Physicochemical and Phytochemical screening of the leaves of *Nyctanthes arbor-tristis* L. (Oleaceae) with reference to its Antioxidant and Antidiabetic activity: *In vitro* & *In vivo* study

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CERTIFICATE OF APPROVAL

This is to certify that the research work embodied in this thesis entitled- **Physicochemical and Phytochemical screening of the leaves of *Nyctanthes arbor-tristis* L. (Oleaceae) with reference to its Antioxidant and Antidiabetic activity: *In vitro* & *In vivo* study** was carried out by **Subham Saha**, (Exam Roll No. M4PHG23035, Registration No.139960 of 2017-2018) for the partial fulfillment of **Degree of Master of Pharmacy**, Jadavpur University, is absolutely based upon his own research project work under my supervision, in the Pharmacognosy & Phytotherapy Research Laboratory, Division of Pharmacognosy, Department of Pharmaceutical Technology, Jadavpur University, Kolkata- 700032.

He has incorporated his findings into this thesis. His thesis has not been submitted before for any degree/diploma or any other academic award elsewhere. I am satisfied that he has carried out his thesis with proper care and confidence to my satisfaction.

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DECLARATION

I hereby declare that the thesis contain literature survey and the original research work entitled- **Physicochemical and Phytochemical screening of the leaves of *Nyctanthes arbor-tristis* L. (Oleaceae) with reference to its Antioxidant and Antidiabetic activity: *In vitro* & *In vivo* study** is carried out by me under the guidance of Prof. (Dr.) Subhash C. Mandal, Department of Pharmaceutical Technology, Jadavpur University, Kolkata-700032.The work is original and has not been submitted in part or in any form to any other university for the award of any Degree or Diploma or Fellowship. The results reported here are the findings of work carried out by me.

I also declare that, as required by the rules and conduct, I have fully cited and referenced all the materials and results that are not original to this work.

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Subham Saha.

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Subham Saha

CONTENTS

List of Figures

List of Tables

List of Abbreviations

Sl No.	Abbreviation	Full Form
1	%	Percentage
2	µg	Microgram
3	ml	Microlitre
4	g	Gram
5	°C	Degree Celsius
6	Fig.	Figure
7	No.	Number
8	Sl.No.	Serial number
9	Abs.	Absorbance
10	ROS	Reactive oxygen species
11	RNS	Reactive nitrogen species
12	cm	Centimeter(s)
13	DPPH	2,2-diphenyl-1-picryl hydrazyl
14	GAE	Gallic acid equivalent
15	IC_{50}	Half maximal inhibitory concentration
16	kg	Kilogram
17	l	Litre(s)
18	m	Metre(s)
19	M	Molarity
20	mg	Milligram(s)
21	min	Minute(s)
22	mM	Millimolar

23	no.	Number
24	QE	Quercetine quivalent
25	pH	Potential of hydrogen
26	SD	Standard deviation
27	SEM	Standard error of mean
28	UV-Vis	Ultra violet visible
29	v/v	Volume by volume
30	NaCl	Sodium Chloride
31	Conc.	Concentrated
32	$FeCl_3$	Ferric chloride
33	H_2SO_4	Sulfuric acid
34	STZ	Streptozotocin
35	WHO	World Health Organization
36	nm	Nanometer
37	µl	Micro liter
38	i.e.	That is
39	*et.al*	Et alia (and the other contributors)
40	w/w	Weight/weight
41	TLC	Thin LayerChromatography
42	HPLC	High Performance Liquid Chromatography
43	ppt	Precipitate
44	CAM	Complementary Medicine and alternative Medicine
45	TM	Traditional Medicine

46	DM	Diabetes Mellitus
47	ADA	American Diabetes Association
48	dl	Decilitre
49	mmol	Millimolar
50	HbA1c	Glycated haemoglobin
51	Hb	Haemoglobin
52	L.	Linneaus
53	Linn.	Linnaeus
54	h	Hour
55	HCl	Hydrochloric Acid
56	Na	Sodium
57	NaOH	Sodium Hydroxide
58	FCR	Folin-Ciocalteu reagent
59	TPC	Total Phenolic Content
60	TFC	Total Flavonoid Content
61	w/v	Weight by volume
62	NAT	*Nyctanthes arbor-tristis*
63	NATME	*Nyctanthes arbor-tristis* methanolic extract
64	β	Beta
65	α	alpha
66	b.w	Body Weight
67	HFD	High Fat Diet
68	STD	Standard
69	EDTA	Ethylenediamine tetraacetic acid

70	THb	Total haemoglobin
71	GHb	Glycosylated haemoglobin
72	R_f	Retention factor
73	TCA	Trichloroacetic acid
74	LPO	Lipid Peroxidation
75	SOD	Super dismutase
76	GSH	Reduced Glutathione
77	TBA	Thiobarbituric acid
78	FBG	Fasting Blood Glucose
79	ANOVA	Analysis of Variance
80	<	Less than
81	±	Plus- minus
82	MDA	Malondialdehyde
83	O_2	superoxide
84	OH	Hydroxyl
85	LD_{50}	Lethal dose
86	ppm	Parts per million
87	e.g.	Exempli gratia (for example)
88	Etc.	Et cetera (and the other things)
89	&	And
90	DNS	3,5-Dinitrosalicylic acid

1. Introduction

Since the beginning of time, traditional medicine and medicinal plants, components of plants, and isolated phytoconstituents have been utilized to prevent and treat a variety of health issues. Between 65 and 80 percent of people in emerging nations use traditional medicine as their main form of healthcare. Additionally, it is becoming more popular in wealthy countries as a low-cost alternative to expensive modern pharmaceuticals for illnesses when modern treatments are unavailable or ineffective, as well as being less toxic and having fewer side effects than modern medicines. Nearly 25% of all drugs given globally contain one of 121 active compounds derived from plants. On the WHO's list of 252 essential medications, only 11 percent consist wholly of plants. Nearly 80% of Indians living in rural areas use traditional medicine and medicinal plants.

Traditional medicine also known as complementary and alternative medicine (TM/CAM) is defined by the World Health Organization (WHO) as the historically used therapeutic modalities used to promote human health and welfare. Traditional medicine has strong cultural and genuine origins, as well as practical experience based on evidence. Nowadays, a variety of conventional, complementary, and alternative therapies from around the globe are becoming more and more popular. The herbal remedies used in traditional medicine have a lengthy history of use, which gives credence to their therapeutic potential. Traditional medicine has its roots in the distant past. WHO has recognized Ayurveda as India's traditional medical system, which dates back more than 5000 years and is still widely used throughout the nation. Ayurveda is widely recognized around the world. It is based on the fictitious knowledge of centuries of observations and experience that about 80% of plants, 12% of animals, and 8% of minerals are used as medicines to treat wide range of diseases(Joshi, 2014).

According to Akerele et al. (1993), 80% of the population of developing countries relies on plant-based medicine as their primary means of medical care. Due to its medicinal equity, the ethnopharmacological evaluation of plants to assess their efficacy, safety, and drug-discovery potential has gained popularity (Karou et al., 2007). Ethnopharmacology is an interdisciplinary study field that involves the incorporation of validated conscientious facet and ethnographically guided research, in addition to the

inclusion of historical perspectives of indigenous knowledge systems (Ningthoujatia et al. 2006), that integrates observation, description, and experimental investigation of traditional medicines and biological effects. It emphasizes the significance of plant-derived medications.

Since antiquity, various health issues have been prevented and treated using plants, plant parts, and plant-based products. About 80% of African and Asian people practice basic medicine using traditional treatments. Around 80% of Indians who live in rural areas employ traditional medical practices or medicinal plants. According to Sahoo et al. (2010), the Indian herbal business uses more than 960 plant species, with 178 of those species producing more than 100 metric tonnes annually.

According to the WHO, a "herbal medicine" is any substance or preparation made from plants that has medicinal or other benefits for human health and contains either raw or processed components from one or more plants. Worldwide, the usage of herbal remedies has increased noticeably in recent years (Zhang et al., 2012). Due to the importance of quality control to maintain the safety and effectiveness of herbal medicines, WHO has developed a set of technical standards for medicinal plants and herbal products that address these issues. By ensuring quality, safety, and efficacy, standardization helps to prevent adulteration from subpar species.

The quest for natural remedies that offer dual benefits in combating oxidative stress and managing diabetes has led to extensive research on various plant-based compounds. One such promising candidate is *Nyctanthes arbor-tristis*, commonly known as the "Night-flowering Jasmine" or "Parijat" in traditional Indian medicine. *Nyctanthes arbor-tristis* is a small to medium-sized tree native to the Indian subcontinent and Southeast Asia. Its leaves have been traditionally used in Ayurvedic medicine for their potential health benefits. Recent scientific investigations have delved into the antioxidant and Antidiabetic properties of these leaves, opening up avenues for potential therapeutic applications.

1.1. Antioxidants

Antioxidant activity refers to the ability of certain molecules to neutralize or counteract harmful molecules known as free radicals. Free radicals are highly reactive compounds that can damage cells, DNA, and other biomolecules, leading to oxidative stress and various health issues. Antioxidants play a crucial role in maintaining the balance between these harmful molecules and the body's defense mechanisms, helping to prevent or mitigate oxidative damage. Common antioxidants include vitamins (like vitamin C and E), minerals (such as selenium), and various phytochemicals found in fruits, vegetables, and other plant-based foods.Antioxidants are substances that prevent oxidation, a chemical process that can result in free radicals and cascade events that could harm an organism's cells. These processes may be inhibited by antioxidants like thiols or ascorbic acid (vitamin C) or thiols, among others. Plants and mammals maintain intricate networks of overlapping antioxidants, including glutathione, to manage oxidative stress.Originally, a substance that blocked oxygen from being consumed was referred to as an antioxidant.Oxidative stress, arising from an imbalance between reactive oxygen species (ROS) production and the body's ability to neutralize them, plays a crucial role in the pathogenesis of various chronic diseases, including diabetes.

The leaves of *Nyctanthes arbor-tristis* contain a plethora of bioactive compounds such as flavonoids, phenolics, and carotenoids, which have shown potent antioxidant activity in various studies.Flavonoids, for instance, are known for their ability to scavenge free radicals and inhibit lipid peroxidation. Quercetin and kaempferol are two major flavonoids identified in Nyctanthes leaves, both exhibiting strong antioxidant potential. These compounds work synergistically to counteract oxidative damage to cellular components, potentially reducing the risk of complications associated with diabetes.

1.2.Diabetes: a brief introduction

Diabetes mellitus (DM) is a developing problem in India, with an estimated 8.7% of the population diabetic between the ages of 20 and 70 (WHO, 2014). In the year 2000, India

(31.7 million individuals) had the highest number of diabetics in the world, followed by China (20.8 million), and the United States (17.7 million) in second and third place, respectively. Diabetes is expected to double globally from 171 million in 2000 to 366 million in 2030, with India seeing the largest increase (Wild et al., 2004). Diabetes mellitus is expected to impact up to 79.4 million people in India by 2030, with considerable rises in the number of people afflicted in China (42.3 million) and the United States (30.3 million) (Whiting et al., 2011). India currently faces an uncertain future in relation to the potential burden that diabetes may impose upon the country. Diabetes is a chronic condition that arises when the pancreas produces insufficient insulin or when the body's insulin is not used effectively. As a result, the concentration of glucose in the blood rises. Hyperglycemia, glycosuria, hyperlipidemia, negative nitrogen balance, and occasionally ketonaemia are all symptoms of this metabolic disease. Thickening of the capillary basement membrane, increased artery wall matrix, and cellular proliferation cause vascular problems such as lumen constriction, early atherosclerosis, glomerular capillary sclerosis, retinopathy, neuropathy, and peripheral vascular insufficiency (Jameson et al., 2018).

In 2016, diabetes was the direct cause of 1.6 million deaths and in 2012 high blood glucose was the cause of another 2.2 million deaths (World Health Organization, Fact Sheet of Diabetes, 2014). Most of the patients can be classified clinically as having either Type 1 or Type 2 Diabetic Mellitus. The other types of diabetes include Peridiabetes, Gestational diabetes.

