify the presence of quinines.

h) Test for Alkaloid- Addition 1 ml of Wagner's reagent to 1 ml of plant sample. The reddish brown color precipitate of the above mixture indicates the presence of alkaloid.

i) **Test for Coumarin**- To 500 µl of plant sample, 1 ml of NaOH was added. Trace of yellowish appearance of solution shows the presence of Coumarin.

j) **Test for Emodin**- To 500 µl of plant sample, 2 ml of NH4OH and 3 ml of benzene was added and mixed. Appearance of red color solution shows that the emodins are present.

k) **Test for Glycoside-** To 1.250 ml of plant sample add 500 µl of glacial acetic acid. Further one drop of Ferric chloride was added to the above solution. In another test tube add 250 µl of concentrated H_2SO_4. The above mixture was then carefully added to 250 µl of H_2SO_4. Appearance of brown ring indicates the presence of glycosides.

l) **Test for Reducing sugar-** To 1 ml of plant sample, addition of 1 ml of Fehling's reagent and then the solution was heated for 15-20 minutes. Formation of red - color precipitate indicates the reducing sugar is present in sample.

m) **Test for Gum and mucilage-** To 500 µl of sample, addition of 1 ml of absolute alcohol was done. White/cloudy precipitate forms in the solution which proofs the presence of gums and mucilage.

n) **Test for Leucoanthocyanin-** To 500 µl of plant extract, addition of 500 µl isoamyl alcohol was done. The upper- layer of the solution changes to red which specifies that leucoanthocyanin is present.

o) **Test for Phlobatanins**- To 500µl of plant extract, addition of 500 µl of 1% aqueous HCL was done. Red color precipitate forms which proofs that the phlobatanins are present.

p) **Test for Anthocyanin-** To 500 µl of sample, 500 µl of 2 N HCl and 500 µl ammonia was added. The solution appears pink-red which then changes to blue- violet which shows that the sample contains anthocyanin.

q) **Test for Phenolic content-** 3 drops of 1% Ferric chloride was added to 2 ml of plant sample. The solution appears deep violet color with the presence of ferric ions that indicates the presence of Phenolic compounds.

B. Quantitative Test:

1. Estimation of Protein

Theory – The phenolic group of tyrosine and tryptophan buildups exhibit in a protein creates a blue purple color complex with Folin-Ciocalteau reagent, which comprises of sodium tungstate molybdate and phosphate. This complex gives maximum absorption in the region of 660 nm wavelength. The potency of the color relies on the amount of these aromatic amino acids present in a protein and fluctuates appropriately with various protein

Reagents –

1) Reagent A: 2% Na_2CO_3 in 0.1 N NaOH
2) Reagent B: 1% NaK Tartrate in H_2O
3) Reagent C: 0.5% $CuSO_4$.5H2O in H_2O
4) Reagent 1: 48 ml of Reagent A, 1 ml of Reagent B, 1 ml of Reagent C
5) Reagent 2: 1 part Folin [2N]: 1part water
6) BSA standard: 1mg/ml

Procedure –

1) 0, 30, 60, 120 and 240 µg/ml of BSA standard solution is taken in 4 test tubes and making up the volume to 1ml using distilled - water.
2) 100 µl each of Sample 1 and Sample 2 taken in 2 test-tubes and making up the volume to 1ml using distilled water.
3) Then Reagent 1 is added to all the test-tubes and then incubated them at room temperature for 10 minutes.
4) Addition of Reagent 2 i.e Folin-Ciocalteau reagent to each of the test tubes and then further incubated it for 30 min at room- temperature.
5) Optical density (O.D) of standard as well as sample is taken at 660 nm.

Table 3: Quantitative method for estimation of protein

Test Tube	BSA/ Sample (µl)	Distilled water(µl)	Reagent 1 (ml)	Incubate for 10 minutes	Reagent 2 (µl)	Incubate for 30 minutes	O.D at 660 nm
Blank	0	1000	4.5		500		
T1	30	970	4.5		500		
T2	60	940	4.5		500		
T3	120	880	4.5		500		
T4	240	760	4.5		500		
S1	100	900	4.5		500		
S2	100	900	4.5		500		

2. Estimation of Reducing sugar

Theory – Many of the reagents are reduced by the reducing property of reducing sugars. Reagent 3, 5- dinitrosalicylic acid (DNS) is reduced to 3 amino 5 nitro salicylic acid in alkaline solution.

Reagents –

1) Preparation of Sodium Potassium Tartrate solution: Weigh 45 g of sodium potassium tartrate and mixed with 75 ml of distilled water.
2) Preparation of 3, 5 dinitrosalicylic acid solution: Weigh 1.5 g of DNS- reagent to which 30 ml of 2M/liter NaOH was added.
3) Preparation of 2 molar NaOH: Weigh 80 g of NaOH and dissolve it in one liter of distilled H_2O.
4) Preparation of DNS reagent: This reagent is freshly prepared by mixing the reagents (1) and (2) and making up the volume to 150 ml with distilled H_2O.

5) Prepare Standard sugar solution:
    a) **Stock solution**: Weigh 250 mg of glucose and then mix it with H_2O and making up the volume to 100 ml.
    b) **Working solution**: It is prepared by taking 10 ml of stock solution and then making up the volume to 100 ml.

Procedure –

1) 6 clean dry test tubes are taken.
2) Standard solution was added in range of 0 - 3 ml in various test - tubes and making up the volume with distilled H_2O in all test tubes upto 3 ml.
3) Then 2 ml of DNS reagent is further added to each test- tube and mixed properly.
4) The test tubes are then allowed to incubate in water-bath for 5 min.
5) After incubation the test-tubes are allowed to be cooled at room temperature.
6) Further absorbance is taken at 540 nm.
7) Prepare standard graph and use it further to estimate the concentration of the unknown.

Table 4: Quantitative method for estimation of reducing sugars

Test Tube	Distilled water (ml)	Sample (ml)	DNS reagent(ml)	Incubate in boiling water bath for 5 minutes and allow to cool	O. D at 540 nm
Blank	3	0	2		
T1	2.9	0.1	2		
T2	2.5	0.5	2		
T3	2.0	1	2		
T4	1.5	1.5	2		
T5	1	2	2		
S1	2.9	0.1	2		
S2	2.9	0.1	2		

3. Estimation of Total phenolic content

Theory – Phenolics have vast assortment of biochemical activities such as antioxidant, antimutagenic, anticarcinogenic & furthermore the capacity to modify the gene expression. Phenolics are one of the biggest phytochemical group that records for the vast majority of the cancer prevention agent action in plants or plant items. The Folin-Ciocalteau reagent (FCR) likewise called the gallic acid equivalence method (GAE), is a blend of phosphomolybdate and phosphotungstate utilized for the colorimetric in vitro examine of phenolic and polyphenolic cancer prevention agents.

Reagents –

1) Gallic acid solution (100 µg/ml): Weigh 0.05 g of gallic acid and add 50 ml of D.W to it.
2) FC Reagent: 0.2 N (2 ml of FC reagent + 18 ml of D.W).
3) Sodium carbonate: 1.5 g of sodium-carbonate in 20 ml of D.W.

Procedure –

1) Gallic acid standard (0, 2, 5, 10, 20 µg/ml) was taken into the clean test tubes.
2) Add water further to make up the volume up to 500 µl.
3) Adding 2.5 ml of FC reagent to every test-tubes.
4) Mixing the solution properly and then incubated the test tubes at room temperature for 5 minutes.
5) After incubation period added 2 ml of 7.5% of sodium carbonate to each of the test tubes and again incubated at room temperature for 1 hour.
6) Absorbance was measured of each sample using spectrophotometer at 765 nm. Using reagent blank absorbance is set to zero.
7) Graph is plotted to know the amount of phenols present in unknown samples.

Table 5: Quantitative method for estimation of gallic acid

Test tube	Distilled water(µl)	Gallic acid/sample(µl)	F.C Reagent(ml)	Incubate at room temperature for 5 minutes	7.5% Na_2CO 3(ml)	Incubate at room temperature for 1 hour	O.D at 765 nm
Blank	500	0	2.5		2		
T1	490	10	2.5		2		
T2	475	25	2.5		2		
T3	450	50	2.5		2		
T4	400	100	2.5		2		
S1	400	100	2.5		2		
S2	400	100	2.5		2		

4. Quantitative estimation of Tannin

Theory: - Tannin are such compounds that reduce phosphotungsto molybdic acid to produce a blue color complex in alkaline solution. This color intensity is proportional to the concentration of tannin present and is measured at 605 nm.

Reagents –

1) Reagent 1: Ferric chloride (0.1M)
2) Reagent 2: Potassium ferocyanide (0.008M)
3) Reagent 3: Hydrochloric acid (0.1N)
4) Reagent 4: Prepared by Mixing reagent 1 (0.811g) + reagent 2 (0.169g) + reagent 3 (95µl) in 50 ml distilled water. Filter 2 times using cotton and 3rd time by using Whatmann filter paper.
5) Tannic acid (1 mg/ml): Dissolve 0.05 g in 50 ml distilled water.

Procedure –

1) Tannic acid standard (0, 0.02, 0.04, 0.06,0.08, 0.1 mg/ml) was taken in clean dry test tubes.
2) Added distilled H_2O to making up of volume to 5 ml.

3) Add 2 ml of reagent 4 in each of the test tubes and incubate it for 10 minutes at room temperature.
4) Absorbance was measured using spectrophotometer at about 605 nm.

Table 6: - Quantitative estimation of Tannin

Test Tubes	Distilled water(ml)	Tannic acid /Sample(ml)	Reagent 4(ml)	Incubation for 10 minutes	O.D at 605 nm
Blank	5	0	2		
T1	4.9	0.1	2		
T2	4.8	0.2	2		
T3	4.7	0.3	2		
T4	4.6	0.4	2		
T5	4.5	0.5	2		
S1	4.5	0.5	2		
S2	4.5	0.5	2		

5. Quantitative estimation of Alkaloid

Procedure – Weighing of 5 g of sample into a 250 ml beaker is done. After that 50 ml of 10% acetic acid in ethanol was included and secured and is permitted to remain for 4 hours. This arrangement was separated and kept in a water bath with the goal that the concentrate gets concentrated to one-fourth of the first volume. Concentrated NH_4OH was added drop wise to the concentrate until the point when it totally gets precipitated. The entire arrangement was permitted to settle and the precipitate was then collected. The buildup left was the alkaloid, which was then dried and weighed.

$$\text{Formula: - Alkaloid content (\%)} = \frac{\text{Weight of extracted material}}{\text{Weight of sample}} \times 100$$

6. Quantitative estimation of Flavonoids

Procedure - 2 g of the plant sample was extracted using 10 ml of 80 % fluid methanol. This arrangement is then stored at room temp for 4 hours. The entire arrangement was sifted through Whatmann filter paper. The filtrate acquired was permitted to dry over-night and was then weighed.

$$\text{Formula: - Flavonoid content (\%)} = \frac{\text{Weight of extracted material}}{\text{Weight of sample}} \text{ X } 100$$

7. Quantitative estimation of Saponin

Procedure – 5 g of powdered sample of the plant was taken into a funnel shaped vessel and 50 ml of 20 % aqueous ethanol was included. The mixture was then kept in a shaker for 30 minutes. After that, the mixture was warmed over a high temp waterbath for 4 hrs at 55˚C. The blend was then separated, and the buildup was re-removed with another 100 ml of 20 % ethanol. The consolidated concentrate was lessened to around 20 ml over water bath at around 90 degree celsius. The mixture was then moved into a 250 ml separatory channel and after that removed twice with 10 ml of diethyl ether. Ether layer was disposed of while watery layer was held. Further addition of 15 ml of n-butanol was done. The n-butanol extricate was washed twice with 5 ml of 5 % watery NaCl. The rest of the arrangement was again warmed in a water bath. After dissipation, the leftover was dried in oven to steady weight and the saponin content was calculated.

$$\text{Formula: - Saponin content (\%)} = \frac{W_2 - W_1}{\text{Weight of sample}} \text{ X } 100$$

W_1 – Wt of evaporating dish

W2 – Wt of evaporating dish + Sample

3.4 Antibacterial activity of plant extract

Preparation of micro-organisms: - The organisms utilized as a part of this investigation include *Escherichia coli, Klebsiella pneumonia, Proteus mirabilis, Staphylococcus aureus.* The strains were kept up on nutrient agar which was inclined at 4˚C. A loop full

of each bacterial strain was inoculated into 50 ml of sterile nutrient broth in 100 ml conical flask. The flask was stored in an incubator for 24 h to activate the strain.

Disc diffusion method

The antibacterial action of *Nyctanthes arbor-tritis* methanol and water extracts were evaluated by agar disc diffusion method. Twenty-four hours' broth cultures of the bacteria used for the assay. The nutrient agar plates were seeded with bacterial suspension and it was spread evenly with a sterile spreader and allowed to dry. Whatman filter paper (no.1) discs of 6mm diameter was dipped in samples (i.e methanol and water extract of leaves) for atleast 2 minutes and further placed on petri-plates with the help of a clean forceps. Discs were little pressed with forceps so that the disc contact with the surface of the medium properly. The standard discs (6mm) impregnated with antibiotics Tetracyclin were used as positive control and water as a negative control. The plates were stored at 37ºC for 24 hr. After incubation the diameter of the inhibition zone (mm) was measured.

3.5 Antifungal activity of plant extract

Preparation of fungal species: - The two fungal species used for this study were *Aspergillus niger* and *Penicillium citrinum*. The fungal specimens are isolated from the soil and identified on the basis of their morphological studies through light microscopy.

Disc diffusion method

The antifungal activity of *Nyctanthes arbor-tritis* methanol and water extracts were evaluated by agar disc diffusion method. The potato dextrose agar media once solidified, then fungal suspension is being poured on media and it was spread evenly with a sterile spreader and allowed to dry. Whatman filter paper (No.1) discs of 6mm diameter was dipped in samples (i.e methanol and water extract of leaves) for at least 2 minutes and further disc was placed on the media with the help of a sterile forceps. Discs has to be pressed with forceps so that contact with the surface of the medium is good. The standard discs (6mm) impregnated with antibiotics Clotrimazole were used as positive control and water as a -ve control. The petri-plates are then stored at 25ºC for 48 hr. After incubation the diameter of the inhibition zone (mm) was measured.

3.6 Synthesis of Cu Nanoparticles Using *Nyctanthes arbor-tritis* leaf extracts

Addition of 1 ml of *Nyctanthes* leaf extract to 100 ml of 1mM (0.03gm) aq. CuSO4.5H2O arrangement is made in a 250 ml beaker. The beaker was then left over-night at room temp. The Cu nanoparticles obtained in this manner was then centrifuged at 7,000 RPM for 15 minutes. This re-dispersion of the pellet is done using distilled H_2O. Once the Cu nanoparticle precipitate is obtained, it is then permitted to dry.