The American Diabetes Association (ADA) developed a criterion for the diagnosis of DM like the occurrence of symptoms like polyuria, polydipsia and unexplained weight loss and a random plasma glucose concentration of greater than 200 mg/dl, a fasting plasma glucose concentration of greater than 126 ml/dl, or a plasma glucose concentration of greater than 200 mg/dl. 2 hours after the ingestion of an oral glucose load (Goldman et al., 2018).

The main function of insulin is to lower blood glucose levels when they rise above normal. When these nutrients, especially glucose, are in excess of immediate needs insulin promotes storage by acting on cell membranes and stimulating uptake and use of

glucose by muscle and connective tissue cells, increasing glycogenesis, especially in the liver and skeletal muscles, accelerating uptake of amino acids by cells, and the synthesis of protein, promoting lipogenesis, decreasing glycogenolysis, preventing the breakdown of protein and fat, and gluconeogenesis (Anne Waugh, Allison Grant 2018). Much of the diabetes burden can be prevented or delayed by behavioral changes favouring a healthy diet and regular physical activity.

Virtually all forms of DM result from a decrease in the circulating concentration of insulin Le insulin deficiency and a decrease in the response peripheral tissues to insulin (insulin resistance).

1.3.Complication

Any form of diabetes increases the chance of long-term effects. The usually appear after a number of years (10-20), but they may be the init symptom in those who have not yet been diagnosed. Damage to blood vessels is one of the most serious long-term effects. Diabetes doubles the risk of cardiovascular disease, and coronary artery disease is responsible for roughly 75% of diabetes deaths in India. Stroke and peripheral vascular disease are two more "macrovascular" illnesses. Damage to the eyes, kidneys, and nerves are the most common microvascular consequences of diabetes. Damage to the eyes, known as diabetic retinopathy, is caused by damage to the blood vessels in the retina of the eye, and can lead to vision loss and blindness over time. Diabetic nephropathy, or kidney damage, can cause tissue scarring, urine protein loss, and eventually chronic kidney disease, needing dialysis or a kidney transplant. Diabetic neuropathy, or damage to the body's nerves, is the most prevalent consequence of diabetes. Numbness, tingling, discomfort, and altered pain feeling are some of the symptoms, which might cause skin damage. Diabetic foot problems (such as diabetic foot ulcers) can emerge and can be difficult to cure, necessitating amputation in certain cases. Proximal diabetic neuropathy also results in severe muscular atrophy and weakening. A relationship exists between cognitive impairment and diabetes. Those

with diabetes have a 1.2 to 1.5-fold higher rate of loss in cognitive function than those without the disease. (World Health Organization, Fact Sheet of Diabetes, 2022).

1.4.Diagnosis

Recurrent or chronic hyperglycemia, a feature of diabetes mellitus, can be diagnosed by any of the following signs and symptoms:

- Fasting plasma glucose level ≥ 7.0 mmol/l (126 mg/dl)
- Plasma glucose ≥ 11.1 mmol/l (200 mg/dl) two hours after a 75 g oralglucose load as in a glucose tolerance test
- Symptoms of hyperglycemia and casual plasma glucose ≥ 11.1 mmol/l(200 mg/dl)
- Glycated hemoglobin(HbA1c) ≥ 6.5%.

1.5.Natural products and Diabetes

Medicine has basically existed from the dawn of human society. Natural products rich in secondary metabolites and chemicals derived from natural products have historically and scientifically produced the majority of new medications (Lahlou, 2013). Humans have employed naturally occurring chemicals for medical purposes for thousands of years. Plants have a vital part in medicine in most cultures. Plants were researched more extensively to determine why they were medically effective with the modernization of chemistry in the early nineteenth century (Beutler, 2009). Many currently accessible medications are obtained either directly or indirectly from plant sources. When we look at the history of plants as medicine, we can see that it dates back to the beginning of time. Natural products have long been a key source of pharmacological substances, and they have played an important part in the evolution of contemporary medicine (Kulkarni, 2014).

Natural products have played an important part in the drug discovery process. Due to the failure of alternative drug discovery strategies to deliver many lead compounds in crucial therapeutic areas such as immunosuppressive, anti-infective, and metabolic illnesses, there has been a revived interest in natural products research recently. Natural product research, on the other hand, must continue to improve in order to remain competitive with other drug discovery methodologies and to keep up with ongoing changes in the drug discovery process (Yuan, 2016).

Natural products have played an important part in the drug discovery process. Due to the failure of alternative drug discovery strategies to deliver many lead compounds in crucial therapeutic areas such as immune suppressive, anti-infective, and metabolic illnesses, there has been a revived interest in natural products research recently. Natural product research, on the other hand, must continue to improve in order to remain competitive with other drug discovery methodologies and to keep up with ongoing changes in the drug discovery process.

Natural products have played an important role in the pharmaceutical business over the last half-century, with various therapeutic classes generated from natural materials and used as templates for synthetic modification in the areas of infectious illnesses and oncology. Between 2000 and 2010, about 40 novel medications derived from terrestrial plants, terrestrial microbes, marine species, and terrestrial vertebrates and were introduced to the market (Kulkarni, 2014). There is a burning urge to discover an effective and cost-effective strategy to control diabetes and associated complications. Scientists are developing new synthetic compounds, but they have a number of drawbacks, including side effects and total treatment costs. Natural products are becoming increasingly popular against this backdrop.

1.6.Anti diabetic

Anti-diabetic activity refers to the ability of a substance, often a natural compound or medication, to help regulate blood sugar levels and manage diabetes. These substances can work by enhancing insulin sensitivity, promoting glucose uptake by cells, inhibiting

glucose production, or other mechanisms. Research, in this area, aims to discover new ways to treat and manage diabetes, which is a chronic condition characterized by high blood sugar levels.Diabetes mellitus, characterized by hyperglycemia due to insufficient insulin production or impaired insulin action, is a global health concern.

Nyctanthes arbor-tristis leaves have been investigated for their potential to manage diabetes through various mechanisms.One significant approach is their ability to enhance insulin sensitivity. Compounds present in these leaves, such as triterpenoids and alkaloids, have been found to stimulate glucose uptake in cells, thereby reducing blood glucose levels. Additionally, the leaves exhibit alpha-amylase and alpha-glucosidase inhibitory activities, which can slow down carbohydrate digestion and absorption, leading to improved glycemic control.

1.7.Synergistic Action:

The antioxidant and antidiabetic activities of *Nyctanthes arbor-tristis* leaves appear to be interconnected. Oxidative stress contributes to insulin resistance, a key factor in type 2 diabetes. By reducing oxidative stress, the leaves may indirectly enhance insulin sensitivity, aiding in glycemic control. Conversely, the antidiabetic effects may also stem from the reduction of oxidative damage to pancreatic beta cells, preserving their function and insulin secretion capacity.

2. Description of Plant

Southeast Asia is habitat to the Nyctanthes genus of flowering plants, which belongs to the Oleaceae family. It is a terrestrial woody perennial with a lifespan of 5 to 20 years(Jain & Pandey, 2016). There are currently only two species recognized as belonging to this genus; the majority of the others have been moved to the Jasminum genus.

Figure 1: *Nyctanthes arbor-tristis* Linn.

Leaves are 4-sided, opposite, shortly petioled, quadrate or oblong, moderately thick, rough,pointed or coarsely serrate, scabrous, dark green in colour,can be easily broken, has abundant veins and the lower surface is thicker than the upper surface (Azma et al., 2021).

The flowers are produced in small clusters of two to seven together. Each of the fruit's two capsule-shaped parts contains a single seed.Flowers open at dusk and finish at

dawn. Fruits are in capsule, orbicular, compressed. Seeds are orbicular and flattened. (Agrawal & Pal, 2013)

2.1.Biological Classification

Name of Plant: *Nyctanthes arbor-tristis* L.

Kingdom: Plantae.

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Lamiales.

Family: Oleaceae.

Genus: Nyctanthes.

Species:N.arbor-tristis.

2.2. Synonyms

Pariaticu Adans.

Omolocarpus Neck.

Bruschia macrocarpa Bertol.

Nyctanthes dentata Blume

Nyctanthes tristis Salisb., nom. superfl.

Parilium arbor-tristis (L.) Gaertn.

Scabritascabra L.

Scabritatriflora L.

2.3. Vernacular names

Bengali: Harsinghar, Sephalika, Seoli, Sheoli.

English: Coral Jasmine, Night Jasmine.

Hindi: Harsinghar, Harsingur, Seoli, Sheoli, Sihau.

Sanskrit: Parijata, Parijatah, Parijataka, Sephalika.

Assamese: Sewali, Shewali, Xewali

Gujarati: Jayaparvati, Parijatak.

Kannada: Goli, Harsing, Parijata.

Konkani: Pardic, Parizatak, Parzonto, Parzot.

Urdu: Gulejafari, Harsingar.

Malay: Seri Gading.

Malayalam: Mannapu, Pavizhamalli, Parijatakom.

Marathi: Kharbadi, Kharassi, Khurasli, Parijatak.

Oriya: Godokodiko, Gunjoseyoli, Singaraharo.

Punjabi: Harsinghar.

Tamil: Manjhapu, Pavala-Malligai, Pavazha-Malligai.

Telugu: Kapilanagadustu, Pagadamalle, Parijat, Sepali.

Indonesian: Srigading (Sundanese, Javanese).

Thai: Karanikaa.

Vietnamese: Iai Tau.

Filipino: Coral Jasmine.

Lao (Tibetan): Salikaa

2.4.Distribution

Nyctanthes arbor-tristis L. is native to Southern Asia, stretching across northern Pakistan and Nepal through northern India to southeast Thailand. It grows at sea level up to 1500 meters altitude, within a wide range of rainfall patterns, from seasonal to seasonal,and is tolerant to moderate shade. In India, it grows in the outer Himalayas and is found in tracts of Jammu and Kashmir, Nepal to east of Assam, Bengal, and Tripura extending through the central region up to Godavari in the south. Flowering usually

occurs from July to October. Nyctanthes prefers secluded and semi seedy places to grow**.** (Rani et al., 2012)

2.5.Climate and Soil

The plant can grow on rocky ground in dry hill shades, dry deciduous forests, or at sea-level up to 1500 m altitude with a wide range of rainfall patterns. It is frequently grown in gardens because of its attractive and unique aroma. This tree grows in a wide range of loamy soils with pH values between 5.6 and 7.5. The plant requires conditions varying from full sunlight to partial shade and needs to be watered regularly but does not require overwatering (Kiew and Bass, 1984).

2.6.Cultivation

It is often cultivated in gardens due to its beautiful flowers and aroma. The shrub can be propagated by cutting as well as by seeds. The seeds have been found to exhibit a poor germination rate because of phenolic compounds leaching out of the imbibed seeds. These inhibitory phenolic compounds were stored in the pericarp assisted by the seed coat. The rate of germination can increases either excluding both. The treating seeds with a solution of antioxidants like polyvinylpyrrolidine and polyvinylpolypyrrolidine prior to germination (Sah & Verma, 2012)

2.7 Traditional usage

Traditional medicine uses different parts like the leaves, bark, roots, and seeds of *Nyctanthes arbor-tristis* to cure a variety of illnesses. Since ancient times, various *Nyctanthes arbor-tristis* parts have been used in the Ayurvedic, Siddha, and Unani systems of medicine.

People claim that *Nyctanthes arbor-tristis* L. has a wide variety of therapeutic uses. In India, Indonesia (Java), and Malaysia, *Nyctanthes arbor-tristis* blossoms are used to promote menstruation, while the tree's bitter leaves have applications as a cholagogue, laxative, diuretic, and diaphoretic (**Agroforestrytreedatabase**).

The plant's opposing, straight forward leaves are 6–12 cm in length and 2–6.5 cm in width.Children with roundworms and threadworms are treated with leaf juice (**Chauhan, 1999**).

The leaf juice is also used as a diuretic and to treat piles, chronic fever, malarial fever, obstinate sciatica, rheumatism, and loss of appetite (**Banerjee et al., 2007**).

Fresh leaf juice when given to newborns with honey mixed with table salt has been touted as a safe purgative. It works as a diuretic and diaphoretic in the form of infusion in doses of two ounces for rheumatism and fever (**Nadkarni, 1982**).

The seed powder is used for alopecia, scalp scurvy, and as an anthelmintic(**Chatterjee et al., 2005**).

The bark is used as a treatment for snake bites and bronchitis (**Chatterjee et al., 2007**).

The tribal people of central India utilize several portions of the Nyctanthes arbor-tristis plant to treat sores, coughs, hiccups, diarrhea, and snake bites (**Jain et al., 2005**).

The inflorescence is used to treat scabies and other skin conditions (**Jain et al., 2005**).

As an anthelmintic, the herb has been utilized in Nepal (**Bhattarai, 1992**).

In Indian traditional medicine, Nyctanthes arbor-tristis is also known to have immuno toxic, antiallergic, antihistaminic, purgative, antibacterial, and ulcerogenic properties in addition to the ones already stated (**Annonymous, 1997**).

Other uses include treating bilious fevers and acting as an expectorant (**Nair et al., 2005**).

Some elderly Buddhist monks in Sri Lanka utilize a hot floral infusion as a sedative (**Ratnasooriya et al., 2005**).

N. arbor-tristis flowers are used to initiate menstruation. The hot floral infusion is employed as a sedative. The flower is effective for treating oral ulcers **(Sasmal et al., 2007**).

In Myanmar, a decoction of dried herb in a dose of 3-6 g or 300-500 ml is used orally for diabetes. Crushed fresh leaves are also applied topically to wounds and sores to lessen inflammation (**Anonymous**).

The Indian Jayantia tribes that live in the vicinity of Myanmar utilize the flower and honey as an anti-spasmodic and the leaf juice as an oral anthelmintic (**Jaiswal, 2010**).

The plant's blooms have historically been used to cure piles and a variety of skin conditions as they are useful as stomachic, carminative, astringent, anti-bilious, expectorant, and hair tonics.According to the bark is used to cure snakebites and bronchitis. (**Aggarwal et al., 2011**).

3. Literature review

Nirmal et al., 2012 Investigated the analgesic and anti-inflammatory potential of the plant and isolate the pure constituent from the leaves responsible for these activities.Analgesic activity of various extracts (petroleum ether, chloroform, ethyl acetate and ethanol) of leaves of the *Nyctanthes arbor tristis* L.were screened by hot plate test and acetic acid-induced writhings and anti-inflammatory activity by carrageenan-induced hind paw edema method at the dose of 50 mg/kg, i.p.The percentage yield of various extracts of leaves was found, petroleum ether extract (7.24%), chloroform extract (6.034%), ethyl acetate extract (4.48%), ethanol extract (8.54%) and aqueous extract (6.95%) respectively. Petroleum ether extract was found to be most active and hence subjected to activity-guided fractionation. The isolated compound was a crystalline solid having melting point at 136–138°C and single spot on TLC with R_f 0.47 (ethyl acetate/hexane, 2:8). It gave positive Liebermann– Burchard test indicating that the compound was a sterol.Results showed thatβ-sitosterol (5, 10 and 20 mg/kg, i.p.) was responsible for the significant and dose-dependent activity comparable with the standard extract. β -sitosterol from *N. arbortristis* leaves might be responsible for analgesic and anti-inflammatory activity.

Vyas and Sarin, 2013 Evaluated the phytochemical content and anti-microbial activity of *Nyctanthes arbor tristis*. The dried leaf extract prepared in ethanol, methanol, petroleum ether and aqueous Solutions and were evaluated for phytochemical screening as well as antimicrobial activity. The antimicrobial activity was evaluated on Gram positive bacteria- *Staphylococcus aureus* (MTCC *3160)*, Gram negative-*Escherichia coli* (MTCC 1652) and *Pseudomonas aeruginosa* (MTCC 647) and fungi- *Aspergillus niger* (MTCC282), *Aspergillus flavus* (MTCC 2456), *Fusarium culmorum*(MTCC349) and *Rhizopus stolonifera* (MTCC 2591). Phytochemical screening of the petroleum ether extract showed the presence of Triterpenoids; Ethanol extract showed the presence of Carbohydrate, Alkaloids, Saponins and Tannins; Chloroform extract showed the presence of Carbohydrate and Flavonoids;Ethyl acetate extract showed the presence of Carbohydrate, Flavonoids, Saponins and Tannins. Results showed that the petroleum ether extract exhibited highest zone of inhibition against *P. aeruginosa* (20.3±0.92 mm)

with low MIC value (24.5 mg/ml). This study indicates that *Nyctanthes arbor-tristis* possess compounds with antimicrobial properties that can be used for plant based antimicrobial agents.

Chitravanshi et al., 1992 Investigated the therapeutic action of *Nyctanthes arbor-tristis* against caecal amoebiasis of rat. The various parts (seeds, leaves, roots, flowers and stem) of *Nyctanthes arbor-tristis* L. were extracted using 50%ethanol and the action of this plant material against trophozoites of *E. hisrolytica in vitro* and in experimentally infected rat caecum was Evaluated. Results showed that the extracts from the leaves, seeds, roots, flowers and stem of the plant were effective in clearing *E. histolytica* infections in a very large number of animals. Among the leaf fractions, the hexane fraction possessed strong efficacy.

Kakoti et al., 2013 Evaluated the analgesic and anti-inflammatory activities of the methanolic Stem Bark Extract of *Nyctanthes arbor-tristis* Linn. The methanolic extract was subjected to preliminary phytochemical test and revealed the presence of alkaloids, amino acids, carbohydrate, flavonoids, glycosides, protein, tannins, and phenolic compounds. The analgesic activity was determined onWistar albino rats by hot plate method, tail flick assay, and tail immersion method using Morphine sulphate as standard drug at a dose of 5mg/kg of body weight. The anti-inflammatory activity was assessed by Carrageenan-induced rat paw oedema using diclofenac sodium as standard drug at a dose of 100mg/kg of body weight. Stem bark extract was given at a dose of 250mg/kg and 500mg/kg of body weight. Results showed that the methanolic extract of the stem bark of *Nyctanthes arbor-tristis* of 500 mg/kg body weight has significant analgesic activity compared to control, standard, and 250 mg/kg body weight methanolic extract. Rats pretreated with the methanolic extract of *Nyctanthes arbor tristis* stem bark showed a significant anti-inflammatory activity and in a dose-dependent manner when compared with standard drug diclofenac sodium.

Saxena et al., 1987 Screened analgesic, antipyretic and ulcerogenic activities of the water-soluble portion of an ethanol extract of the leaves of *Nyctanthes arbor tristis* L.

For Analgesic activity methods were selected for screening both morphine-like as well asaspirin-like analgesic activities. Result showed that the extract exhibited significant aspirin-like antinociceptive activity but failed to produce morphine-like analgesia. It was also found to possess antipyretic activity against brewer's yeast-induced pyrexia in rats. The extract also produced gastric ulcers following oral administration for six consecutive days in rats.

Priya and Ganjewala, 2007 Investigated the antibacterial potential of *Nyctanthes arbor-tristis* was evaluated on gram- positive (*Steylococcus aureus*) and gram-negative (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*) bacteria. For the preparation of extractdried leaf, flower, fruits and seed were extracted using ethyl acetate and chloroform. These extracts were used to assess their antibacterial potential in terms of zone of inhibition of bacterial growth. Extracts were subjected to preliminary phytochemical test and revealed the presence of alkaloids, flavonoids, glycosides, saponins, tannins, and phenolic compounds.100, 200 and 300 µl of each extract was used for antimicrobial screening. Both, ethyl acetate and chloroform extract (volume 300 µl) have shown significant antibacterial activity against the microorganisms tested. The results revealed that the gram negative (*Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) bacteria were more sensitive as compared to those of gram positive bacteria to all the extracts.

Khatune et al., 2001 Evaluated the Antibacterial activity and cytotoxicity of *Nyctanthes arbor-tristis* flowers. Successive petroleum ether, $CHCl_3$ and EtOAc the percentage yields was found 4.94, 7.22 and 1.57%, respectively. Petroleum ether and $CHCl_3$ extracts gave positive test for steroids while $CHCl_3$ and EtOAc extracts gave positive results for glycosides. The antimicrobial activity was evaluated on Gram positive bacteria *Bacillus subtilis, Bacillus cereus, Bacillus megaterium, Staphylococcus aureus, Streptoccus sp., Sarcinalute*and Gram negative bacteria *Escherichia coli, Shigella dysenteriae, Shigella shiga, Shigella boydii, Shigella sonnei, Pseudomonas aeruginosa* in terms of Zone of inhibition diameter (mm). Antibacterial activity was evaluated by disc diffusion method and Cytotoxicity by *Artemia salina* (Brine shrimp) lethality. The

results showed that Chloroform and ethyl acetate extracts of *Nyctanthes arbor-tristis* flowers in the order effectively inhibited the growth of all gram-positive and gram-negative tested bacteria; petroleum ether extract showed no antibacterial activity. On the contrary, all tested extracts showed significant cytotoxic activity.

Aggarwal and Goyal, 2013 Investigated the Antimicrobial properties of leaves of *Nyctanthes arbor-tristis*. The different solvent extracts (petroleum ether, chloroform, Ethanol, Benzene, hot water) were prepared on the basis of polarity in soxhlet apparatus. On phytochemical screening of various solvent extract of *Nyctanthes arbor-tristis* Chloroform extract showed the presence of Alkaloids, Saponins and Tannins; Ethanolic extract showed the presence of Alkaloids, Glycosides, Saponins, Tannins, Flavonoids and Steroids; hot water extract showed the presence of Alkaloids, Saponins, Tannins, Flavonoids and Steroids while the Petroleum ether and Benzene extracts gave negative results for these tests. Antimicrobial activity of various solvent extracts of *Nyctanthes arbor-tristis* was evaluated against *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Aspergillus niger*. The results showed that the test plant material was susceptible to different microorganism. *Nyctanthes arbor-tristis* possessed a broad spectrum of activity against a panel of bacteria responsible for some common microbial disease in human and in plants.

Pandey, 2012 Evaluated the Anti-oxidant activity *of Nyctanthes arbor tristis* Linn. The flower of *Nyctanthes arbor-tristis* Linn. was extracted with n-butanol. Total phenolic content of the extracts was determined by Prussian blue method was found to be 1220 mg catechin is equivalent to 100 g of flower powder. The antioxidant activity of the extracts was evaluated through in vitro model systems such as DPPH Radical scavenging, total anti-oxidant activity and reducing power. The % Inhibition by extract (86%) as well as BHT is comparable by considering 1% of BHT is 100%. Total antioxidant activity was found to be 248 mg/100 g. Reducing power in terms of EC50 value of the extract is excellent (12.10), which is comparable with that of BHT (12.26).

Khanapur et al., 2014 Investigated the antioxidant and anti proliferative activities of flower extracts of Nyctanthes *arbor-tristis* Linn. The dried flowers were extracted with

95% ethanol under sonication and the antioxidant activities were investigated using in vitro assays along with the determination of phytochemical constituents (total polyphenol and total flavonoid). Arborside C and β-monogentiobioside ester of α-Crocetin were identified in crude active extracts through LCMS/MS analysis. The antiproliferative activity was carried out by MTT assay by employing different human cancer cell lines. The lowest IC50 value of 24.56 ± 6.63 μg/ml was observed against Colo 205 cell line.

Rathee et al., 2007 Evaluated the antioxidant activity of *Nyctanthes arbor tristis* leaf extract. The dried and powdered *N. arbor-tristis* leaves (100 g) were extracted successively with petroleum ether, chloroform, ethyl acetate, methanol, and water (each 1 l) for four days using a Soxhlet apparatus. Ethyl acetate extract was sub-fractionated by successive extraction with petroleum ether, chloroform, ethyl acetate, acetone, and methanol (each 2 × 50 ml) at room temperature.The antioxidant activity of the extracts was evaluated through DPPH Radical scavenging, Anti-lipid peroxidation (LPO) assay, Hydroxyl radicals scavenging assay, Hydrogen peroxide scavenging assay, Superoxide radicals scavenging assay, DNA strand break assay, Assay of reducing power of NEA along with the determination of phytochemical constituents (total polyphenol and total flavonoid). The results showed impressive antioxidant activity, due to its ability to scavenge various biologically relevant reactive oxygen species and inhibit lipid peroxidation and DNA strand breaks.