3.7 Synthesis of Fe Nanoparticles Using *Nyctanthes arbor-tritis* leaf extracts

Addition of 5 ml of *Nyctanthes* leaf extract to 10 ml of 1M (0.16gm) aqueous $FeCl_3$ solution was done in a 250 ml beaker. The beaker was then left outside overnight at room temp. The Fe nanoparticles solution obtained in this way was then purified through centrifugation at 7,000 RPM for 15 minutes. This re-dispersion of the pellet in done using distilled H_2O. Then the Fe nanoparticles were allowed to dry.

3.8 Characterization of Fe and CU nanoparticles

The green synthesis of nanoparticles was portrayed by Scanning Electron Microscope (SEM). The solvent of the Fe and Cu NPs fluid sample was evaporated, and the powder was scattered onto a glass film before SEM examination. The scanning electron microscope (SEM) focusses light emission vitality electrons on surface of sample for scanning and besides create a combination of signals at the surface of solid specimens. The signals that convey from this electron-sample gives collaboration data about the sample for instance, outside morphology (surface), chemical composition, and crystalline structure and introduction of materials making up the sample.

3.9 Antibacterial activity of Fe and Cu nanoparticles

The antibacterial activity of Fe and Cu nanoparticle was assessed by agar well diffusion technique. Twenty-four hours' broth cultures of the bacteria utilized for the measure. On nutrient agar media once solidified, bacterial (100 µl) suspension was poured on it and spread uniformly with a clean spreader and permitted to dry. A well of 6mm diameter was made by utilizing a sterile cork borer. One of the well-received 100µl of Fe/Cu nano-

particle solution, other well contained Ampicillin (positive control), water (negative control), and the sample. The plates were stored at 37ºC for 24 hr. Once incubation is over, the diameter of the inhibition zone (mm) was measured.

3.10 Antifungal activity of Fe and Cu nanoparticles

The antifungal activity of Fe and Cu nanoparticle was assessed by agar well diffusion technique. Twenty-four hours' broth cultures of the fungus utilized for the assay. On potato dextrose agar (PDA) media once it is solidified, fungal (100 µl) suspension was poured on it and is then spread uniformly with a clean spreader and permitted to dry. A well of 6mm diameter was made by utilizing a sterile plug borer. One of the well-received 100µl of Fe/Cu nano-particle solution, other well contained Clotrimazole (positive control), water (negative control), and the sample. The plates were stored at 25ºC for 48 hrs. Once incubation time is over the diameter of the inhibition zone (mm) was measured.

3.11 Extraction of essential oil

Essential oil can be extracted by four different methods such as steam distillation, water distillation, solvent extraction and soxhlet extraction using various solvents. The steam distillation method was investigated using a Clevenger apparatus. The Clevenger Apparatus conducts the distillation process by boiling, condensing and decantation to separate the oil. Clevenger apparatus consists of a round bottom flask, condenser and receiver. The round bottom flask is made up of glass, which have a capacity of 2000 ml.

The process of distillation consists of loading of 50g powdered dried leaf extract into the round bottom flask, adding sufficient amount of water. The steam formed by heating the distilled water in the round bottom flask then moves through the extraction container where it causes the volatile components from the leaves to evaporate and travel with the steam through the connecting tube. Further the vapor is condensed in the condenser and collecting the oil in the receiver and the temperature is maintained by heater. The distillation is continued for 3-4 hours.

Fig 4: - Clevenger Apparatus

3.12 Thin Layer Chromatography (TLC) of Crude extract

The principle rule of partition is adsorption. Compounds to be segregated are placed on a thin layer of adsorbent set on a chromatographic plate. The mobile phase solvent moves through TLC plate as a result of narrow activity i.e capillary action (against gravitational power). The components move along the TLC plate as demonstrated by their affinities towards the adsorbent. The portion of sample with greater fondness towards the stationary phase moves slower. The part with lesser fondness towards the stationary phase travels quicker. In this way components can be isolated using thin - layer chromatographic plate in view of affinity towards the stationary phase.

Thin layer chromatography is considered as fast, effective and cheap method and is one of the best known technique of plant biochemistry. TLC is mainly used for preliminary separation and determination of plant constituents. It is helpful for proper identification and authentication of phytoconstituents. The collected water and methanol leaf extract was applied as a spot 1cm from edge of the plate by using capillary tube. The plate was then placed in closed container containing solvents i.e. Isopropanol: Acetone in the ratio of 5:5 as a mobile phase. It shows good separation of components. After developing the plate up to two third of the length of plate it was removed from the container and allowed to dry. After dryness they were examined in ultra violet chamber and Rf values were calculated.

Fig 5: - TLC sheet

3.13 Spectroscopic scanning

Spectroscopic scanning was carried out using UV/ Vis spectrophotometer, which is used to measure the absorbance of UV/Vis light by a sample, either for a solitary wavelength or over a range in the spectrum. For scanning, sample preparation was done. For which 5g dry leaf extract was taken and mixed with 20-25 ml methanol, and then kept the solution for shaking for 3-4 hours on a shaker. The extract was filtered and centrifuged at 5000rpm for 15-20 minutes. After filtering the solution, the sample was diluted 10,000 times. Sample taken in cuvette was scanned for from 200-800 nm using UV/Vis spectrophotometer instrument.

3.14 HPLC Analysis of Saponins

HPLC was performed for Saponins with a Thermo Scientific UHPLC (Chromeleon Dionex UltiMate 3000) system. The chromatographic separation of samples was accomplished by a reversed-phase HPLC column (C18, 250mm×4.6mm, 5µ particle size) using acetonitrile and acidified water (70:30) mobile phase with flow rate of 1.0mL/min, and the detection wavelength set was 294 nm. The extracted saponins were mixed with HPLC grade methanol

and diluted the extract 100 times. The sample was filtered utilizing 0.2µm cellulose acetate syringe filter (15mm) and before injecting 10µl.

Fig 6: - HPLC Instrument

3.15 Amino acid Profilling

Amino acid analysis was carried out using Hitachi Amino Acid Analyzer (L-8900, Japan). Injection volume taken for analysis was 20µl. 30 mg sample was taken to carry out hydrolysis (using HCl) for analyzing all amino acids (except Tryptophan) before injecting the sample into analyzer. For analyzing Tryptophan amino acid, 30mg sample was taken to carry out hydrolysis using Methanesulfonic acid.

Protocol for all amino acids (except Tryptophan) Hydrolysis with HCl: -

Take the sample in hydrolysis tube (30mg). Addition of 6N HCl (10mL) is done and then this mixture is blend to the nitrogen 3 – 4 min (for dissipation of oxygen and making the condition idle). Close the tube firmly and guarantee that there will be no any air spillage from the tube. Further, kept the tube in an Oven at 110 °C for 22 h. After hydrolysis bring the tube at room temperature. Open the tube and move the sample in 25 ml volumetric flask and keep up the volume with distilled water up to the mark. Then filter the sample with Whatman filter

paper (110 mm,Cat No. 10001-110). Take the sample (5 mL) in the nitrogen Evaporator (18 – 20 psi, 60 – 70 °C). Drying of sample is done to eliminate out HCl. Wash the dried sample with 5ml distilled water (3 times) to evacuate acid. Dilute the dried sample and alter the volume with 0.02N HCl (8.4 ml) to acquire the sample. Further the sample is filtered with syringe filter Whatman 0.20 µm PES Filter (Cat .No. 6794 – 2512). At last put the sample (1.5 mL) in Glass vial of Hitachi L-8900 AAA auto sampler rack. The final concentration of protein ought to be 0.5 mg protein/ml in test. While after hydrolysing, the sample must be 3mg or less in 1 mL of 6N HCl.

Protocol for tryptophan (Trp) Hydrolysis with Methanesulfonic acid: -

Measure the sample (30mg) for the hydrolysis. Addition of 4 N methanesulfonic acid (10ml) with 0.2 % 3-(2-aminoethyl) indole in Digestion tube is done. Expose this mixture to the nitrogen 3 – 4 min (for vanishing of oxygen and making the condition idle). Close the tube firmly and guarantee that there will be no any air spillage from the tube. Put the sample in Hot Air Oven to Hydrolyse the sample at 110°C for 22hrs. After the deterioration add 4N NaOH to change the pH to around 2. For pH balance utilize pH test paper. Alter the volume by 0.02N HCl as a sample (0.5 mg protein/ml). Channel the sample with syringe filter Whatman 0.20 µm PES Filter (Cat .No. 6794 – 2512). Put the sample (1.5 mL) in Glass vial of Hitachi L-8900 AAA auto sampler rack.

3.16 Fatty acid Profilling

Fatty acids were quantified using Gas chromatography. Sample volume taken for GC was 1.000000 µl and the sample amount (1.0000) was injected automatically at 12.5000 pts/s sampling rate. The sample was run for 75 minutes.

4 RESULTS AND DISCUSSION

The present investigation carried on the *Nyctanthes arbor-tristis* uncovered the presence of therapeutic active constituents. The phytochemically active compounds of *Nyctanthes arbor – tristis* were qualitatively and quantitatively investigated for leaves and the outcomes are displayed in Table 7 and 8.

4.1 Qualitative analysis

Phytochemical studies show the presence of secondary metabolites such as saponins, flavonoids, tannins and carbohydrates whereas alkaloid, steroid, quinine, emodins, and anthocyanin are absent. Terpenoids and gums & mucilages are detected in water extraction of leaves and was absent in solvent (methanol) extraction. Protein, leucoanthocyanin and glycoside are detected in solvent extraction of leaves, but was absent in water extraction. Coumarin and phlobatannins may be present in plant.

Table 7: - Qualitative phytochemical analysis

Test	Water extraction	Solvent extraction
Saponin	+++	+++
Flavonoid	+	+
Tannin	+	+
Alkaloid	-	-
Protein	+/-	+/-
Carbohydrate	+++	+++
Steroid	+/-	-
Coumarin	+/-	-
Terpenoid	+	-
Quinine	-	-
Emodin	-	-
Phenol	-	-
Glycoside	-	+
Gum and mucilage	-	-
Phlobatanins	+/-	-

Leucoanthocyanin	+/-	++
Anthocyanin	-	-

+ = indicates presence of phytochemicals, - = indicates absence of phytochemicals

+/- = phytochemicals may be present, +++ = shows high concentration of phytochemicals

Chemical substances present in plants have therapeutic esteem that plays a physiological activity on the human body. Diverse phytochemicals are found to have an extensive variety of activities, which may help in protection against intense ailments. For instance, alkaloids assume a noteworthy part to ensure against some chronic ailments. Saponins secure against hypercholesterolemia and anti-infection properties. Steroids have (analgesic) pain- relieving properties. The saponins and steroids are likewise in charge of focal sensory system (central nervous system) activities. Phytochemical screening of water and solvent extract of *Nyctanthes arbor-tristis* leaves were utilized to study the presence of saponins, tannins, flavonoids, alkaloids and steroids and furthermore have different medicinal properties, for example, calming, against diabetic and pain- relieving activities and furthermore for focal sensory system activities.

Fig 7- Saponin Test (Water)

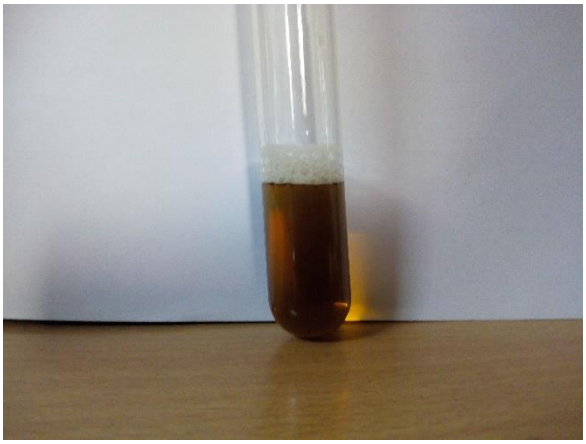


Fig 8- Saponin test (Methanol)

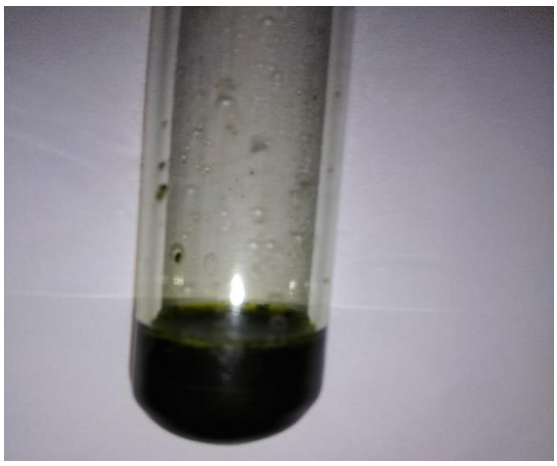


Fig9: - Tannin test (Water)

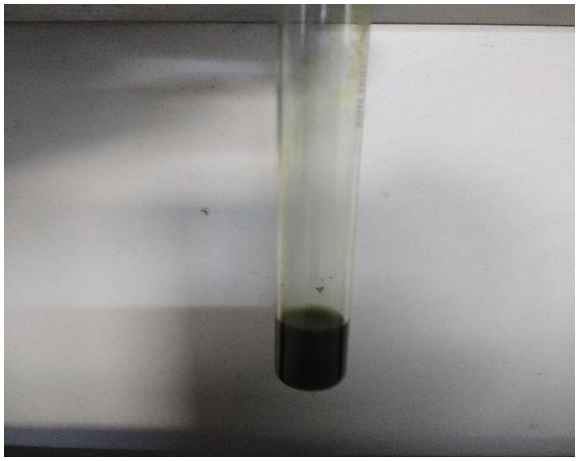


Fig10: - Tannin test (Methanol)

Fig 11: - Flavonoid test (water)

Fig 12: - Flavonoid test (Methanol)

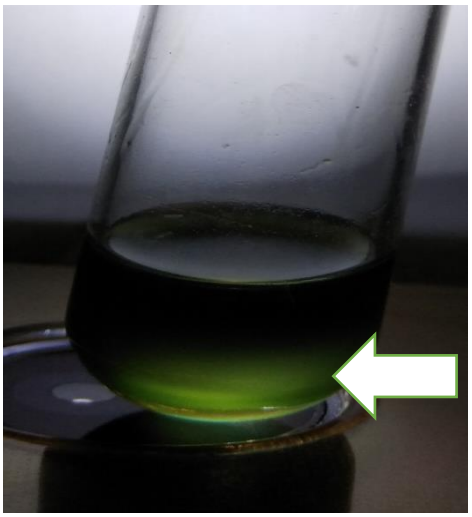


Fig 13- Glycoside test (Methanol)

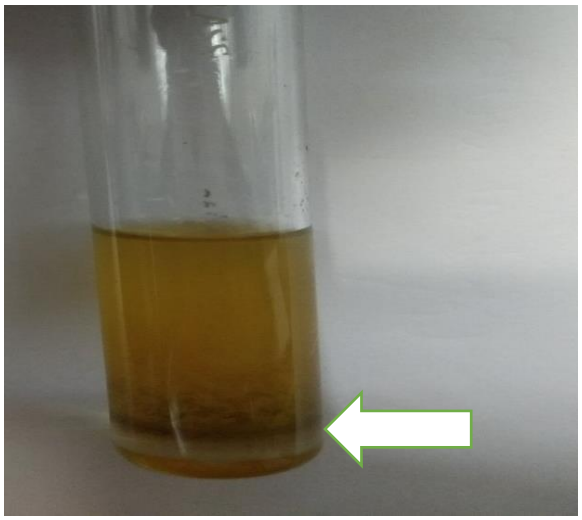


Fig 14- Terpenoid test (Water)