Sasikumar et al., 2010 Investigated the in vitro antioxidant activity of methanol extract and flavonoid fraction of leaves of *Nyctanthes arbor-tristis* using 1, 1 –Diphenyl Picryl Hydrazyl (DPPH) quenching assay and ferric reducing power capacity. The samples were tested at 1000, 500, 250, 125, 62.5 and 31.75 µg/ml concentrations. The results showed that methanol extract and flavonoid fraction exhibited potential DPPH scavenging activity with IC_{50} values of 63.6±0.29 and 61.9±0.15 µg/ml respectively and strong reducing power activity (0.969±0.008 and 1.502±0.0210 at 1000 µg/ml respectively).The results of the study revealed that the flavonoid fraction had strong antioxidant activity, achieved by quenching capacity against DPPH radical, and reducing power.

Jamdagni et al., 2018 Evaluated green synthesis of zinc oxide nanoparticles using flower extract of *Nyctanthes arbor-tristis* and their antifungal activity.Flower extract was used as the biological reduction agent for synthesizing zinc oxide nanoparticles from zinc acetate dihydrate. Synthesis conditions were optimized for maximal and narrow size range synthesis of zinc oxide nanoparticles. The resultant nano powder was characterized using various analytical techniques, such as UV–Visible spectroscopy, Fourier Transform Infrared spectroscopy, X-ray diffraction, Dynamic Light Scattering and Transmission Electron Microscopy. The nano powder was stored in dried form and was found to be stable after 4 months. The size range of nanoparticles obtained upon synthesis at optimum conditions was 12–32 nm as reported by TEM. X-ray diffraction studies confirmed the crystalline nature of the nanoparticles indicating particle size within the range provided by electron microscopy data. Nanoparticles were tested for their antifungal potential and were found to be active against all five tested phytopathogens with lowest MIC value recorded as16 µg/ml.

Saxena et al., 1984 Investigated the water soluble portion of the alcoholic extract of the leaves of *Nyctanthes arbor-tristis* for the presence of anti-inflammatory activity. NAT inhibits the acute inflammatory oedema produced by different phlogistic agents, viz. carrageenin, formalin, histamine, 5-hydroxytryptamine and hyaluronidase in the hind paw of rats. The acute inflammatory swelling in the knee joint of rats induced by turpentine oil was also significantly reduced. In subacute models, NAT was found to check granulation tissue formation significantly in the granuloma pouch and cotton pellet test. Acute and chronic phases of formaldehyde induced arthritis were significantly inhibited. NAT was also found to inhibit the inflammation produced by immunological methods, viz. Freund's adjuvant arthritis and PPD induced tuberculin reaction. The LD_{50} was found to be 16 g/kg.

Lad and Bhatnagar, 2017 Evaluated the antioxidant effects of *Nyctanthes arbor-tristis* leaves extract on the liver of Freund's complete adjuvant (FCA) induced arthritis (RA) rat model. 50% ethanolic extract of *N. arbor-tristis* leaves (NATE) was evaluated for its phytoconstituents (total phenolic, total triterpenoids and total flavonoid content) and in vitro antioxidant activity such as free radical scavenging activity (FRSA), metal

chelation activity (MCA), reducing power (RP) and plasmid nicking assay. Effects of NATE treatment on the oxidative stress markers along with histopathology of liver in arthritic rats were studied. The results revealed the presence of phenolic, triterpenoid and flavonoid compounds in hydroethanolic extract of *N. arbor-tristis*, which were strongly and positively correlated with the antioxidant activities. NATE significantly modulated oxidative stress markers of liver in favor of reducing the oxidative stress.

Uroos et al., 2017 Evaluated the antiarthritic potential of NAT using Freund's adjuvant-induced arthritic rat model**.** Methanolic, ethyl acetate, and n-hexane extractswere used for this study.Macroscopic arthritic scoring and water displacement plethysmometry were used to evaluate arthritic development. Hematological and biochemical parameters were investigated and ankle joints were processed for histopathological evaluation. Qualitative phytochemical analysis and GC-MS analysis were conducted for identification of constituent. Different phytochemicals tests revealed the presence of various constituents like Carbohydrates, Glycoside, Tannins, Terpenoids, Phenols and Acids. NAT extracts suppressed arthritic scoring, paw edema, infiltration of inflammatory cells, pannus formation, and bone erosion. The plant extracts ameliorated total leukocytes and platelet counts and nearly normalized red blood cells (RBC) counts and hemoglobin (Hb) content. The extracts were found safe in terms of hepatotoxicity and nephrotoxicity as determined by aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, and urea levels. Comparative analysis showed that ethyl acetate extract produced the highest inhibition of paw edema. Results showed that *Nyctanthes arbor-tristis* ameliorated experimental rheumatoid arthritis and ethyl acetate extract possessed the highest inhibitory activity.

Rathore et al., 2007 Studied the modulation effect of pro- and anti-inflammatory cytokines following long term use of water soluble ethanol extracts from different organs (Fruits, seeds, and leaves) of *Nyctanthes arbor-tristis* (NAT) in mouse model of arthritis.Arthritis was induced in mice by two injections of Freund's complete adjuvant on days 0 and 12 in the sub-planter surface of the right hind paw. Results revealed that theoral administration of leaf and fruit extracts in arthritic mice reduced joint homogenate levels of tumor necrosis factor-α, interleukin-1β, and interleukin-6 on days

2, 14, and 47 in comparison to untreated arthritic mice. Interleukin-10 level was elevated in the inflamed joint on days 2, 14, and 47 in comparisons to untreated arthritic mice. Evidence of lesser inflammation of the footpad and joint and associated histological observation support the therapeutic benefit of leaf and fruit extracts from *Nyctanthes arbor-tristis***.**

Rangika et al., 2015 Investigated the Hypoglycemic and hypolipidemic activities of aqueous extract of flowers from *Nyctanthes arbor-tristis* L. in male mice. Dose of aqueous extract of flowers (AEF) was given at 250, 500 and 750 mg/kg body weight.To understand themechanisms of actions of AEF, animals were orally administered with 500 mg/kg or the vehicle (DW) and glucose tolerance was performed before and after glucose challenge. For further studies, in vitro alpha-amylase assay and glucose absorption from the gastrointestinal tract were performed using 500 mg/kg of the extract. Additionally, glycogen content in the liver and skeletal muscles, a complete lipid profile assay, and toxicological and biochemical parameters were conducted after a chronic study. The results showed that 500 mg/kg and 750 mg/kg body weight of AEF significantly reduced fasting blood glucose levels respectively by 49 % and 39 % at 4 h post-treatment, while 500 mg/kg of AEF also decreased the random blood glucose level significantly by 32 % at 4 h post-treatment. AEF significantly inhibited glucose absorption by 85 % from the intestine and increased diaphragm uptake of glucose by 64 %. The extract also exhibited inhibition (16.66 %) of alpha-amylase enzyme activity. It also decreased the level of total cholesterol (by 44.8 %), triglyceride (by 53 %) and increased (by 57 %) the high-density lipoprotein cholesterol.

Das et al., 2021 Studied the formulation and evaluation of the antidiabetic, hypolipidemic and antioxidant potential of the tablets of Arbortristoside-A from the seeds of *Nyctanthes arbor-tristis* Linn. Arbortristoside-A was chemically isolated from the seeds' ethanolic extract of *N. arbor-tristis*. The antioxidant property of arbortristoside-A was screened in-vitro by the help of DPPH methodology. The granules of arbortristoside-A with suitable excipients were prepared by wet granulation method. The tablets were prepared by the help of rotating punch tablet machine and subjected to standard evaluation process. Its antidiabetic property was evaluated by using

streptozotocin induced diabetic wistar rats of either sex. This study also included a serum biochemical assays and pancreatic and hepatic histopathological modification. Results revealed that daily oral treatment with tablets of arbortristoside-A and glibenclamide (10 mg/kg) for three weeks reduced the plasma glucose, cholesterol, triglycerides, AST and ALT levels significantly whereas improvement in HDL-cholesterol level was observed as that of the diabetic control animals. Significant recovery of the pancreas and liver was observed with the tablets administration which was supported by its significant antioxidant activity.

Patil et al., 2010 Investigated the Larvicidal activities of six plants(*Nyctanthes arbor-tristis* and 5 others) extracts against two mosquito species, *Aedes aegypti* and *Anopheles stephensi* Chloroform, dichloromethane and methanol extracts of the leaves and roots were used for this study. The larval mortality was observed after 24 h of exposure. The results revealed that all extracts showed moderate larvicidal effects. However, the highest larval mortality was found in methanol extracts of *P. zeylanica* roots and *B. aegyptica* roots against *Ae.aegypti* (LC50 169.61 mg/lit, 289.59 mg/lit) and An.stephensi (LC50 222.34 mg/lit, 102.29 mg/lit), respectively. The methanol extracts of plants were more effective than the other extracts. This is an ideal eco-friendly approach aid for the control of mosquito species, *Ae. aegypti*, and *An.stephensi*.

Mathew et al., 2009 Investigated the Larvicidal activity of *Saraca indica, Nyctanthes arbor-tristis,* and *Clitoriaternatea* extracts against three mosquito vector species viz., *Aedes aegypti*, *Culex quinquefasciatus*, and *Anopheles stephensi.* The powdered plant parts (leaves, bark, roots, and seeds) wereextracted with suitable solvent such as petroleum ether, chloroform, and methanol using a Soxhlet extractor. Results showed that In the case of S. indica/asoca, the petroleum ether extract of the leaves and the chloroform extract of the bark were effective against the larvae of *C. quinquefasciatus* with respective LC_{50} values 228.9 and 291.5 ppm. The LC_{50} values of chloroform extract of *N. arbor-tristis* leaves were 303.2, 518.2, and 420.2 ppm against *A. aegypti, A. stephensi, and C. quinquefasciatus*, respectively. The methanol and chloroform extracts of flowers of *N. arbor-tristis* showed larvicidal activity against larvae of *A. stephensi* with the respective LC_{50} values of 244.4 and 747.7 ppm. Among the methanol

extracts of *C. ternatea* leaves, roots, flowers, and seeds, the seed extract was effective against the larvae of all the three species with LC_{50} values 65.2, 154.5, and 54.4 ppm, respectively, for *A. stephensi, A. aegypti, and C. quinquefasciatus.*

Shukla et al., 2012 Evaluated the effect of iridoid glucosides, isolated from *Nyctanthes arbor tristis*, on redox homeostasis of Leishmania parasite. These compounds led to an increase in reactive oxygen species by inhibiting a crucial enzyme of redox metabolism of the parasite. Our experiments clearly showed that these compounds are highly active as antileishmanial agents. The *in vitro* experiments on intra-macrophageal amastigotes showed significant killing of parasite even at very low concentration. Determination of mechanism of action of iridoid glucosides showed that increased ROS level leads to oxidative stress, cell membrane damage and apoptosis of Leishmania sp. Cellular toxicity assays on Human embryonic kidney (HEK 293) and mouse macrophage (J774A.1) cell lines showed these compounds to be very safe for therapeutics application.

Puri et al., 1994 Investigated thestrong stimulation of antigen specific and non-specific immunity of *Nyctanthes arbor-tristis*. Increased in humoral and delayed type hypersensitivity (DTH) response to sheep red blood cells (SRBC) and in the macrophage migration index (MMI), has been demonstrated in mice. Shade dried seeds, seed kernel, seed coat, leaves, stem bark, roots and fresh flowers were treated with 50% ethanol separately. The results showed that maximum activity was found in the seeds in which the active principle(s) appear to be mainly associated with lipids. In flowers and leaves, however, the major activity was found in the aqueous fraction of the 50% ethanol extract.

Kannan et al., 2007 Investigated the immune modulatory potential of an Indian medicinal plant, *Nyctanthes arbor-tristis* L. (Oleaceae). Ethanolic extract of *N. arbor-tristis* (NAEE) was screened in rats for humoral and cell-mediated immune responses. Oral administration of the NAEE to rats at a dose of 50, 100, 150 and 200 mg/kg significantly enhanced the circulating antibody titre when challenged with sheep red blood cells (SRBC) and heat-killed Salmonella antigens. The chronic administration of

NAEE increased the total counts of white blood cells (WBC) and potentated the delayed-type hypersensitivity (DTH) reactions.