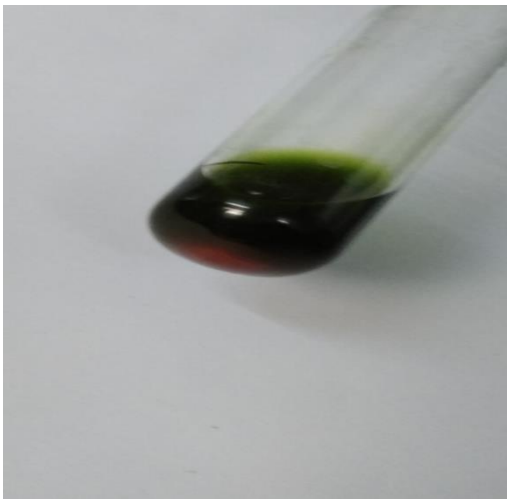


Fig 15: - Carbohydrate test (Water)

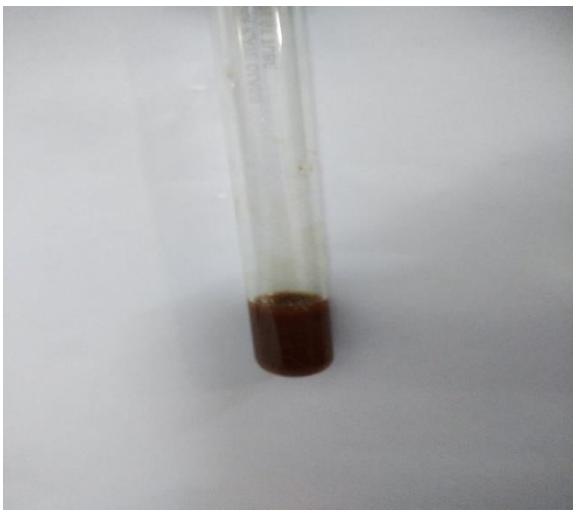


Fig 16- Carbohydrate test (methanol)

Fig 17: - Leucoanthocyanin test (Methanol)

4.2 Quantitative analysis

The results of quantitative analysis on seven major groups of phytochemical constituents in the medicinal plants is summarized and shown in Table 7.

Table 8: - Quantitative analysis of phytochemical constituents

Test	Water extraction (%)	Solvent extraction (%)
Protein	19.226	19.285
Reducing sugar	7.4	2.7
Phenol	1.076	1.832
Tannin	9.4	13.1
Alkaloid	8	0.6
Flavonoid	3.83	2
Saponin	1.2	2.8

The result showed that alkaloid content was present in the leaf exract of *Nyctanthes arbor – tristis* (Parijat). Percentage of alkaloid present in water extraction from leaves of Parijat plant was 8%, and solvent extraction from this Parijat leaves was 0.6%.

The flavonoid content was also present in the leaf extract of *Nyctanthes arbor – tristis* (Parijat). Percentage of flavonoid present in water extraction from leaves of Parijat plant was 3.83%, and solvent extraction from this Parijat leaves was 2%.

The saponin content was present in the leaf extract of *Nyctanthes arbor – tristis* (Parijat). Percentage of saponin present in water and solvent extraction from leaves of Parijat plant was 1.2% and 2.8%.

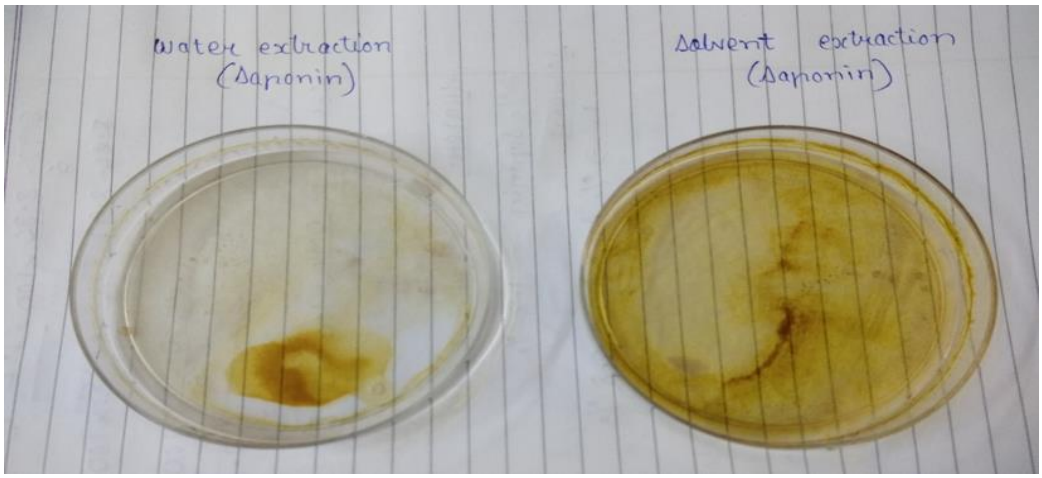


Fig 18: Quantitative estimation of Saponins

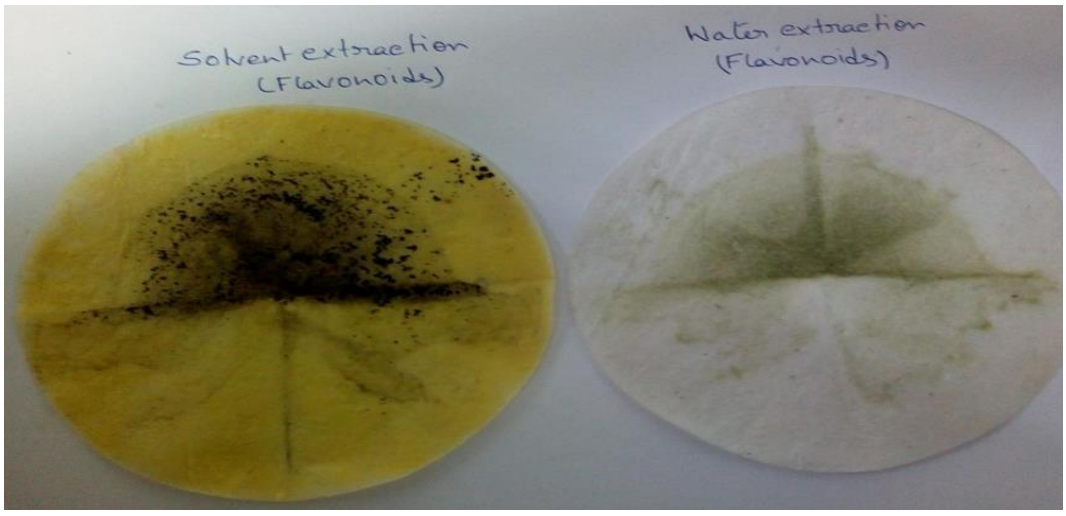


Fig 19: - Quantitative estimation of Flavonoids

Table 9: - Quantitative estimation of protein

Test Tube	Concentration(µg/ml)	Optical Density (nm)
Blank	0	0
T1	30	0.157
T2	60	0.356
T3	120	0.523
T4	240	0.834
S1	192.26	0.726
S2	192.85	0.728

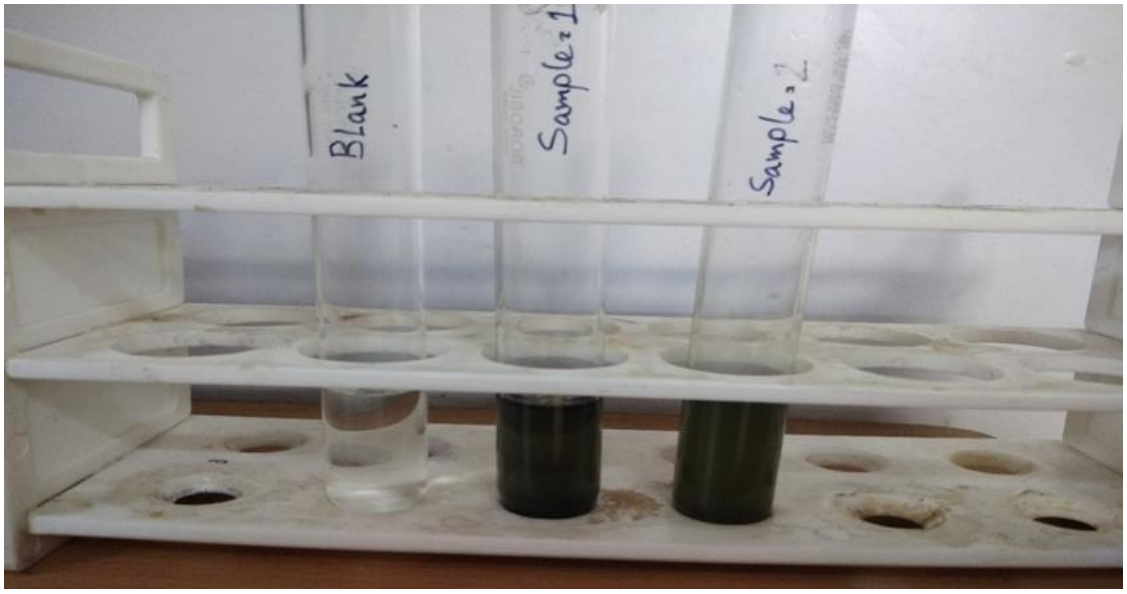
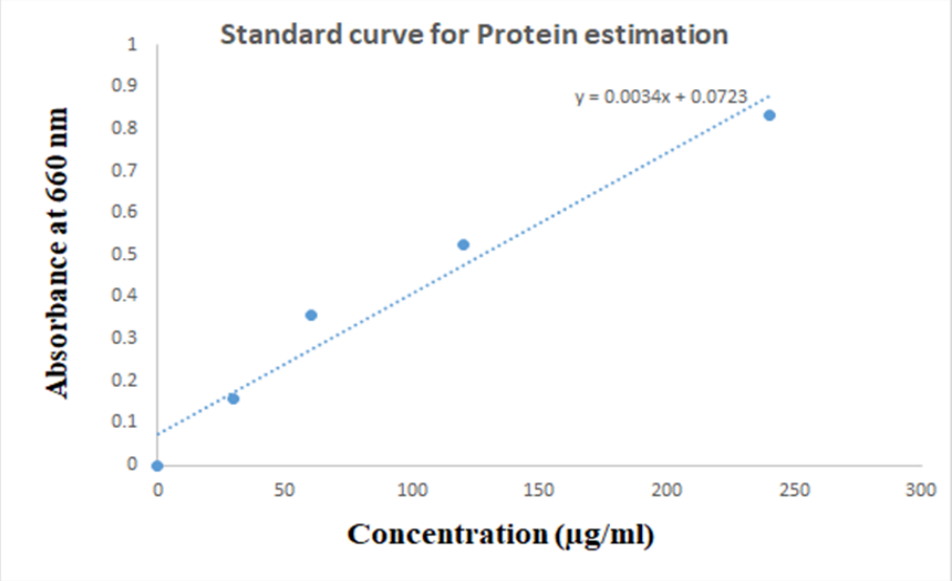


Fig 20: - Test for protein (Water and Methanol extract)

Graph 1: Standard curve for Protein estimation



The amount of protein in leaves of *Nyctanthes arbor-tristis* plant was contemplated by spectroscopic technique. The protein substance of leaves in water separate was observed to be 19.226% and the methanolic extricate was found to contain 19.285%.

Table 10: - Quantitative estimation of reducing sugar

Test Tube	Concentration (mg/ml)	Optical Density (nm)
Blank	0	0
T1	0.03	0.324
T2	0.06	0.775
T3	0.09	1.097
T4	0.12	1.384
T5	0.15	1.611
S1	0.074	0.855
S2	0.027	0.345

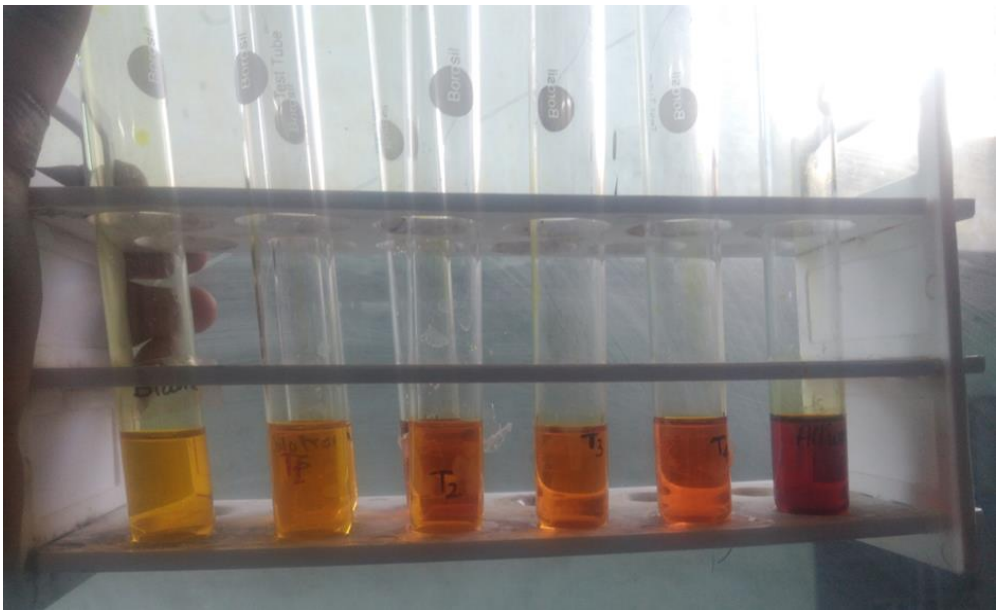


Fig 21: Test for Reducing sugar (Standard)

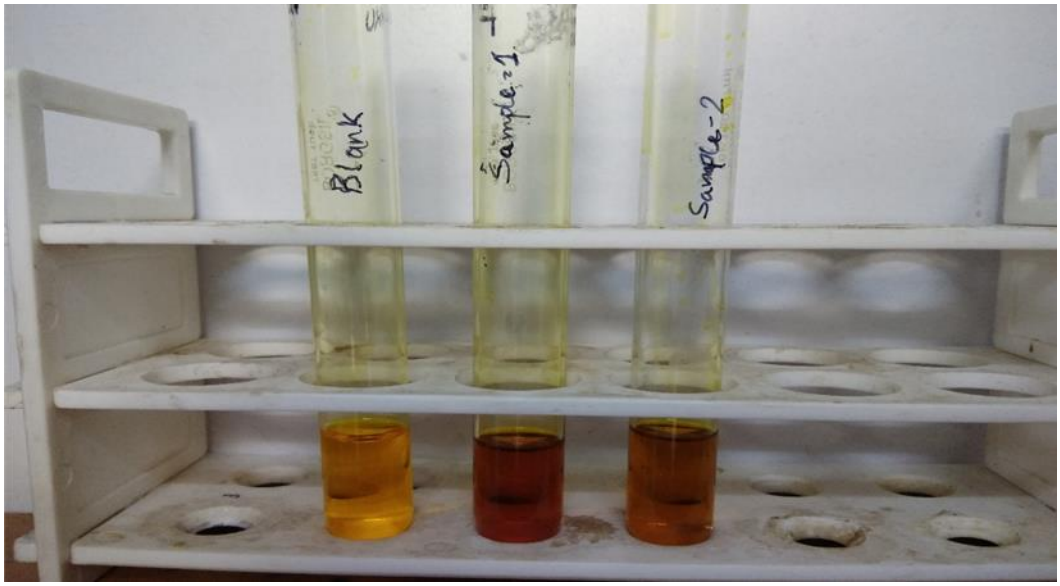
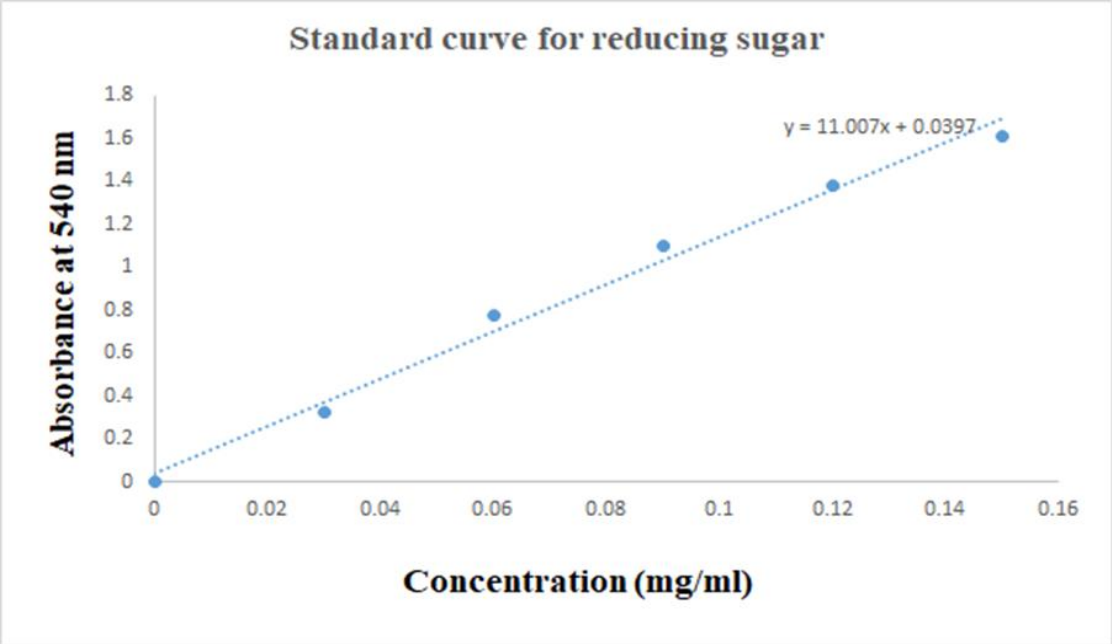


Fig 22: - Test for reducing sugar (Water and Methanol extract)

Graph 2: Standard curve for estimation of reducing sugar



The reducing sugar content of leaves of *Nyctanthes arbor-tristis* plant was contemplated by spectroscopic technique. The reducing sugar substance of leaves in water extricate was observed to be 7.4% and the methanolic separate was found to contain 2.7%.

Table 11: - Quantitative estimation of phenolic content

Test Tube	Concentration (µg/ml)	Optical Density (nm)
Blank	0	0
T1	2	0.128
T2	5	0.182
T3	10	0.560
T4	20	1.510
S1	10.76	0.731
S2	18.32	1.304

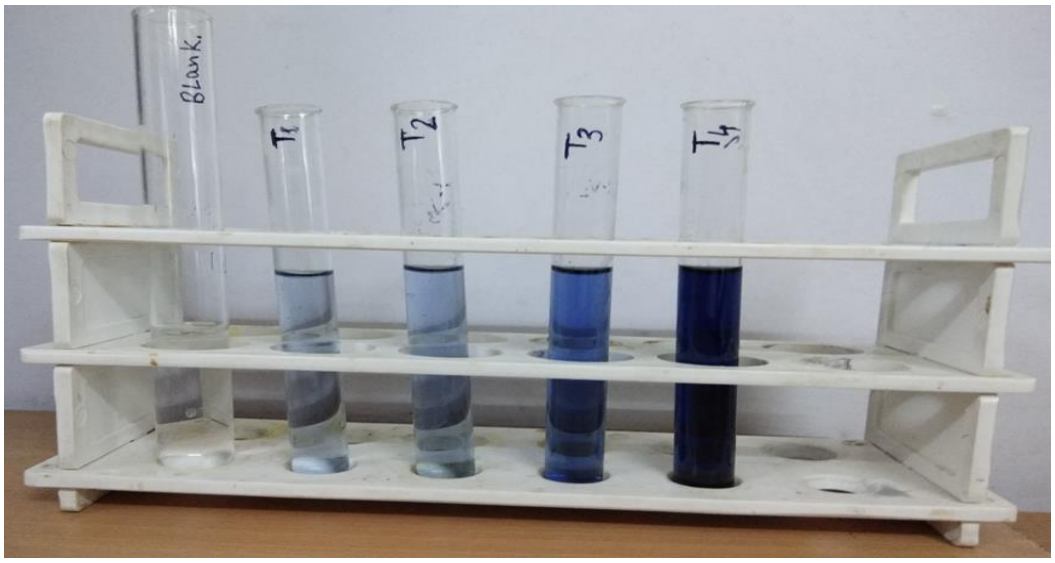


Fig 23: Test for phenolic content (Standard)

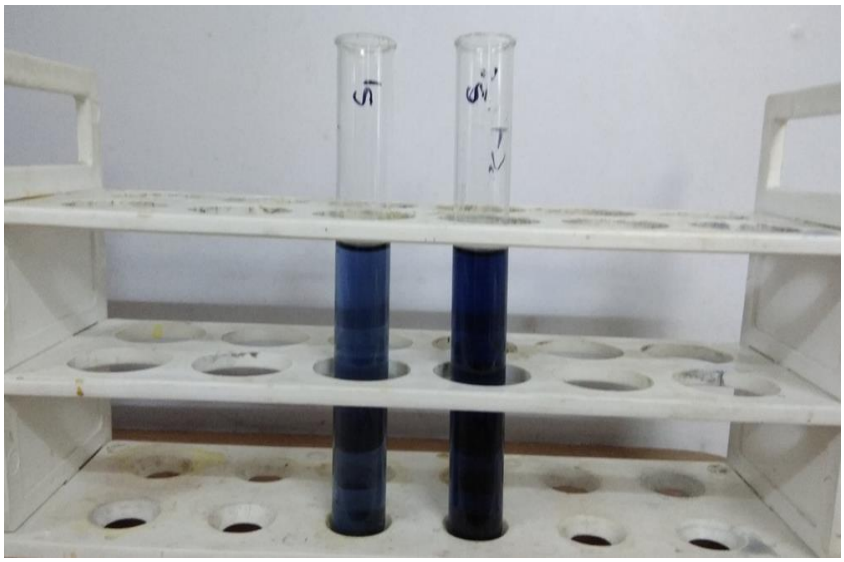
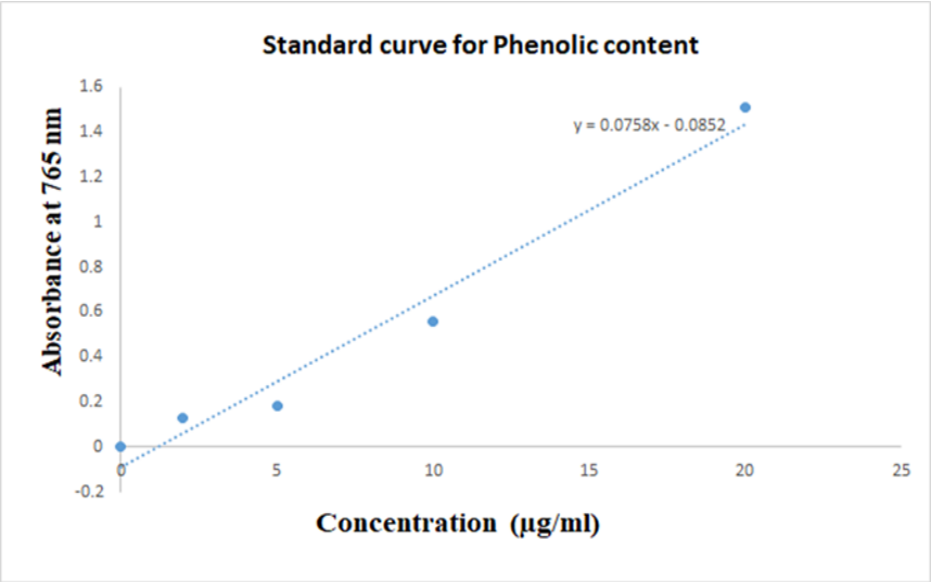


Fig 24: - Test for phenolic content (Water and Methanol extract)



Graph 3: Standard curve for estimation of phenolic content

The phenolic substance of leaves of *Nyctanthes arbor-tristis* plant was examined by spectroscopic technique. The phenolic substance of leaves in water separate was observed to be 1.076% and the methanolic extricate was found to contain 1.832%.

Table 12: - Quantitative estimation of Tannin

Test Tube	Concentration (mg/ml)	Optical density (nm)
Blank	0	0
T1	0.02	0.155
T2	0.04	0.246
T3	0.06	0.401
T4	0.08	0.561
T5	0.1	0.700
S1	0.094	0.655
S2	0.131	0.909

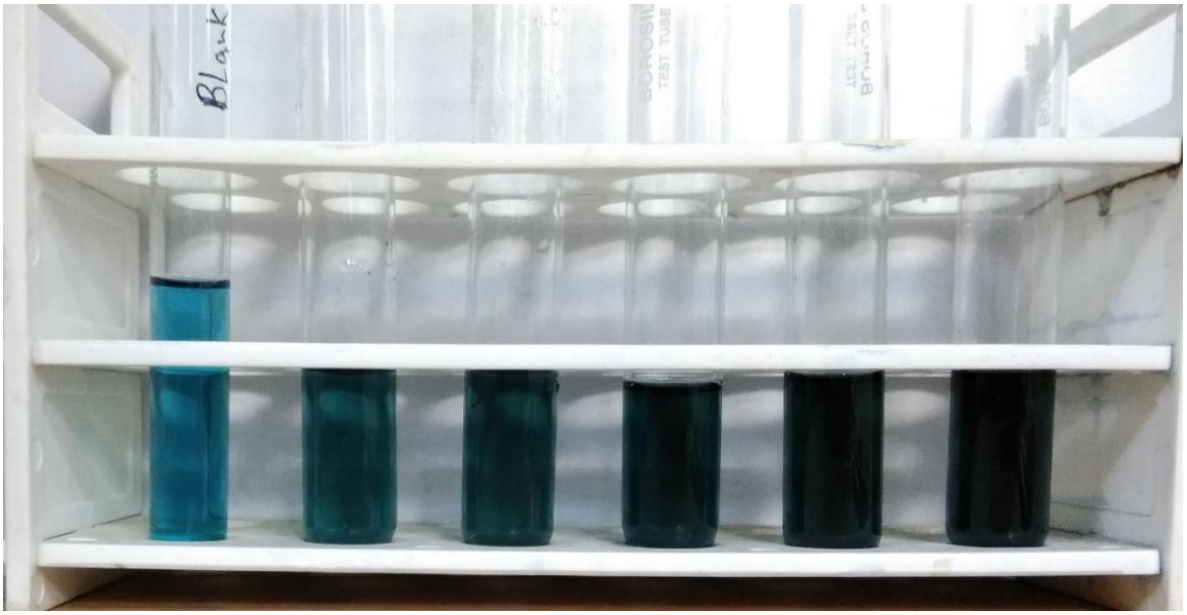


Fig 25: - Test for Tannin (Standard)

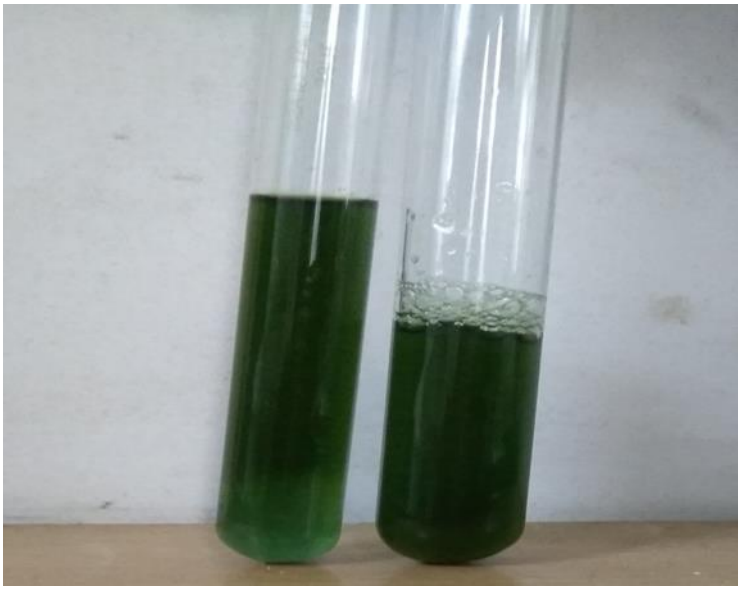
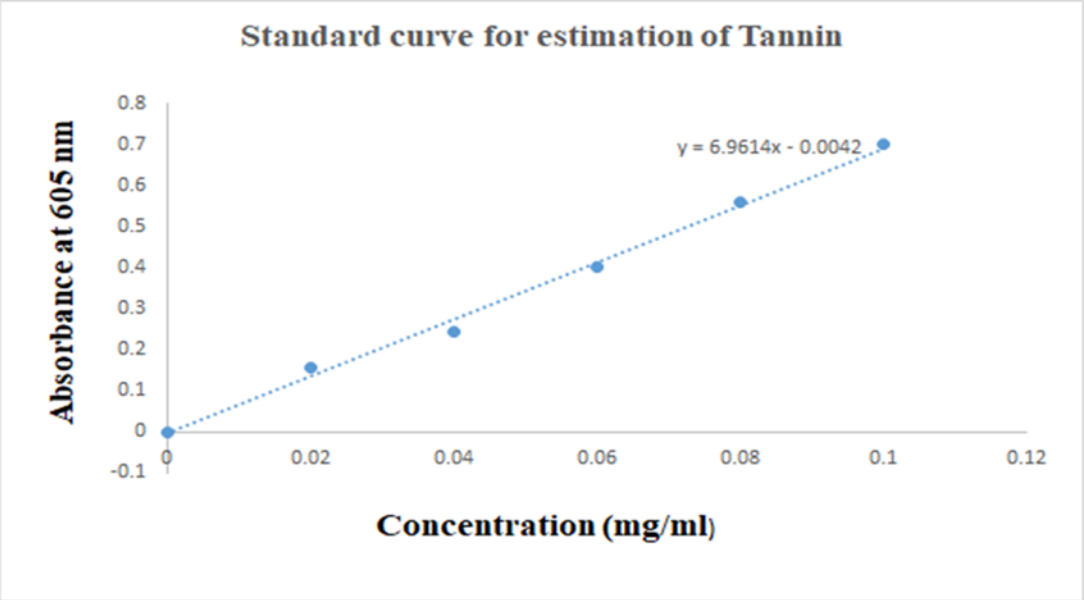


Fig 26: - Test for Tannin (Water and Methanol extract)

Graph 4: Standard curve for estimation of Tannin



The tannin compound of leaves of *Nyctanthes arbor-tristis plant* was contemplated by spectroscopic technique. The amount of tannin present in leaves in water separate was observed to be 9.4% and the methanolic remove was found to contain 13.1%.