Ahmed et al., 2016 Evaluated the anthelmintic activity of *Nyctanthes arbor-tristis* leaves on Indian earth worms *Phereima posthuma*. The powdered leaves of *Nyctanthes arbor-tristis* was extracted with chloroform, Acetone and hot water in soxhlet apparatus and used for further evaluations. The result revealed that all tested extract of *Nyctanthes arbor-tristis* shown anthelmintic activity in a dose dependant manner. The potency of test sample was found to be inversely proportional to time taken for paralysis or death of worms. The activities were comparable with reference drug Piperazine Citrate. Among the tested extract, the Acetone extract was found to possess promising Anthelmintic activity in comparison with other extract and standard.

4. Aims and objectives:

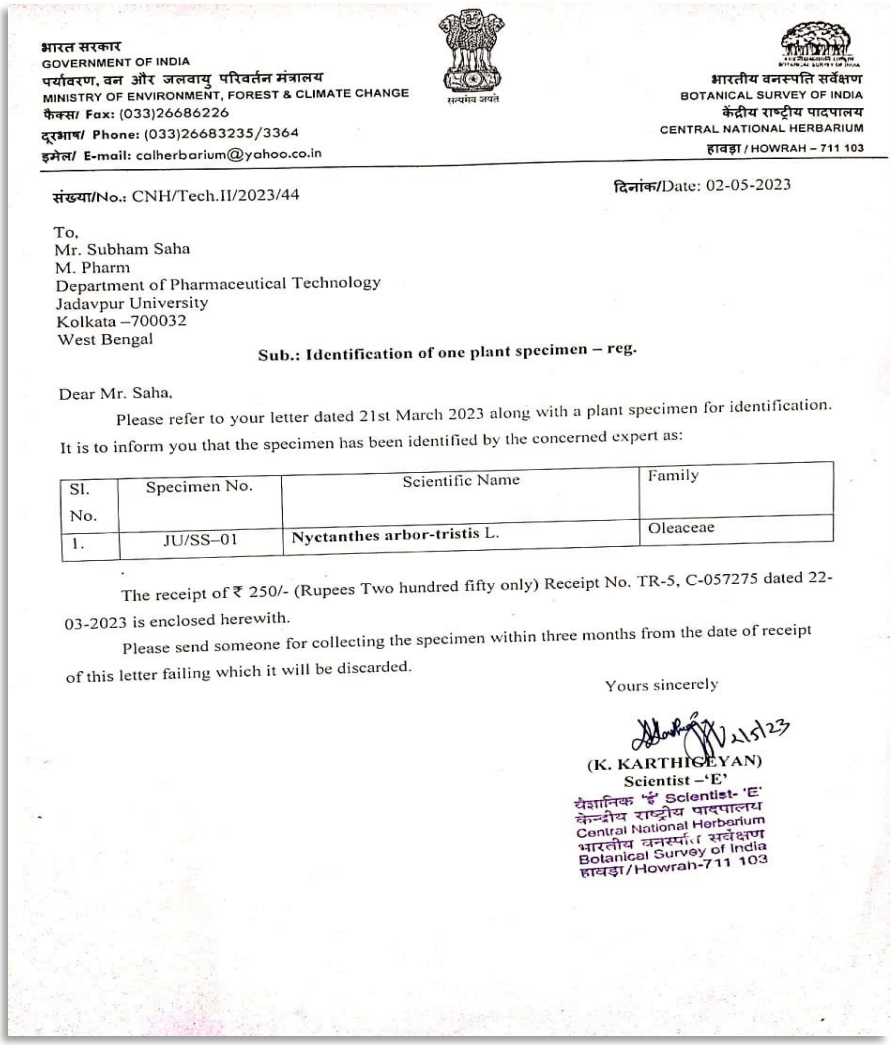
The present research work was under taken to evaluate the Pharmacognostic, Physicochemical & Phytochemical screening of the leaves of *Nyctanthes arbor-tristis* L. (Family: Oleaceae) as well as the Antioxidant and Anti-diabetic activity. The research work reported in this thesis was performed in a systemic manner as follows:

1. Collection, drying and preparation of leaves powder
2. Authentication of the plant specimen
3. Preparation of plant extract with suitable solvent
4. Basic Pharmacognostic study of the leaves of *Nyctanthes arbor-tristis* L.
5. Physico-chemical study of the powdered leaves
6. Phytochemical screening of the plant extract
7. TLC and HPLC of the plant extract
8. Preparation and characterization of tincture
9. Fluorescence characterization of powdered leaves on treatment with various chemical reagents
10. *In-vitro* antioxidant study
11. *In-vitro* anti-diabetic study
12. *In-vivo* anti-diabetic study in High fat diet rat model

5. Plant Material

The mature leaves of *Nyctanthes arbor-tristis* L. were collected from the CoochBehar district of West Bengal (India) during the month of September and October. Taxonomical identification of the plant (Reference No. CNH/Tech.II /2023/44) was established by the Central National Herbarium, Botanical Survey of India, Shibpur, Howrah.

The leaves were washed thoroughly with tap water then the leaves were shade-dried for 4-5 weeks. The dried leaves were pulverized in a mechanical grinder to obtain a fine powder and stored in a closed container for further use.



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सत्यमेव जयते

भारतीय वनस्पति सर्वेक्षण
BOTANICAL SURVEY OF INDIA
केंद्रीय राष्ट्रीय पादपालय
CENTRAL NATIONAL HERBARIUM
हावड़ा / HOWRAH – 711 103

संख्या/No.: CNH/Tech.II/2023/44

दिनांक/Date: 02-05-2023

To,
Mr. Subham Saha
M. Pharm
Department of Pharmaceutical Technology
Jadavpur University
Kolkata –700032
West Bengal

Sub.: Identification of one plant specimen – reg.

Dear Mr. Saha,

Please refer to your letter dated 21st March 2023 along with a plant specimen for identification. It is to inform you that the specimen has been identified by the concerned expert as:

Sl. No.	Specimen No.	Scientific Name	Family
1.	JU/SS–01	**Nyctanthes arbor-tristis** L.	Oleaceae

The receipt of ₹ 250/- (Rupees Two hundred fifty only) Receipt No. TR-5, C-057275 dated 22-03-2023 is enclosed herewith.

Please send someone for collecting the specimen within three months from the date of receipt of this letter failing which it will be discarded.

Yours sincerely

2/5/23

(K. KARTHIGEYAN)
Scientist –'E'
वैज्ञानिक 'ई' Scientist- 'E'
केन्द्रीय राष्ट्रीय पादपालय
Central National Herbarium
भारतीय वनस्पति सर्वेक्षण
Botanical Survey of India
हावड़ा/Howrah-711 103

Figure 2: Taxonomical identification certificate of the plant from BSI

6. Materials and methods:

6.1. Prarmacognostic evaluation:

6.1.1. Morphological Character:

The morphological characters were observed on the basis of visual analysis i.e. observation of the color, smell, taste, texture, shape, and size of the plant material was done with naked eyes, and perform sensory evaluation.

6.1.2. Microscopic Character:

Microscopical study of leaves of *Nyctanthes arbor-tristis* L. was performed according to the standard procedures of Kokate et al., 2019, Khanet al., 2013

For microscopic evaluation of the leaf constants like stomata,transverse sections were performed.

6.1.3. Powder microscopy

The microscopic examination of powdered leaf material was performed to detect and to establish various peculiar microscopic characters in order to differentiate between the adulterated and the substituted powdered or intact leaves supply. Slides of powdered leaf material was prepared and seen under microscope. (Kokateet al., 2019; Khan et al, 2013)

6.2. Physicochemical evaluation

The methods described in the WHO guidelines(WHO, 2011); Kokateet al.,2019; Mukharjee, 2002; Mandal et al.,1999; Khandelwal,2006 on quality control methods for medicinal plant materials were used for physicochemical evaluation.

6.2.1. Loss on drying:

A dried shallow weighing bottle was filled with 2g of powdered sample. The sample was distributed uniformly by shaking the bottle. The sample bed height was not more than 10 mm. Then they dried the sample to a constant weight in a hot air oven at 105° C. After drying, the shallow weighing bottle was cooled in a desiccator at room temperature Then weighed and calculated the loss on drying in terms of percentage w/w.

Formula:

% loss on drying = Loss I weight/ Weight of the drug (in grams)* 100

6.2.2. Ashvalues:

a. Total ash

The 2g powdered sample was placed in a silica crucible which was previously tarred and Incinerated at 450°C until it was free from carbon. The total ash was cooled in a desiccator at room temperature and weighed. The total ash value percentage (w/w) was calculated with reference to the dried sample.

Formula:

% of total ash = X-Y/Z*100

X= Weight of the dish+ Ash (after complete incineration)

Y= Weight of the empty dish

Z= Weight of powdered drug taken

b. Water soluble ash:

Total ash containing silica crucible weighed and boil with 25ml of water for 5 minutes. Then filtered it with ash less filter paper, filtered ash was washed with hot water. Then filtrate was dried, ignited at 450°C for 15 minutes, cooled and weighed. The insoluble matter weight was subtracted from total ash weight; the weight difference represents

water soluble ash. Water soluble ash percentage (w/w) was calculated with reference to dried sample.

Formula:

% of water-soluble ash = X-Y/Z*100

X= Weight of the dish+ Ash (after complete incineration)

Y= Weight of the empty dish

Z= Weight of powdered drug taken

c. Acid insoluble ash:

The total ash containing silica crucible is weighed and boiled with 25ml of hydrochloric acid for 5 minutes. Then it was filtered with ash-less filter paper. The filtered ash was washed with hot water. Then the filtrate was ignited at 450°C for 15 minutes, cooled and weighed. The acid insoluble ash percentage (w/w) was calculated using the dried sample.

Formula:

% of acid insoluble ash = X-Y/Z*100

X= Weight of the dish+ Ash (after complete incineration)

Y= Weight of the empty dish

Z= Weight of powdered drug taken

6.2.3. Extractive value:

Extractive values are used for the evaluation of a crude drug. It gives an idea about the nature of the chemical constituent present in a crude drug.

There are two types of Extractive value that we can determine-

a. Cold Maceration

b. Hot Extraction

In this study, we have performed the cold maceration of the powdered leaves to determine the extractive value.

Cold Maceration

1. Determination of Water soluble extractive value

5 g of coarsely powdered air-dried plant leaves were macerated with 100 ml of water in a closed flask for 24 hours, and it was shaken frequently during the first 6 hours and allowed to stand for 18 hours. Then it was filtered, 25 ml of the filtrate was evaporated in a flat shallow dish, and dried at 105℃ and weighed. The percentage (w/w) of water-soluble extractive value was calculated with reference to the air-dried plant leaves.

Formula:

%of Water soluble extractive= X-Y/Z*100

X= Initial weight of petri dish

Y= Final weight of petri dish

Z= Weight of powdered drug

2. Determination of Alcohol (ethanol) soluble extractive value

5g of coarsely powdered air-dried plant leaves were macerated with 100 ml of absolute ethanol in a closed flask for 24 hours, and it was shaken frequently during the first 6 hours and allowed to stand for 18 hours. Then it was filtered, 25 ml of the filtrate was evaporated in a flat shallow dish, and dried at 105℃ and weighed. The percentage (w/w) of alcohol soluble extractive value was calculated with reference to the air-dried plant leaves.