4.3 Antibacterial assay

We found that plant extract in water gave more steady antimicrobial activity contrasted with those extracted in organic solvent (methanol) in the case of *Klebsiella pneumonia* (K.P), a gram negative microbes and *Staphylococcus aureus* (Sa), a gram positive microorganisms. *Nyctanthes* plant extracts were absolutely inactive against *Escherichia coli* (gram – ve) and *Proteus mirabilis* (gram – ve). The outcomes got affirm the therapeutic strength of *Nyctanthes* arbor-tritis plant utilized as a part of customary drug. Likewise, these outcomes frame a decent reason for determination of applicant plant species for assist phytochemical and pharmacological examination. The aftereffects of the present investigation bolster the utilization of the contemplated plants and propose that the plant extracts have compounds with antibacterial properties that can be utilized as antimicrobial agents in new medications for the treatment of irresistible infections caused by pathogens. The most dynamic

concentrates can be subjected to isolation of the remedial antimicrobials and experience advance pharmacological assessment.

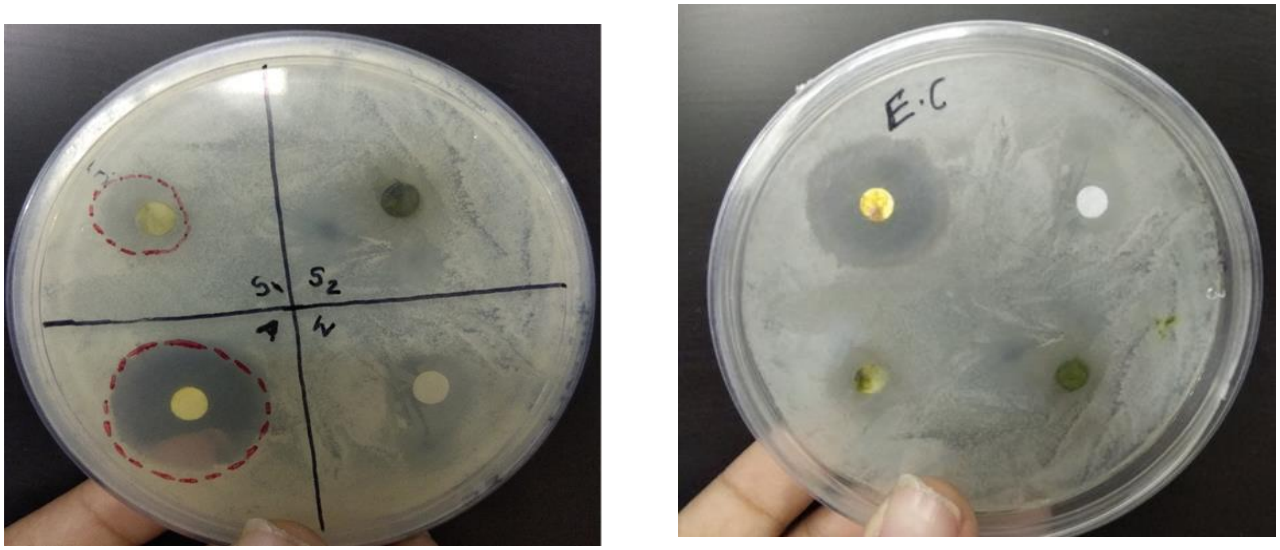


Fig 27: - Antibacterial effect of *E. coli*

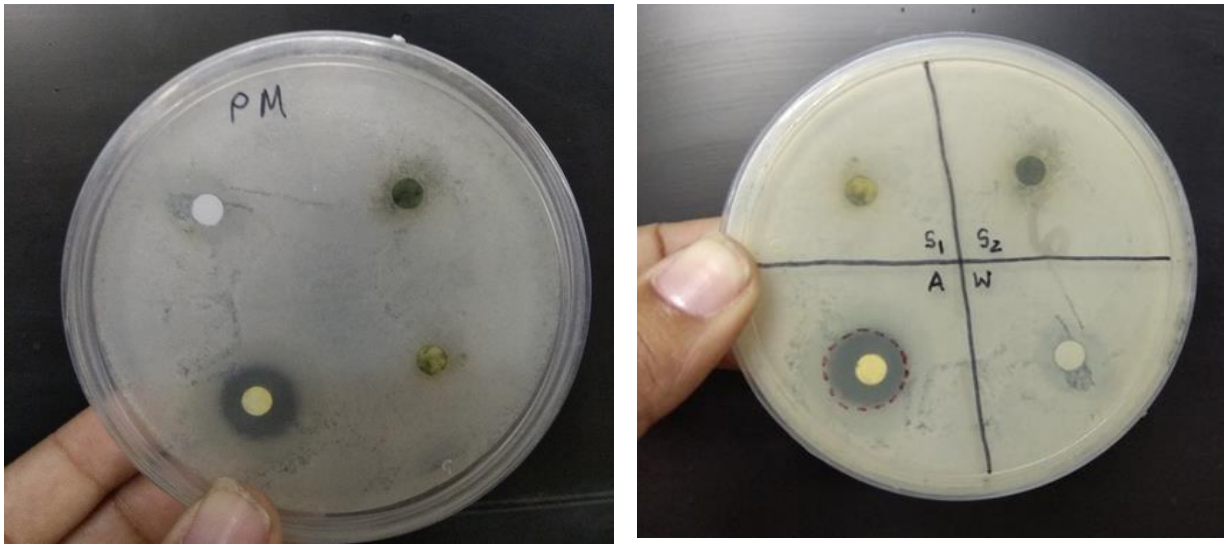


Fig 28: Antibacterial effect of *Proteus mirabilis*

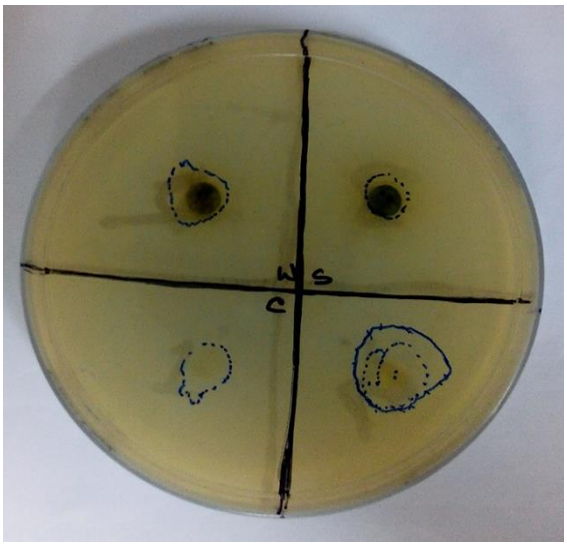


Fig 29: - Antibacterial effect of *Klebsiella pneumonia*

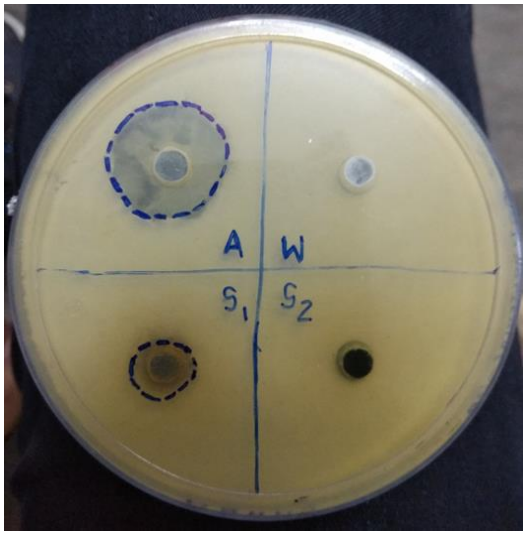


Fig 30: - Antibacterial effect of *Staphylococcus aureus*

4.10 Antifungal assay

In present investigation antifungal activity was shown by disc diffusion method and found that very little activity is being shown in case of both water and solvent (methanol) extract by *Pencillium citrinum* and no activity is shown in case of *Aspergillus niger*. The across the board utilization of the *Nyctanthes* plant in conventional arrangement of drug for different ailment is bolstered by our examination giving extraordinary significance to its pharmacological assessments. In spite of the fact that plant is safe and have no harmful impact, however the powerful therapeutic impacts of the plant against specific human disease should be confirmed by more controlled and thorough clinical preliminaries.

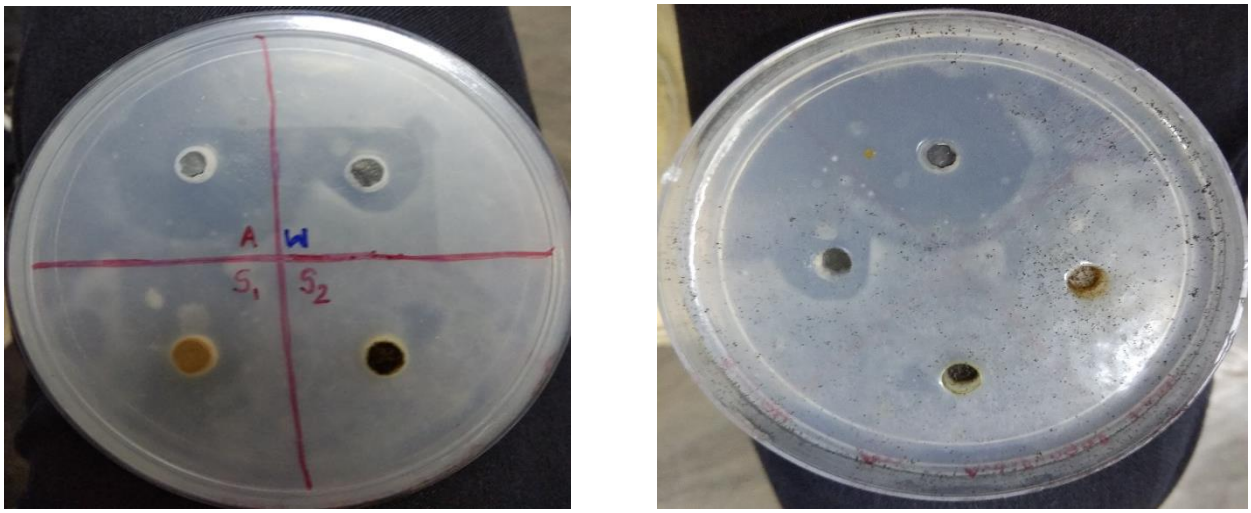


Fig31: - Antifungal effect of *Penicillium citrinum*

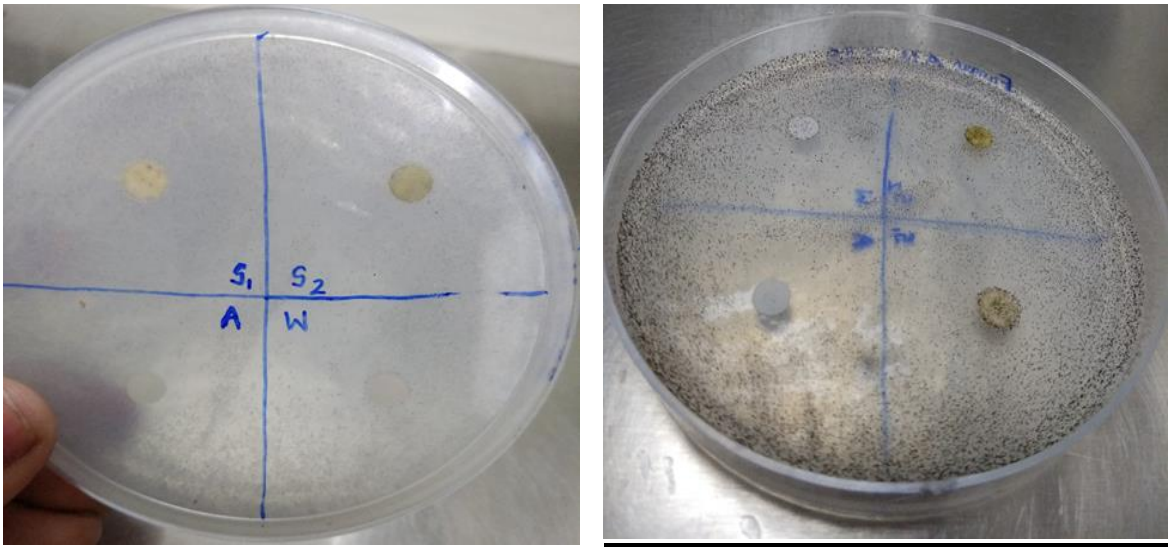


Fig32: - Antifungal effect of *Aspergillus niger*

4.5 SEM Analysis: -

Fe and Cu nanoparticle were analyzed using Scanning electron microscopy. SEM images (Fig 33,34) shows results of Fe and Cu nano particles where the powder is deposited on a carbon strip and allowed to dry. Further NPs were coated with gold for proper visualization. The Fe NP sample was observed at 100.0kx magnification and size was found in the range of 48.37nm - 54.41nm. The Cu NP was also viewed at 100.0kx magnification and size of Cu NP through SEM analysis was found in the range of 26.76nm – 54.73nm.

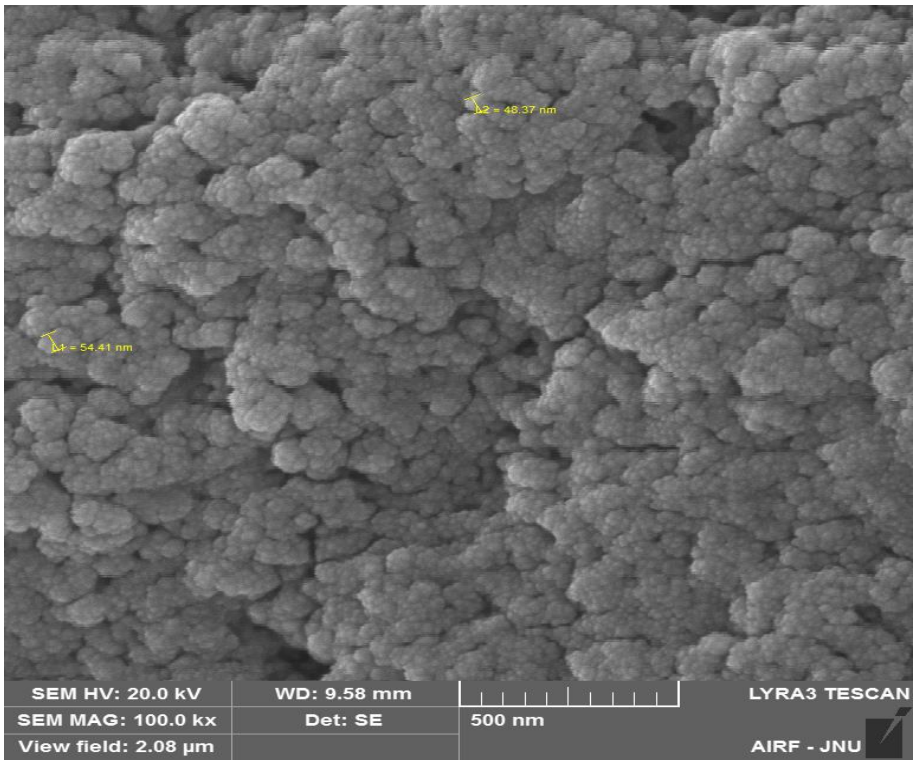


Fig 33: - SEM image of Fe nano particle

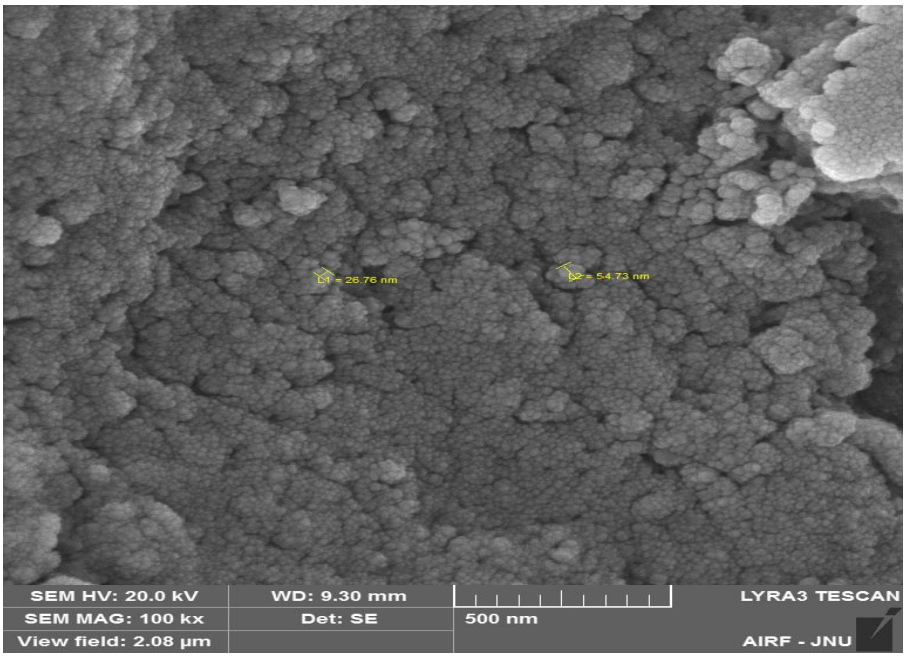


Fig 34: - SEM image of Cu nano particle

4.6 Antibacterial effect of Fe and Cu nanoparticle

In view of the perceptions, it can be deciphered that high rate of activity against the bacterial strains was accounted by Fe nanoparticles, and in this way Fe NPs show high antibacterial action. It has been seen that less action against the bacterial strain was accounted by Cu nanoparticles, and henceforth it displays slight antibacterial activity. The presence of an inhibition zone obviously demonstrates that the component of the biocidal activity of nanoparticles includes destroying of membrane. Extend of inhibition relies upon the concentration of nanoparticles and also on the underlying bacterial fixation. Fe nanoparticles can in this manner be considered as potential antibacterial agent.

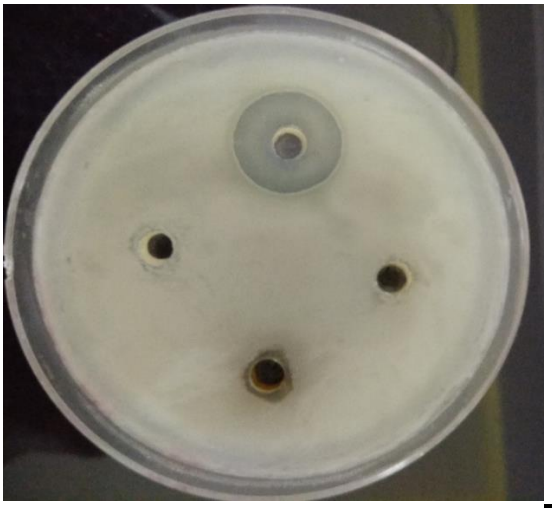


Fig 35: Antibacterial activity of Cu nano particle

Fig 36: Antibacterial activity of Fe nano particle

4.6 Antifungal effect of Fe and Cu nanoparticle

In view of the perceptions, it can be deciphered that high rate of action against the fungal strains was accounted by Fe NPs and consequently Fe NPs show high antifungal activity. It has been seen that less activity against the fungal strain was accounted by Cu nanoparticles, and subsequently it displays slight antifungal action. Therefore, it could be utilized for creating antifungal agents for business use in the field of agribusiness. This investigation subsequently reports as an eco-friendly methodology for synthesis of Fe and Cu

nanoparticles. Such investigations have extraordinary potential for growing great fungicidal formulations having nanoparticles.

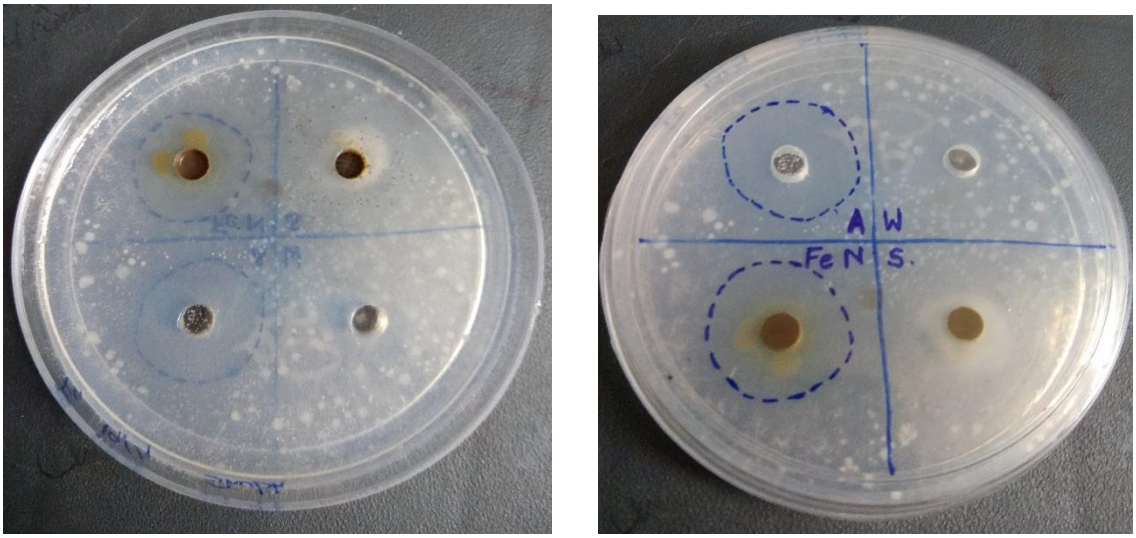


Fig37: - Antifungal effect of Fe NPs

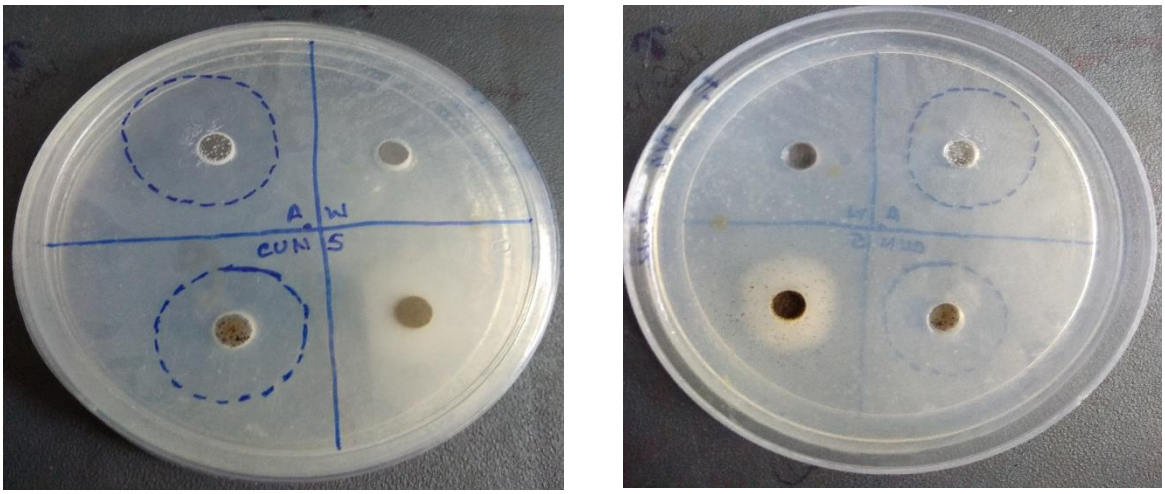


Fig38: - Antifungal effect of Cu NPs

4.7 Chemical composition of essential oil

Trace of essential oil was acquired from dried leaves of *Nyctanthes arbor-tristis* by steam distillation technique using Clevenger device. The yield of fundamental oil got was only 250µl from 100g of dried leaf extract. Prabodh Satyal, Prajwal Paudel, Ambika Poudel and William N Setzer announced that the basic oil comprise of linalool, hexenyl benzoate, terpineol, methyl salicylate,hexadecanoic acid, octadecanoic acid,eugeno land hexenol. The

fundamental oil additionally has various therapeutic properties such as anti-arthritic, antimicrobial, anti-inflammatory, antiviral, hostile to leishmanial, against pyretic, immunostimulant, antinociceptive, hostile to hypersensitivity, against plasmodial, hepatoprotective and narcotic.

Fig39: - Oil extracted from Clevenger apparatus

4.8 Spectroscopic scanning analysis

The methanol extract of *Nyctanthes arbor-tristis* were scanned from range 200 nm to 800 nm by using UV/Vis spectrophotometer.The wavelength and absorbance of various peaks is shown below in fig and table.

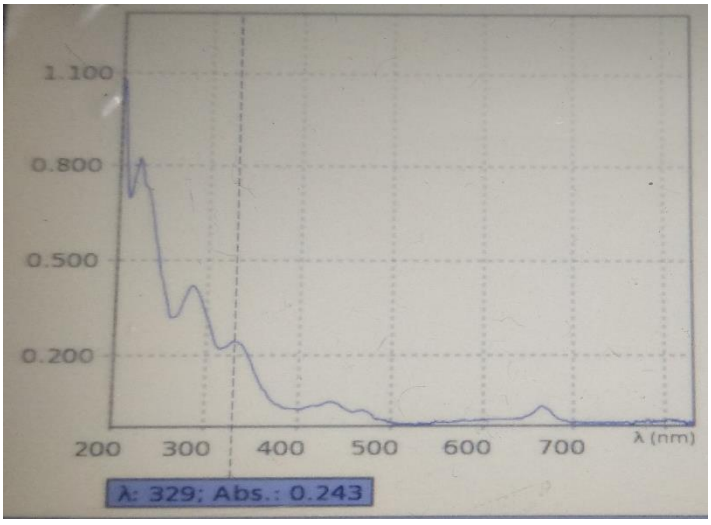


Fig 40: - Spectroscopic scanning of methanol extract of *Nyctanthes* (without using filter)

λ	Abs
203	1.079
223	0.821
233	0.708
283	0.418
331	0.241
437	0.054
469	0.029
665	0.040

Table13 – Absorption spectrum data sheet (without using filter)

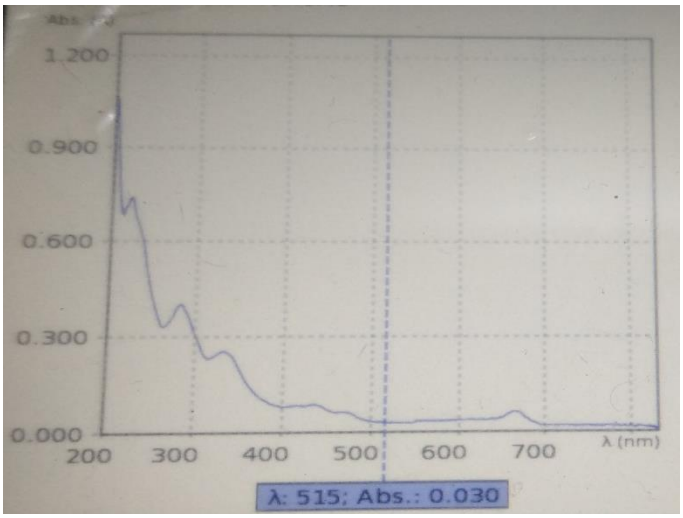


Fig 41: - Spectroscopic scanning of methanol extract of *Nyctanthes* (using 0.2micron nylon filter)

λ	Abs
203	1.014
223	0.737
235	0.616
281	0.398
331	0.250
437	0.084
473	0.060
545	0.028
665	0.060

Table14: - Absoption spectrum data sheet (using 0.2micron nylon filter)

4.9 Thin Layer Chromatography analysis

Thin Layer Chromatography (TLC) has been carried out for water and solvent (methanol) extract using Isopropanol: Acetone (mobile phase) in the ratio of 5:5. The patch obtained by TLC was viewed under UV light chamber as shown in fig and the Rf values were calculated and is shown in table. The methanol extract gave 2 spots on TLC plate, of which one is blue and other is dark green. The aqueous extract gave 2 spots on TLC of which one is blue and other is light blue.