Formula:

%of Water soluble extractive= X-Y/Z*100

X= Initial weight of petri dish

Y= Final weight of petri dish

Z= Weight of powdered drug

6.2.4. Determination of crude fiber:

2 g of the dried leaves powder was boiled with 200 ml of 1.25% sulfuric acid with constant stirring. Filter the solution and wash the filtrate with hot water. Then the filtrate was treated with 200ml of 1.25% sodium hydroxide solution and boiled for 30 minutes. Filter the solution and wash the filtrate with hot water. The filtrate is then incinerated at 110 °C to a constant weight. The percentage of crude fiber value was calculated with reference to the dried sample

Formula:

% of crude fiber = X-Y/Z*100

X=Weight of crucible + filtrate (before incineration)

Y= Weight of crucible + filtrate (after incineration)

Z= Weight of the powdered drug

6.2.5. Determination of Swelling Index:

1g of dried sample was placed in a 25ml measuring cylinder, 25ml of water was added, and the cylinder was shaken every 10 minutes for 1 hour. Then allow it to stand for 3 hours: Measure the volume (in ml) occupied by the dried sample

6.2.6. Determination of the Foaming Index

1g of dried sample was transferred to a 500 ml. conical flask containing 100 ml of boiling water Then, cool and filter into a volumetric flask and add sufficient water to make the volume up to 100ml. (V1) Ten stopper test tubes should be obtained and marked. Successive portions of 1, 2 ml up to 10 ml of the drug have to be put into separate tubes and the remaining volume adjusted with water up to 10 ml. After closing the tubes with stoppers, shaking is portions performed for 15s and then allowed to stand for 15 min, followed by the height of each tube being measured.

If the height of the foam in every tube is less than 1 cm, the foaming index is less than 100.

If a height of foam of 1 cm is measured in the 1st to 10^{th} tubes, the volume of the dried sample solution decoction in this tube (a) is used to determine the index.

If this tube is the 1^{st} or 2^{nd} tube in a series, prepare an intermediate dilution in a similar manner to obtain a more precise result.

If the height of the foam is more than 1 cm in the 1^{st} to 10^{th} tube, the foaming index is over 1000.

In this case, repeat the determination using a new series of dilutions of the decoction in order to obtain a result.

The following formula was used for the calculation of the foaming index.

Foaming index = 1000/a

a=the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1 cm is observed.

6.2.7. Determination of Bitterness value

In accordance with WHO recommendations, the bitterness of herbal material was assessed by contrasting the threshold bitter concentration of an extract with that of a diluted solution of quinine hydrochloride (WHO, 1998). The amount of bitterness is represented in units that are equivalent to the amount of bitterness in a 2000 ml solution having 1.0 g of quinine hydrochloride. After thoroughly cleaning the mouth with drinking water, 10 ml of the most diluted solution was used for swirling it in the mouth for 30 seconds, preferably near the base of the tongue. If the bitter taste was no longer felt in the mouth after 30 seconds, the solution was dumped and left for a minute to see if delayed sensitivity was to blame. Then the drinking water is used to rinse the mouth.It was not less than at least 10 minutes the next highest concentration was tasted.The lowest concentration at which a substance still causes a bitter sensation after 30 seconds is known as the threshold bitter concentration. The drinking water for mouthwashing and all solutions were kept at 20 to 25 °C. The bitterness was calculated using the following formula.

(2000 ×c)/ (a× b)

Where a = concentration of the stock solution (mg/ml)

b = volume of the plant drug with the threshold bitter concentration

c = quantity of quinine hydrochloride (in mg) in the tube with the threshold bitter concentration

6.3. Extraction methodology:

Powdered leaves of *Nyctanthes arbor-tristis* L. were extracted by a Soxhlet extractor with Petroleum ether and Methanol.

Figure 3: Sohxlet extractor assembly

75g of powdered leaf was taken for successive extraction by Soxhlet apparatus. The extraction process was continued till all constituent were extracted ant it was confirmed by taking sample from the shiphon tube of Soxhlet extractor. Then it was examined by TLC with the respective solvent which was used for the extraction process Complete extraction was indicated by the absence of spot-on TLC plate in iodine chamber. The extract was concentrated by vacuum distillation, dried in open air conditions, and stored in an airtight container.

The dried extracts were weighed and percentage yields were calculated for each extract.

6.4. Test for Phytochemical Screening

Phytochemical screening of extracts was performed by using the standard protocol (Kokate et al., 2019; Mandalet al., 2015; Banarjee et al., 2014)

1. Detection of Carbohydrates

a. Molisch test:

Two mi of extract solution were treated with a few drops of Molisch reagent in a test tube and two ml of concentrated Sulphuric acid were added in test tubes. The formation of a purple colour ring at the junction of the two layers indicates the presence of carbohydrate in the extract sample.

b. Fehling's test:

Added equal volumes of extract and Fehling's solution (equal vol. of Fehling's solution A and B) and boiled. The appearance of a brick red precipitate indicates the presence of reducing sugar

c. Barfoed's test:

1 ml of extract is treated with Barfoed's reagent and heated for 1-2 min. If the solution turns red, monosaccharide is present.

2. Test for Proteins and amino acids:

a. Biuret test

When 2 ml of extract is treated with 2 ml of biuret reagent, the violet colour indicates the presence of protein.

b. Ninhydrin test:

To 2 ml of extract solution, add 2 drops of ninhydrin solution and boil for a fewminutes. The violet colour indicates the presence of amino acids.

c. Mallon's test

The extracted solution was treated with 2 ml of Mellon's reagent. A white precipitate indicates the presence of amino acid.

3. Test for Steroids

a. **Salkowski test:**
   An equal volume of extract and chloroform are treated with a few drops of concentrated Sulphuric acid Steroids are indicated by the presence of a reddish-brown colour in the Lower layer

b. **Liberman Burchard test:**
   The extract solution was first treated with acetic anhydride, and then a few drops of concentrated sulfuric acid were added along the side of the test tube. The presence of steroids was indicated by the presence of a colour

c. **Sulfur powder test**:
   Add a pinch of Sulphur powder to the test solution; it will sink to the bottom

4. Test for Terpenoids

a. **Noller's test:** The extract solution was treated with Noller's reagent (0.1% stannic chloride in thionyl chloride). The presence of terpenoids was indicated by the presence of a red colour

5. Test for Alkaloids

a. **Mayer's test:**

Two ml of extract were treated with 0.2 ml of dilute HCL and a few drops of Mayer's reagent were added. The formation of a yellow precipitate indicates the presence of alkaloids.

b. **Dragendorff's test:**

Two ml of extract were treated with 0.1 ml of dilute HCL and a few drops of Dragendorff's reagent. The formation of an orange-brown precipitate indicates the presence of alkaloids

c. **Hager's test:**

Two ml of extract were treated with 0.1 ml of dilute HCL and added a few drips of Hager's reagent. The formation of a yellow precipitate indicates the presence of alkaloids

d. **Wagner's test:**

Two ml of extract were treated with 0.1 ml of dilute HCL and added a few drops of Wagner's reagent. The formation of a reddish-brown precipitate indicates the presence of alkaloids.

6. Test for Glycosides

a. Cardiac Glycosides:

(i) Keller-killiani test:

Two mi of extract and glacial acetic acid, a few drops of 5% $FeC1_3$, and conc. sulphuric acid were added in a test tube. The formation of a reddish-brown ring

at the junction of two layers and the upper layer showing a bluish green colour indicates the presence of glycosides.

(ii) Legal's test:

1 ml of Na-Nitroprusside is mixed with equal volumes of Pyridine solution and extract. Pink colour indicates the presence of glycosides

(iii) Raymond's test:

Two ml of extract solution were treated with a solution of dinitrobenzene in hot methanolic alkali. The presence of glycosides is indicated by the presence of pink or red color.

b. Anthraquinone glycosides:

(i) Bontrager's test:

For 5 minutes, boil 2 ml of extract with dilute sulfuric acid for 5 minutes, then filter it Cool the filtrate and shake with an equal volume ofchloroform. Separate the chloroform layer and shake it with half of its volume of dilute ammonia. The ammoniacal layer turns red or pink to indicate the presence of glycosides.

(c) Saponin test:

(i) Foam test: Shake the extract vigorously with water. Stable foam indicates presence of saponin.

7. Test for Flavonoids:

a. Shinoda's test:

The extract is dissolved in 5 ml (95%) ethanol to which is added a few drops of concentrated HCI and 0.5 g of magnesium chloride. It gives pink, orange, and red colour.

b. Ferric chloride test:

To the extract, add a few drops of 10% $FeC1_3$. It gives a green color,which

indicates the presence of flavonoids.

c. Sodium Hydroxide test:

To the extract, add sodium hydroxide solution. It gives a yellow colour which disappears after the addition of acid

8. Test for Tannin and Phenolics:

a. Gelatin test:

To the test solution, add 1% gelatin solution and treat with 10% NaCl A precipitate is formed

b. $FeCl_3$ test:

When the extract was treated with a 5% $FeCl_3$ solution, a dark green or bluish black colour appeared.

c. 10% NaOH test:

The extract was treated with 4 ml of 10% NaOH solution and shaken well. An emulsion was formed to indicate the presence of tannin.

6.5. Chromatographic analysis of the prepared extracts

Preparation of plates

Slurry was prepared by thoroughly mixing 18 grams of Silica Gel G with 40 ml of distilled water. After stirring for 30 seconds the mixture was then transferred to 6 clean glass plates of 10 cm × 22 cm in size. The mixture was then drawn across their surface with the help of an application in order to obtain a thin uniform film of Silica Gel G of 250 µ on the glass surface.

The plates were them allowed to set for overnight and then activated in a hot air oven at 105^0C - 110^0C for 30 minutes. After cooling the plates to room temperature (27^0C) they were stored in a desiccator until required for use.

Application of extractive

Petroleum ether and methanolic extract was dissolved in Petroleum ether and methanol respectively. With the help of a capillary tube the extract was applied on the plate for the experiment.

Solvent System Used

The solvent system used for TLC where as follows-

For Petroleum Ether extract the solvent system was

Petroleum Benzene : Ethyle Acetate = 15.5 : 4.5

For Methanolic extract the solvent system was

Benzene : Chloroform : Methanol = 13 : 7 : 2

6.6. HPLC analysis of methanolic extract of *Nyctanthes arbor-tristis* L. leaf.

The methanolic extract of *Nyctanthes arbor-tristis* L. leaves was subjected to HPLC analysis which revealed the presence of different phenolics and flavonoids as the major phytoconstituents.

Initial separation condition:

1. Column for separation : Acclaim C18 (250mm x 4.6mm, 5µm)
2. Instrument: Dionex Ultimate 3000 (Thermo Sci. USA)
3. Detector: Photo Diode Array (PDA)
4. Pump: Quaternary system LPG 3400 SD
5. Injection volume: 20µl (loop 20µl)
6. Detection wavelength: 260nm, 280nm, 310nm
7. Flow rate: 1ml/min
8. Mobile phase: Methanol (Solvent A) , 0.5% aqueous acetic acid in water (Solvent B)
9. Gradient Programme: The gradient elusion was 90 % solvent B and 10% solvent A and flow rate was settled from 1ml/min to 0.7 ml/min in 27 min, from 10 to

40 % solvent A with flow rate 0.7 ml/min for 23 min, 40% solvent A and 60% B with flow rate 0.7 ml/min primarily for 2 min and then flow rate altered from 0.7 to 0.3 ml/min in 65min, from 40 to 44% solvent A with flow rate 0.3 to 0.7ml/min in 70 min, 44% solvent A with flow rate 0.7 to 1ml/min for 10 min duration, solvent A changed from 44% to 58 % with flow rate 1ml/min for 5 min, 58 to 70% solvent A in 98 min at constant flow rate 1 ml/min . Then 10% A in 101min and equilibrated for 4min for next injection.

10. Data acquisition software: Chromeleon 6.8
11. Identification and quantitation: External standard method. Reference standards protocatechuic acid, gentisic acid, chlorogenic acid, p-hydroxy benzoic acid, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, sinapic acid, salicylic acid, gallic acid and ellagic acid, catechin, rutin, myricetin, quercetin, naringin, apigenin and kaempferol were used for identification. Standard curves were performed for quantitation of each component.

6.7. Development of the manufacturing process and characterization of tincture from the leaves of *Nyctanthes arbor-tristis* L.

The leaves of *Nyctanthes arbor-tristis* L**.** were shade dried. Then the leaves were powdered by a mechanical grinder and passed through a 40 mesh sieve. 21 Amber coloured bottles were taken. In each bottle 1 g of the powdered drug were taken. 10 ml of alcohol of 7 different strengths that is 40% v/v, 50% v/v, 60%v/v, 70% v/v, 80% v/v, 90% v/v and absolute alcohol were successively poured in each bottle. Further these bottles were made into separate three sets having 7 bottles in each set.