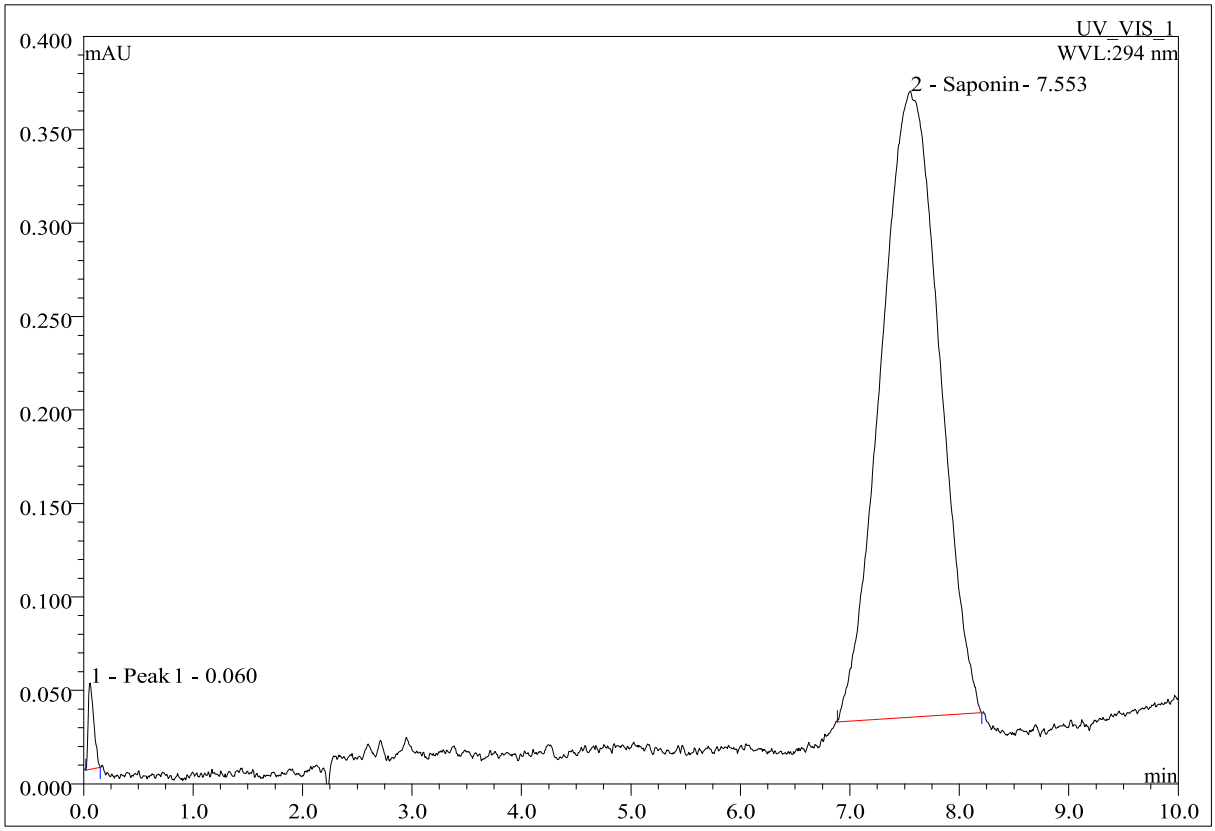
Fig 42: - TLC plate viewed in Ultraviolet Chamber

Table 15: - TLC pattern of Extracts of *Nyctanthes arbor-tristis*

Extracts	No of spots	Color	Rf values
Water	2	Blue Light blue	0.74 0.78
Methanol	2	Blue Dark green	0.72 0.8

4.10 HPLC analysis

Analysis of Saponins was done using Thermo Scientific UHPLC (Chromeleon Dionex UltiMate 3000) system. The chromatographic separation of samples was successfully carried out by a reversed-phase HPLC column (C18, 250mm×4.6mm, 5µ particle size) and acetonitrile: acidified water (70:30) mobile phase with flow rate 1.0mL/min, and saponins were detected at a set wavelength of 294 nm. Under these conditions the retention time of Saponins was 7.55min. The sample showed a major peak at the same retention time i.e 7.5 min indicating the presence of saponin with a peak height of at 0.335mAU and the area of saponin peak was calculated as 0.208mAU/min (Fig 43). The area under the saponin peak in the sample covered 98.69% of all the peaks. This clearly reflected the extraction and purification process yielded a pure preparation of saponin and confirmed the preliminary test results.



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	0.06	Peak 1	0.046	0.003	1.31	n.a.	BMB
2	7.55	Saponin	0.335	0.208	98.69	n.a.	BMB
Total:			0.381	0.210	100.00	0.000	

Chromeleon (c) Dionex 1996-2006
default/Integration Version 6.80 SR15b Build 4981 (268185)

Fig 43: - HPLC chromatogram of sample for detection and confirmation of saponin

4.11 Amino acid analysis

The values of amino acid contents in the leaves of *Nyctanthes arbor-tristis* are shown in Table 16-18. The study revealed that leaves of *Nyctanthes* contain 34 AA (Fig 44), which includes 9 essential, 10 non-essential and 15 non-proteinogenic amino acids. The highest content of EAA present was Lysine and in case of NEAA highest content present was Glutamic acid. The concentration of non-proteinogenic amino acids was very low in leaves of *Nyctanthes arbor-tristis*. However, *Nyctanthes* leaves can be considered as a very good protein source as they contain various essential, non-essential and non-proteinogenic amino acids. Hence it can also be used as food supplement, since it also has various medicinal property.

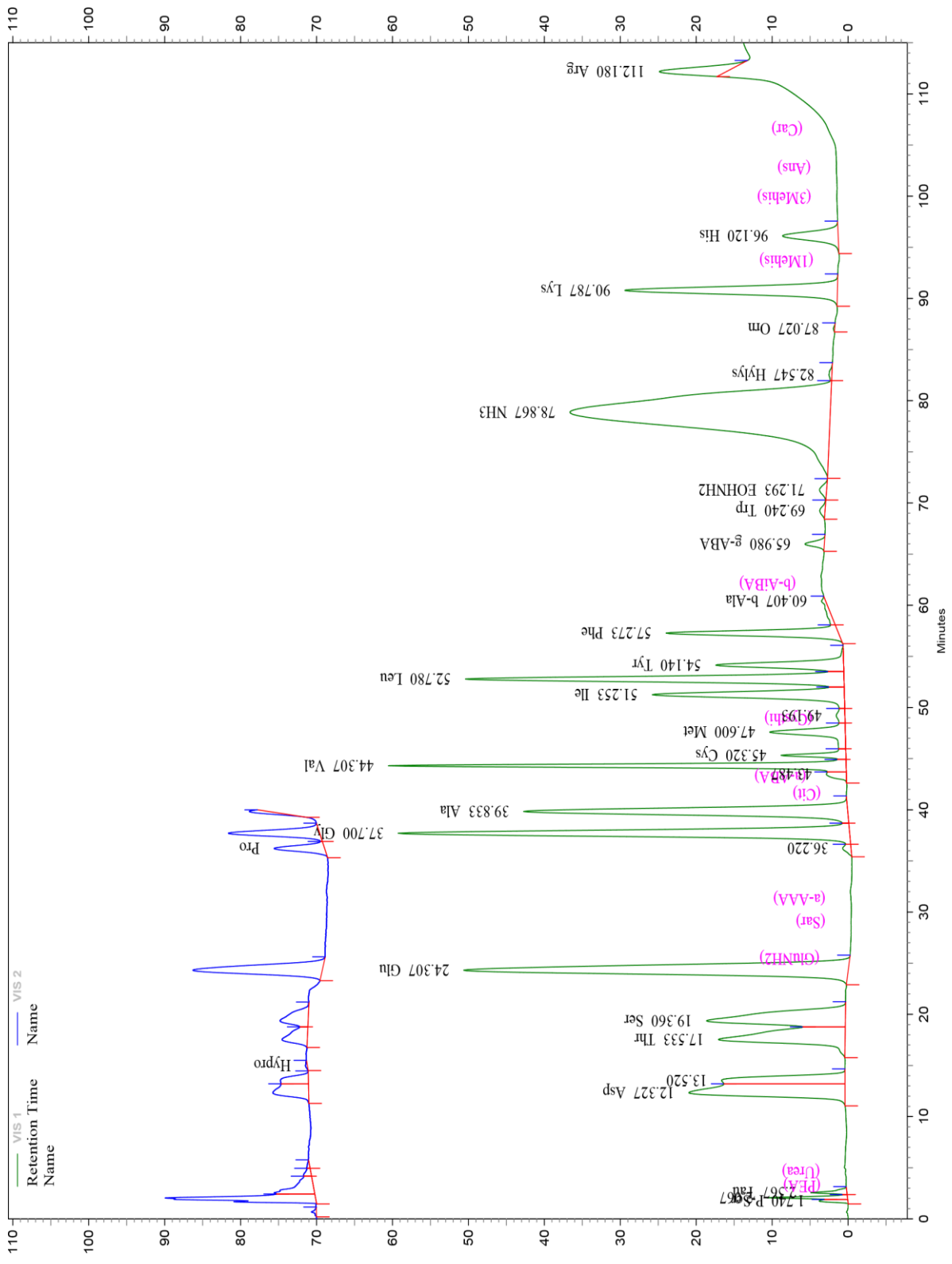


Fig 44: Chromatogram showing various amino acids in the sample. Hitachi L-8900 amino acid analyzer, Japan, was used for the analysis.

Table 16: - Essential amino acids (EAA) profile of *Nyctanthes arbor tristis* in leaves

Amino acid	General formula	Molecular weight (g/mol)	Concentration (g/100g)
Histidine (His)	$C_6H_9O_2N_3$	155.16	0.258 ± 0.032
Isoleucine (Ile)	$C_6H_{13}O_2N$	131.17	0.612 ± 0.006
Leucine (Lue)	$C_6H_{13}O_2N$	131.17	1.101 ± 0.028
Lysine (Lys)	$C_6H_{14}O_2N_2$	146.19	0.791 ± 0.122
Methionine (Met)	$C_5H_{11}O_2NS$	149.21	0.246 ± 0.044
Phenylalanine (Phe)	$C_9H_{11}O_2N$	165.19	0.715 ± 0.014
Threonine (Thr)	$C_4H_9O_3N$	119.12	0.613 ± 0.048
Tryptophan (Trp)	$C_{11}H_{12}N_2O_2$	204.23	0.030 ± 0.000
Valine (Val)	$C_5H_{11}O_2N$	117.15	0.741 ± 0.008

Table 17: - Non- essential amino acids (NEAA) profile of *Nyctanthes arbor tristis* in leaves

Amino Acid	General Formula	Molecular weight (g/mol)	Concentration (g/100g)
Alanine (Ala)	$C_3H_7O_2N$	89.09	0.718 ± 0.054
Arginine (Arg)	$C_6H_{14}N_4O_2$	174.2	0.361 ± 0.010
Asparatate (Asp)	$C_4H_7O_4N$	133.11	1.075 ± 0.153
Cysteine (Cys)	$C_6H_{12}O_4N_2S$	240.29	0.218 ± 0.010
Glutamic Acid (Glu)	$C_5H_9O_4N$	147.13	2.043 ± 0.132
Glutamine (Glu NH2)	$C_5H_{10}N_2O_3$	146.14	0.004 ± 0.000
Glycine (Gly)	$C_2H_5O_2N$	75.07	0.776 ± 0.073
Proline (Pro)	$C_5H_9O_2N$	115.13	0.671 ± 0.015
Serine (Ser)	$C_3H_7O_3N$	105.09	0.591 ± 0.035
Tyrosine (Tyr)	$C_9H_{11}O_3N$	181.19	0.491 ± 0.103

Table 18: - Non-proteinogenic amino acids profile of *Nyctanthes arbor-tristis* in leaves

Amino Acid	General Formula	Molecular weight (g/mol)	Concentration (g/100g)
Phosphoserine (p- Ser)	$C_3H_8NO_6P$	185.07	0.025 ± 0.000
Taurine (Tau)	$C_2H_7NO_3S$	125.15	0.033 ± 0.017
Phospho ethanol amine (PEA)	$C_2H_8NO_4P$	141.063	0.009 ± 0.000
Sarcosine (Sar)	$C_3H_7NO_2$	89.093	0.011 ± 0.000
α Amino adipic acid (a- AAA)	$C_6H_{11}NO_4$	161.156	0.005 ± 0.000
α Amino-n- butaric acid (a-ABA)	$C_4H_9NO_2$	103.12	0.012 ± 0.000
Cystathionine (Cysthi)	$C_7H_{14}N_2O_4S$	222.263	0.046 ± 0.001
β -Alanine (b-Ala)	$C_3H_7NO_2$	89.09318	0.056 ± 0.012
β -Amino isobutyric acid (b-AiBA)	$C_4H_9NO_2$	103.12	0.006 ± 0.000
ϒ –Amino- n- butyric acid (g- ABA)	$C_4H_9NO_2$	103.12	0.058 ± 0.014
Ethanol amine (EOHNH2)	C_2H_7NO	61.08	0.020 ± 0.002
Hydroxylysine (Hylys)	$C_6H_{14}N_2O_3$	162.187	0.015 ± 0.002
Ornithine (Orn)	$C_5H_{12}N_2O_2$	132.16	0.013 ± 0.001
1 Methylhistidine (1 Mehis)	$C_7H_{11}N_3O_2$	169.184	0.041 ± 0.003
3 Methylhistidine (3 Mehis)	$C_7H_{11}N_3O_2$	169.1811	0.013 ± 0.004
Hydroxy proline (Hypro)	$C_5H_9NO_3$	131.13	0.087 ± 0.015

Citrulline (Cit)	$C_6H_{13}N_3O_3$	175.2	0.015 ± 0.002

4.12 Fatty acid analysis

Fatty acids were quantified using Gas chromatography. Sample volume taken for GC was 1.000000 µl and the sample amount (1.0000) was injected automatically at 12.5000 pts/s sampling rate. The sample was run for 75 minutes. The highest content present in *Nyctanthes* leaf extract was C16:0 Methyl palmitate i.e 6% from the graph can also be seen (Fig 45). List of fatty acids present in leaves of *Nyctanthes* is shown in Table 19.