One such set of 7 bottles containing the leaves powered sample with 7 different strength of alcohol were kept for maceration for 7 days. Another two such sets of 7 bottles were macerated for 14 days and 21 days. These bottles were shaken from time to time. After completion of the requisite days the materials were strained off through filter paper. (Mandal et al, 1996)

Figure 4: Tinctures of different alcohol concentration after maceration for different period of time.

A. pH of the tincture

The pH of the tinctures was measured with the help of pH meter and the pH observed in each dilution of alcohol and under the number of days macerated in each case have been note down.

B. The content of total solids in the tincture (% w/v)

The term "total solids" is applied to the residue obtained when the Preparation is dried to constant weight under the condition specified in the British Pharmacopoeia 1980.

Method:

The filtered were pipette out and taken in a beaker. It was allowed to evaporate at low temperature until the ethanol was removed. Then the beaker was heated on a water bath until the residue was apparently dry. After drying to constant weight, the beaker with its content was kept in desiccators.

The amount of the solid material obtained in each dilution of alcohol and under the number of days macerated in each case have been note down.

C. Specific gravity of the tincture

The specific gravity of the different strength of different batches was also measured using pycnometer.

6.8. Fluorescence characterization of powdered leaves of NAT on treatment with various chemical reagents

The leaves of *Nyctanthes arbor-tristis* L**.** were shade dried. Then the leaves were powdered by a mechanical grinder. Small amount of powdered leaves were taken in a watch glass and treated with various chemical reagents. Colour of the powdered leaves on treatment with various chemical reagents was observed by naked eye and UV in both short wavelength (254 nm) and long wavelength (366 nm). (Mandal et al., 1996)

6.9. *In-vitro* Antioxidant studies

6.9.1. Determination of Total phenolic content

Total soluble phenolics in the extracts were determined according to the method used by Miliauskas et al., 2004; Mandal et al., 2009 with trivial modification using gallic acid as a standard phenolic compound. The phenol reacts with Phosphomolybdic acid in presence of alkaline medium to produce blue colour complex known as molybdenum blue complex.

Chemicals used in this assay method

Gallic acid

Folin-Ciocalteu reagent

Sodium carbonate

Preparation of gallic acid standard for calibration curve

10mg of gallic acid was dissolved in 10ml of distilled water to make a solution of 1mg/ml. From this solution it was further diluted to get concentration of 20, 40, 60, 80 and 100µg/ml.

Preparation of test sample solution

10mg of extract was dissolved in 10 ml of distilled water to make a solution of 1mg/ml. From this solution it was further diluted to get concentration of 20, 40, 60, 80 and 100 µg/ml.

Preparation of 40 ml of 0.2N Folin-Ciocalteu reagent

4ml of Folin-Ciocalteu was dissolved in 3tml of distilled water.

Preparation of 100 ml of 75 g/L of Sodium carbonate

7.5g of sodium carbonate was dissolved in 100ml of distilled water.

Protocol for estimation of total phenolic content

1ml of each concentration of gallic acid was mixed with 5ml of Folin-Ciocalteu reagent (diluted 10 fold) and 4ml of sodium carbonate. Absorbance was measured spectrophotometrically in triplicates at 765 nm and calibration curve was plotted. 1ml of each concentration of extract solution was mixed with 5 ml of Folin-Ciocalteu reagent (diluted 10 fold) and 4ml of sodium carbonate. Absorbance was measured spectrophotometrically in triplicates at 765nm.

1ml of distilled water was mixed with 5ml of Folin-Ciocalteu reagent and 4ml of sodium carbonate. This was control and absorbance was measured spectrophotometrically at 765 nm.

The total phenolic content in the extract expressed in Gallic acid equivalents (GAE) was calculated by the following formula:

T=C*(V/M)

Where,

T=Total phenolic contents, mg/g plant extract, in Gallic Acid Equivalent (GAE)

C=Concentration (mg/ml) of Gallic acid obtained from calibration curve.

V=Volume of extract (ml).

M- Weight (mg) of plant extract.

6.9.2. Determination of Total Flavonoid Content

The total soluble flavonoid content of the fractions was determined with aluminium chloride using Quercetin as the standard according to the method of Halliwell and Gutteridge, 1985 with trivial modifications in the method.

Chemicals used in the assay

Quercetin

Aluminium chloride

Potassium acetate

Preparation of standard solution

10mg of quercetin was dissolved in 10ml of methanol to prepare concentration of 1mg/mlor 1000 µg/ml and finally diluted to 20, 40, 60, 80, 100 µg/ml.

Preparation of test sample solution

10mg of extract was dissolved in 10ml of methanol to prepare concentration of 1mg/ml or 1000ug/ml and finally diluted to 20, 40, 60, 80, 100 µg/ml.

Preparation of 10% Aluminium chloride and 1M Potassium acetate

2g of Aluminium chloride was dissolved in 20ml of distilled water to prepare 10%, Aluminium chloride.

0.98g of Potassium acetate was dissolved in 10ml of distilled water to prepare 1M of potassium acetate.

Protocol for estimation of total flavonoid content

To 1ml of each different concentration of quercetin, 2 ml of methanol was added to each concentration. Then it was mixed with 0.2 ml of aluminium chloride and 0.2ml of

potassium acetate and finally 5.6ml of distilled water was added to each concentration. To 1ml of extract, 2ml of methanol was added. Then it was mixed with 0.2ml of aluminum chloride and 0.2ml of potassium acetate and finally 5.6ml of distilled water was added to each concentration. All the samples were incubated for 30 minutes at room temperature and absorbance was measured at 415nm against control. The total flavonoid content in the fractions was determined as µg quercetin equivalent by using the standard quercetin graph and the following formula:

T=C*(V/M)

Where,

T=Total flavonoid content, mg/g plant extract, in Quercetin Equivalent (QE).

C=Concentration (mg/ml) of Gallic acid obtained from the calibration curve.

V=Volume of extract (ml).

M- Weight (mg) of plant extract.

6.9.3 Following are the methods carried out to evaluate the in vitro antioxidant activity of *Nyctanthes arbor-tristis* L.

Determination of DPPH radical scavenging activity

The antioxidant potential of any compound can be determined on the basis of its scavenging activity of the stable DPPH (1. 1-diphenyl-2-picrylhydrazyl hydrate). Radical scavenging activity of the hydro-alcoholic leaves extract of *Nyctanthes arbor-tristis* against stable DPPH determined spectrophotometrically using the method as described by Blois, in 1958 with trivial modifications in the method. The absorption maximum of a stable DPPH radical in methanol was at 517nm. When DPPH reacts with an antioxidant, which can donate hydrogen, it gets reduced. (Blois, 1958)

Chemicals used in this assay method

1.1-diphenyl-2-picrylhydrazyl hydrate

Ascorbic acid

Methanol

Preparation of standard solution

Ascorbic acid was used as standard forthisassay; 10 mg of ascorbic acid was dissolved in 10ml of methanol to give concentrations of 20, 40, 60, 80 and 100µg/ml

Preparation of test sample solution

Stock solutions of samples were prepared by dissolving 10mg of extract in 10ml of methanol to give concentrations of 20, 40, 60, 80 and 100 µg/ml.

Preparation of 0.3 mM DPPH solution

11.82mg of DPPH was dissolved in 100ml of methanol and it was kept protected from light by covering the test tubes with aluminium foil.

Protocol for estimation of DPPH scavenging activity

Antioxidant activity of the extract was determined as per the method described by Blois,1958with trivial modifications. 1ml of 0.3mM DPPH solution was added to 2ml of each different concentrations of standard solution and incubated at dark for 30min at room temperature after it has been shaken vigorously. This was the preparation of standard solution of different concentrations. 1ml of 0.3mM DPPH solution was added to 2ml of each different concentrations of sample solution and incubated at dark for 30 min at room temperature after it has been shaken vigorously. This was the preparation of test solution of different concentrations.

1ml of 0.3 mM DPPH solution was added to 2ml of methanol and this solution was taken as control and allowed to incubate at dark for 30mins at room temperature. After 30 min, absorbance was measured 517 nm taking methanol as blank using UV-Visible spectrophotometer. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples. All the tests were performed in triplicate. Ascorbic acid was used as a reference compound. The capability to scavenge the DPPH radical was calculated as the inhibition percentage of free radical by the sample/ standard using the following formula:

Inhibition of DPPH scavenging activity= $(A_0-A_t)/ A_0 * 100$

Where,

A_0 is the absorbance of the control reaction and

$A_t$is absorbance of test/standard.

The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} values were calculated by linear regression of plots, where the abscissa represents the concentration of the tested plant extracts and the ordinate represents the average percent of scavenging capacity.

The IC_{50} value is defined as the concentration (in µg/ml) of extract that inhibits the formation of DPPH radicals by 50% (Prasad et al., 2009).

6.10. *In vitro* evaluation of antidiabetic activity(Telagari and Hullatti, 2015; Velmaniand Mandal, 2016)

6.10.1 Inhibition of α amylase assay

Materials and methods

Chemicals and reagents required

1. Alpha-amylase enzyme
2. Phosphate buffer
3. Starch
4. DNS
5. Sodium dihydrogen phosphate
6. Sodium chloride
7. Sodium hydroxide
8. Sodium potassium tartrate
9. Acarbose (standarddrug)

Preparation of Phosphate buffer solution

1.2 Gram of sodium dihydrogen phosphate and 0.195 gram sodium chloride was dissolved in 500 ml distilled water. pH was maintained at 6.9 at 20 ^{0}C .pH was adjusted using 1 M HCl for acidic pH and 1 M NaOH for basic pH.

Preparation of Starch solution

For the preparation of the starch solution, 100 mg of starch was dissolved in 10 ml of phosphate buffer. The prepared solution was solubilized by boiling it in a water bath.

Preparation of 2 M NaOH

800 mg of NaOH was weighed and dissolved in 10 ml of distilled water.

Preparation of 5.3 M potassium sodium tartrate solution

14.96 gram of potassium sodium tartrate was dissolved in 10 ml of 2 M NaOH.

Preparation of 96 Mm 3, 5-dinitrosalicylic acid solution (DNSsolution)

0.438 gm of DNS solution was dissolved in 20 ml distilled water.

Preparation of color reagent

12 ml of warm distilled water was mixed with 8 ml of 5.3 M potassium sodium tartrate and 20 ml of 96 mM DNS solution.

Preparation of alpha-amylase solution

0.0015 gram of alpha-amylase enzyme was dissolved in 100 ml phosphate buffer.

Preparation of Standard stock solution

The standard stock solution of acarbose was prepared by dissolving 0.05 g of the standard drug in 50 ml distilled water to make the concentration of the stock 1000 µg/ml.

Preparation of stock solution for the test sample

0.05 g of the extract was dissolved in 50 ml to make the concentration as 1000 µg/ml.

Preparation of dilutions

From the prepared stock solutions of both standard and test sample, 5 dilutions were made. The concentrations of dilutions were made as 20 µg/ml,40 µg/ml,60 µg/ml,80 µg/ml and 100 µg/ml.

Methods

Inhibition of the alpha-amylase activity

1 ml of phosphate buffer was added in 5 different test tubes. To these, 0.2 ml alpha-amylase solution was added. To the above mixture, 0.4 ml of standard drug solution was added. The above mixture from each of the test tubes was incubated at 37^0C for 20 minutes. To these incubated mixtures of each test tube, 0.4 ml of starch solution was added and the mixture was then again incubated for 30 minutes at a temperature of 37^0C in a water bath. After 30 minutes, 2 ml of DNS solution was added to each test tubes and boiled for 10 minutes. After that, the absorbance of the sample was measured at 540 nm. The measurement of absorbance of control was conducted using a similar method and by replacing standard drug solution with the vehicle.

A Similar method was used to measure the absorbance of the test sample by using extract solution in place of standard drug solution.