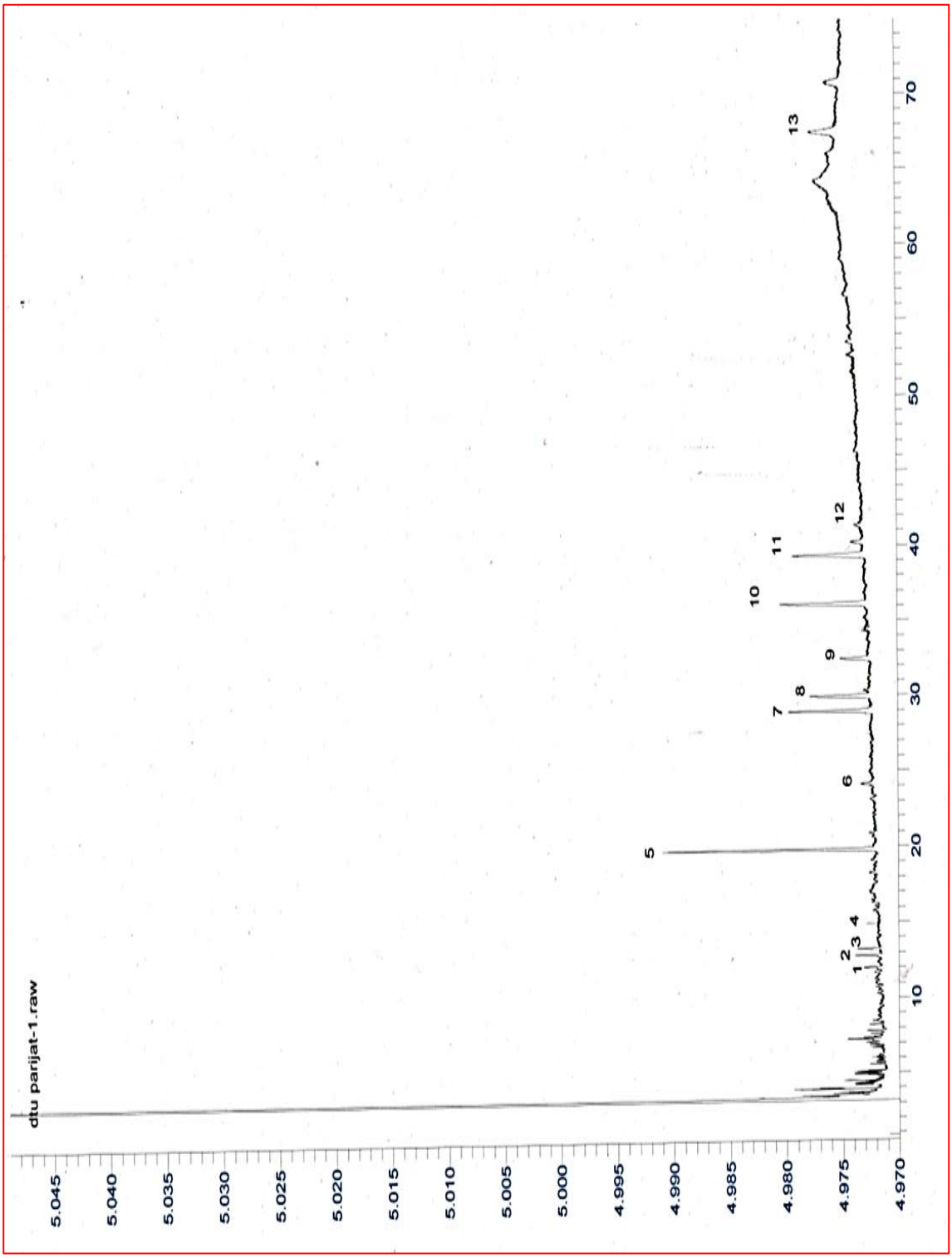


Fig 45: - GC chromatogram of fatty acids

Table 19: - List of fatty acid present In *Nyctanthes* leaf extract

Fatty acids	Concentration (%)
C4:0 Methyl butyrate	4
C6:0 Methyl caproate	4
C8:0 Methyl caprylate	4
C10:0 Methyl decanoate	4
C11:0 Methyl undecanoate	2
C12:0 Methyl dodecanoate	4
C13:0 Methyl tridecanoate	2
C14:0 Methyl myristate	4
C14:1 (cis-9) Methyl myristoleate	2
C15:0 Methyl pentadecanoate	2
C15:1 (cis-10) Methyl pentadecenoate	2
C16:0 Methyl palmitate	6
C16:1 (cis-9) Methyl palmitoleate	2
C17:0 Methyl heptadecanoate	2
C17:1 (cis-10) Methyl heptadecenoate	2
C18:0 Methyl stearate	4

C18:1 (trans-9) Methyl octadecenoate	2
C18:1 (cis-9) Methyl oleate	4
C18:2 (all-trans-9,12) Methyl linolelaidate	2
C18:2 (all-cis-9,12) Methyl linoleate	2
C18:3 (all-cis-6,9,12) Methyl linolenate	2
C18:3 (all-cis-9,12,15) Methyl linolenate	2
C20:0 Methyl arachidate	4
C20:1 (cis-11) Methyl eicosenoate	2
C20:2 (all-cis-11,14,) Methyl eicosadienoate	2
C20:3 (all-cis-8,11,14) Methyl eicosatrienoate	2
C20:3 (all-cis-11,14,17) Methyl eicosatrienoate	2
C20:4 (all-cis-5,8,11,14) Methyl arachidonate	2
C20:5 (all-cis-5,8,11,14,17) Methyl eicosapentaenoate	2
C21:0 Methyl heneicosanoate	2
C22:0 Methyl behenate	4
C22:1 (cis-13) Methyl erucate	2
C22:2 (all-cis-13,16) Methyl docosadienoate	2
C22:6 (all-cis-4,7,10,13,16,19) Methyl docosahexaenoate	2
C23:0 Methyl tricosanoate	2
C24:0 Methyl lignocerate	4
C24:1 (cis-15) Methyl nervonate	2

Table 20: - Fatty acid report of *Nyctanthes* leaf extract

Peak (#)	Component name	Retention time (min)	Area (uV*sec)	Area (%)
1	C14:0	11.993	5.639032	1.0891
2	C14:1	12.811	10.692074	2.0650
3	C15:0	13.220	10.223319	1.9745
4	C15:1	14.893	0.582763	0.1126
5	C16:0	19.787	134.237977	25.9265
6	C17:1	24.088	6.478290	1.2512
7	C18:0	28.959	74.687449	14.4250
8	C18:1 Cis	29.952	51.923113	10.0283
9	C18:2 Cis	32.451	24.229337	4.6796
10	Gamma C18:3 n3	36.113	81.411448	15.7236
11	C20:1	39.340	76.076197	14.6932
12	Alpha C18:3 n3	41.360	3.484518	0.6730
13	C24:1	67.479	38.099046	7.3584

5. CONCLUSION

As indicated by the aftereffect of fundamental secondary phytochemical screening it is inferred that the leaves of *Nyctanthes arbor-tristis* contained considerable number of secondary bioactive compounds; for example, alkaloid, flavonoid, steroid, phenol, tannin, saponin, glycoside and terpenoid. These secondary metabolites were identified using various techniques and can be utilized to cure different sicknesses customarily and used to tranquilize plants by pharmaceutical enterprises. Quantitative estimation revealed higher quantities of Protein (19.22%) and Tannin (13.1%) than other compounds in leaf extracts. Fe and Cu nanoparticles were synthesized using the leaf extracts. These nanoparticles exhibited better antibacterial and antifungal activities. The *Nyctanthes arbor-tristis* Fe-Cu nanoparticles can be used for the development of antibacterial agents or fungicidal formulations. This application of nanoparticles is just one, but various avenues for their applications are still open for research.

6. REFERENCES

1 Maiti R, Rodriguez HG, Kumari A. Nutrient Profile of Native Woody Species and Medicinal Plants in Northeastern Mexico: A Synthesis. J Bioprocess Biotech Vol 6, pp 283 (2016).

2 Jerezano Alberto VD, Ríos Saúl A, Tepancal-Gomez E, et al. Some Traditional Medicinal Plants of North Region from Puebla, Mexico: Uses and Potential Pharmacological Activity of Rumex spp. Nat Prod Chem Res Vol 4, pp 223 (2016).

3 Getasetegn M, Tefera Y. Biological Activities and Valuable Compounds from Five Medicinal Plants. Nat Prod Chem Res Vol 4, pp 220 (2016).

4 Gupta A, Shah AP, Chaphalkar SR. Extraction of Proteases from Medicinal Plants and their Potential as Anti-Viral Targets. J Biotechnol Biomater Vol 6 pp 228 (2016).

5 Norkulova K, Safarov J, Sultanova S. Research on Aerodynamics Separator for Medicinal Plants. J Food Process Technol Norkulova et al., J Food Process Technol Vol 7, pp 586 (2016).

6 Alothyqic N, Almalki M, Ebqa'ai M, et al. In Vitro Antibacterial Activity of four Saudi Medicinal Plants. J MicrobBiochem Technol Vol 8, pp 83-89 (2016).

7 Kumar M. Rural Communities on Ethno Medicinal Plants, Uses and their Conservation. Med Aromat Plants Vol 3, pp 160 (2016).

8 Opinde HR, Gatheri GW, Nyamache AK. Antimicrobial Evaluation of Crude Methanolic Leaf Extracts from Selected Medicinal Plants Against *Escherichia coli*. J Bacteriol Parasitol Vol 7, pp 272 (2016).

9 Tiwari SS. Medicinal Plants-Perspectives and Needs. J Pharmacogn Nat Prod Vol 2, pp 105 (2016).

10 Bouyahya A, Abrini J, El-Baabou A, et al. Determination of Phenol Content and Antibacterial Activity of Five Medicinal Plants Ethanolic Extracts from North-West of Morocco. J Plant PatholMicrobiol Vol 7, pp 342 (2016).

11 UNESCO. Culture and Health, Orientation Texts – World Decade for Cultural Development 1988 – 1997, Document CLT/DEC/PRO – 1996, Paris, France (19960.

12 Mahato SB, Sen S Advances in triterpenoid research, Phytochemistry (1997) Vol 44, pp 1185-1236 (1997).

13 Kappers IF, Aharoni A, van Herpen TW, Luckerhoff LL, Dicke M, et al. Genetic engineering of terpenoid metabolism attracts bodyguards to Arabidopsis. Science Vol 309, pp 2070-2072. (2005).

14 Hérouart D, Sangwan RS, Fliniaux MA, Sangwan-Norreel BS Variations in the Leaf Alkaloid Content of Androgenic Diploid Plants of Datura innoxia. Planta Med Vol 54, pp 14-17 (1988).

15 Gibson EL, Wardel J, Watts CJ. Fruit and Vegetable Consumption, Nutritional Knowledge and Beliefs in Mothers and Children. Appetite; Vol 31, pp 205-228 (1998).

16 Mathai K. Nutrition in the Adult Years. In Krause's Food, Nutrition, and Diet Therapy, 10th ed.ed. L.K. Mahan and S. Escott-Stump, Vol 271, pp 274-275. 2000

17 Walton NJ, Mayer MJ, Narbad A. Molecules of Interest: Vanillin. Phytochemistry, Vol 63, pp 505-515 (2003).

18 Dai J, Mumper R. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules, Vol 15, pp 7313-7352 (2010).

19 Tapas AR, Sakarkar DM, Kakde RB. Flavonoids as Nutraceuticals: A Review.Tropical Journal of Pharmaceutical Research, Vol 7, pp 1089-1099 (2008).

20 De Bruyne T, Pieters L, Deelstra H, Vlietinck A. Condensed vegetables tannins: biodiversity in structure and biological activities. Biochemical System Ecology, Vol 27, pp 445–59 (1999).

21 Dolara P, Luceri C, De Filippo C, Femia AP, Giovannelli L, Carderni G, Cecchini C, Silvi S, Orpianesi C, Cresci A. Red wine polyphenols influence carcinogenesis, intestinal microflora, oxidative damage and gene expression profiles of colonic mucosa in F344 rats. Mutation Research, Vol 591, pp 237–46, (2005).

22 Gyamfi MA, Aniya Y. Antioxidant properties of Thonningianin A, isolated from the African medicinal herb, Thonningia sanguine. Biochemical Pharmacology, Vol 63, pp 1725–37 (2002).

23 Molyneux RJ, Nash RJ, Asano N. Alkaloids: Chemical and Biological Perspectives, Pelletier SW, ed. Pergamon, Oxford, Vol 11, pp 303 (1996).

24 Saxena, R.S, B. Gupta, K.K. Saxena, R.C. Singh and Prasad, Study of Anti-inflammatory Activity in the leaves of *Nyctanthes arbor tristis* Linn. An Indian Medicinal Plant. J Ethanopharmcol, Vol 1(3), pp 319-30 (1984).

25 Puri A, Saxena R, Saxena RP, Saxena KC, Srivastava V, Tandon JS, Immunostimulant activity of *Nyctanthes arbor-tristis* L. J Ethnopharmacol, Vol 42(1), pp 31-7 (1994).

26 Mathew, N., Anitha, M.G., Bala, T.S., Sivakumar, S.M., Narmadha, R., Kalyanasundaram, Larvicidal activity of Saraca indica, *Nyctanthes arbor-tristis* and Clitoria ternatea extracts agzainst three mosquito vector species. Parasitol, Vol 104(5), pp 1017-25 (2009).

27 Bansal S, Bharatiand AJ, Bansal YK. Efficient In-vitro Regeneration of a Medicinal Plant Harsinghar (*Nyctanthes arbor-tristis* Linn.). Plant Tissue Cult. & Biotech Vol 22(2), pp 137-142 (2012).

28 Prabodh Satyal, Prajwal Paudel, Ambika Poudel, William N. Setzer. Chemical composition and biological activities of essential Oil from leaf and bark of *Nyctanthes arbortristis* Linn.from Nepal. Open Access J Medicin. & Arom. Plants.; Vol 3(1), pp 1-4 (2012).

29 Sonu Sharma, Bhawana kapoor, Mani bhusal, Karisma Sharma. Anti –bacterial evaluation of plant *Nyctanthes arbor-tristis* linn. Int. J. Advanc. Pharma. Res.; Vol 3(7), pp 1004-1009 (2012).

30 Repon Kumer Saha, Srijan Acharya, Syed Sohidul Haque Shovon, Apurba Sarker Apu, Priyanka Roy. Biochemical investigation and biological evaluation of the methanolic extract of the leaves of *Nyctanthes arbor tristis* in vitro. Asian Paci. J Trop. Biomed.; Vol S15, pp 34 (2012).

31 Kannan P, Ganjewala D. Preliminary Characterization of Melanin Isolated from Fruits and Seeds of *Nyctanthes arbor-tristis*. J Sci. Res.Vol 1(3), pp 655-661 (2009).

32 Kannan P, Ganjewala D. Preliminary Characterization of Melanin Isolated from Fruits and Seeds of *Nyctanthes arbor-tristis*. J Sci. Res.: Vol 1(3), pp 655-661 (2009).

33 M. Sumdrarajan, S. Gowri, Department of Industrial chemistry, Green chemistry Lab, Alagappa University, Vol. 8, pp 447-451 (2011).

34 Bhavya Bandi, K. Venkatesan, Ujwala Mannarapu, M. Keerthi. Isolation and partial characterization of alkaloids from stem bark of *Nyctanthes arbor-tristis* ISSN 0976-0350; (2011).

35 Bhadouria RS, Bhargava S, Pancholi SS. Isolation and characterization of two alkaloids from the ethanolic extract of *Nyctanthes arbor-tristis* linn. Leaves. Advance. Res. Pharma. & Biolog.; Vol 2(4), pp 342-344 (2012).

36 Meghashri S, Gopal S. Biochemical characterization of radical scavenging polyphenols from *Nyctanthes arbor tristis*. J. Pharm. Bioallied. Sci, Vol 4(4), pp 341-344 (2012).

37 Harborne JB, 'Phytochemical methods: A guide to modern technique of plant analys i', Champman and Hall, London, 1998.

38 Kokate, C. K., Purohit, A. P. and Gokhale, Pharmacognosy, Nirali Prakashan, New Delhi, India, Vol 13, pp 1-14 (2006).