Calculation of Inhibition concentration

The concentration of the plant extract required to scavenge the radicals was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition was calculated by

% Inhibition = (Ac – As) / Ac * 100

Where,

Ac = Absorbance of the control

As = Absorbance of the sample

6.11. *In vivo* antidiabetic studies

Preparation of 0.1M sodium citrate buffer solution (4.5pH)

To prepare 100ml of 0.1M citrate buffer 270.278 mg of Sodium citrate dihydrate and 207.7 mg of citric acid to 80 ml of HCl or NaOH were mixed. The final volume was made up to 100 ml withdistilled water. The solution was adjusted to desired p H using distilled water. (Gomori, 1955)

Preparation of Streptozotocin solution

A solution of STZ was prepared by dissolving the weighed quantity of streptozotocin in 0.1M freshly prepared ice-cold citrate buffer (pH 4.5) solution.

Preparation of 5% Dextrose solution

5gm of dextrose is weighed and dissolved in 100 ml of distilled water.

Preparation of Standard (Metformin HCI) solution: (Dose: 50mg/kg)

50 mg of Metformin HCI is dissolved in 1ml of distilled water (Mandal et al., 1997; Chakraborty et al., 2018)

Preparation of test solution:

Solution of methanolic extract of *Nyctanthes arbor-tristis* L. was prepared by dissolving the extract in distilled water.

Housing of animals

Wistar albino rats weighing 150-200g used for all experiments studied. Animal experiment protocol (JU/IAEC-22/38) was duly approved by Institutional Animal Ethics Committee (IAEC), Department of Pharmaceutical Technology, Jadavpur

University, Kolkata-700032. Animals collected randomly from State centre for Laboratory Animal Breeding West Bengal Livestock Development Corporation Limited, Buddhapark, Kalyani, Nadia,PIN-741235 separate cage undercontrolled conditions of temperature (22 ± 2°C). All animals weregiven high fat diet and water *ad libitum*. Animalskept at light/dark cycle of 12 hrs. Animals divided in five groups with six animals in each group. Institutional Animal Ethics Committee (Regd. No. 1805/CPCSEA) approved the research protocol and all experimental procedures followed in the study.

Table 1: Composition of high fat diet.

Corn Starch (g/100g)	**25 gm**
Whole wheat flour (g/100g)	**24.5 gm**
Vegetable oil (g/100g)	**2 gm**
Casein (g/100g)	**11 gm**
Refine wheat flour (g/100g)	**22 gm**
Coconut Oil (g/100g)	**23 gm**
Fructose (g/100g)	**15.5 gm**
Vitamin and mineral mix (g/100g)	**2 gm**

Streptozotocin induced hyperglycemia ((Mandal, et al., 1997; Chakraborty et al., 2018)

The acute experimental hyperglycemia produced in rats by the single dose of

streptozotocin (STZ) injection (40 mg/kg b.w.i.p). The selected animals, weighing between 150-200g fasted overnight were administered with Streptozotocin (35 mg/kg b.w) intraperitoneal (1.0 ml/100 g). Fasting blood sugar levels were determined on 5th day after administering STZ to confirm stable hyperglycemia. The diabetic rats after confirmation of stable hyperglycemia divided into different groups of 6 rats each. That

day considered as the 0^{th} day. Metformin hydrochloride at a dose 50 mg/kg b.w. used as a standard drug fortreatment. After seven days, rats with glycemia ≥ 170 mg/dl were selected for further experimentation. Drug and doses administered accordingly as mentioned below:

1. Group 1 (Normal control): without any drug treatment

Received only vehicle/distilled water.

2. Group 2 (Positive control): Diabetes (STZ-injected rats)

3. Group 3 (Standard drug treated): Diabetes (STZ-injected rats) treated with standard drug -metformin hydrochloride (50 mg/kg b.w.).

4. Group 4 (Low dose treated): Diabetes (STZ-injected rats) treated with 200 mg/kg bodyweight with *Nyctanthes arbor-tristis* methanolic extract

5. Group 5 (High dose treated): Diabetes (STZ-injected rats) treated with 400 mg/kg bodyweight with *N. arbor-tristis*methanol extract.

Methanolic extract of *N. arbor-tristis* and standard drug metformin was orally administered for 28 days.Body weights and blood glucose levels of overnight-fasted rats measured weekly. At the end of the experimental period, the animals were sacrificed under the influence of anaesthetic and pancreas, kidney, liver were collected for histopathological examination.

Calculation of Doses: (Mandal et al., 1997; Chakraborty et al., 2018)

For test sample

The LD_{50} was found from the acute toxicity studies. LD_{50} values of the Methanolic extract of *N. arbor-tristis* were found to be safe up to 2000 mg (Bharti et al. 2011). The in vivo biological studies were done on serum and tissues of male wistar rats at the doses of 200 mg and 400 mg/kg body weight.

For Streptozotocin: 35mg/kg b.w.

For standard (Metformin HCL): 50 mg/kg b.w.

Evaluation of hypoglycaemic activity: [Mandal et al., 1997; Chakraborty et al., 2018]

i. Fasting Blood Glucose (FBG) analysis

To quantify the levels of blood glucose, blood samples were aseptically taken from the ends of mice's tails and placed on glucose test strips. Blood was collected from the tail of overnight fasted rats with the help of a syringe and fasting blood glucose concentration noted down on days 0, 7, 14, 21 and 28 using ACCU-CHEK Guide Glucometer.

ii. Estimation of Glycosylated Haemoglobin

Glycosylated haemoglobin is an important parameter to be checked into the diabetic patients. This is identified as unusual haemoglobin in patients with diabetes, due to increased glucose level it binds with haemoglobin. It is used to measure long term monitoring of diabetes. Glycosylated hemoglobin (HbA1c) level was determined from whole blood sample by using commercially available assay kits (Coral Clinical System. Tulip diagnostics Pvt. Ltd.,India). It was estimated by hemolyzed preparation of whole blood and continuously mixed with a weakly binding cation exchange resin for 5 min. The unstable fraction was eliminated during the hemolysate preparation and during the resin binding. During mixing in the vortex, HbA1c binds to the ion exchange resin present in resin tube and GHb became free in the supernatant. Later the mixing period, filter separator was used to remove the resin from the supernatant. The percentage of glycosylated haemoglobin in sample was determined by measuring absorbance of the glycosylated haemoglobin (GHb) fraction & the total haemoglobin (THb) fraction. The percent of glycosylated hemoglobin of the sample was estimated by the ratio of the absorbance of the Glycosylated haemoglobin & the Total hemoglobin fraction of the control and the test. Test methods was followed by collection of blood from each group (n= 6) in a EDTA vial and mixed with 0.5 ml lysing reagent and mix until complete

lysis take place and another tube is prepared by using 0.1 ml of reconstituted control which is used as control for this test. Then both tubes were allowed to stand for 5 min.

Ion-exchange resin tubes were marked as control and test and hemolysate quantified as 0.1 ml of was added into the resin tubes. Resin separator tube was positioned over each ion-exchange tube so that the rubber sleeve was remaining approximately 1 cm above the liquid level of the resin suspension. The whole mixture was then vortexes continuously for 5 min. When settling of resin, resin separator was pushed into the tubes until the resin was tightly packed. Supernatant was aspirated from each tube and kept into cuvette followed by measured the absorbance against distilled water.

THb was measured by dispensed 5 ml of distilled water into tubes labelled as control and test. After that 0.02 ml of hemolysate was added in the labelled tubes and after proper mixing absorbance was measured against distilled water [Kazemi, 2019].

Calculations:

Ratio of Control (R_C) = Absorbance of Control GHb/ Absorbance of Control THb

Ratio of Test (R_T) = Abs. of Test GHb/ Abs. of THb

GHb% = Ratio of Test (R_T)/Ratio of Control (R_C) * 10 (Value of Control)

iii. Estimation of Total Protein

Proteins are the constitute of muscle, enzymes, hormones and other structural and functional entities of the body. Main plasma proteins like albumin and globulin fractions vary widely depend upon various disease conditions. In this study, plasma protein level was estimated by using commercially available assay kit (Arkray Healthcare Pvt. Ltd., Mumbai, and Maharashtra). Biuret reagent, which was provided in kit, was used for the detection of serum protein. Serum was collected from each group, where n= 6. Three test tubes were labelled as blank, test and standard where blank contains only biuret reagent and distill water, standard tube contains biuret reagent and protein standard and sample tube contains serum sample and biuret reagent. Samples of

each tube were mixed properly and followed byincubation at 37°C for 10min. Absorbance was measured for test and standard tubes at 550 nm against within 60 (Alimohammadi et al., 2013.; Chaudhary et al.,2016)

Calculations:

Total proteins (g/dl) = Abs. Test/Abs. Sample x 8

iv. Estimation of serum lipid profile:

Total cholesterol, triglycerides, high density lipoproteins are the main lipids found in serum. Certain pathophysiological conditions, such as hypercholesterolaemia, hyperlipidaemia, hypothyroidism, uncontrolled diabetes, nephrotic syndrome and cirrhosis result increased levels of serum lipid. Malabsorption, malnutrition, hyperthyroidism, anemias and liver diseases are the causes of lower level of serum lipid. Cholesterol kit uses CHOD/PAP method to determine cholesterol activity in serum or plasma. All the reagents were ready to use.

For cholesterol determination three tubes were prepared and labelled as blank, sample and test. Blank tubes constituted with working reagent and distill water, standard tubes constituted with working reagent and standard and test tubes constituted with serum sample and working reagent. All the contents were mixed properly and incubated at 37°C for 5 min, followed by measuring the absorbance at 505 nm against blank.

Calculations:

Cholesterol in mg/dl = Abs. of test / Abs. of standard * 200

For estimation of triglycerides in serum sample, procedure was same, only standard tubes contained standard triglyceride with working reagent.

Triglycerides in (mg/dl) = Abs. of test/ Abs. of Standard × 200

v. Determination Tissue (Liver and Kidney) antioxidant parameter:

The livers and kidneys of sacrificed animals were extracted, washed in ice cold normal saline and accompanied by cold 0,15 M Tris HCl (pH 7.4), blotted with tissue paper for

drying and weighed. A 10% w/v of tissue homogenate was prepared in 1.15% potassium chloride (pH 7.4) by using Teflon-glass homogenizer and divided into two portions. The first portion of homogenate was treated with of 0.02 M EDTA and kept on ice bath for 10 min: then precipitating proteins with 50% w/v trichloroacetic acid (TCA) followed by centrifugation at 3000 g for 15 min, the supernatant was used for the estimation of reduced glutathione and lipid peroxidation. After that second part of the tissue homogenate was centrifuged at 5000 g at 4°C for 15 min; supernatant was collected after centrifugation and glutathione-S- transferase, superoxide dismutase, catalase and total protein were estimated from supenatant. The supernatants were collected, and lipid peroxidation (LPO) [malondialdehyde (MDA)], GSH, and superoxide dismutase (SOD) were estimated by using commercially available reagent kits (Erba Diagnostics, Mumbai, Maharashtra, India) [Du Hao et al., 2017; Đorđević et al., 2017].

a. Lipid peroxidation:

Thiobarbituric acid reactive substances (TBARS) by product of lipid peroxidation in the liver tissue were measured by method (Ohkawa et al., 1979). 0.2 ml of supernatant was mixed with 0.2 ml of 8.1% sodium dodecyl sulphate (SDS), 1.5 ml of 20% acetic acid (pH 3.5) and 1.5 ml of 0.8% thiobarbituric acid (TBA). The total reaction mixture was made up to 4 ml with distill water heated at water bath at 95°C for 60 minutes. All the tubes were incubated and cooled at room temperature and volume of the tube was made up to 5 ml with distilled water. Each tube contained 5.0 ml of n-butanol: pyridine mixture (15: 1) was added and the reaction mixture was vortexed thoroughly for 2 minutes. All the tubes were centrifuged at 30009 for 10 minutes, the upper organic layer was separated and absorbance was measured at 532 nm against an appropriate blank without the tissue sample. TBARS level were expressed as µmoles of malondialdehyde (MDA)/g of liver tissue by using extinction co-efficient of 1.56 x $105M^{-1cm-1}$.

b. Reduced Glutathione (GSH):

GSH is predominant in liver tissue, GSH level was determined as per reported method (Ellman, 1959). To 1 ml of protein free supernatant, 2 ml of Tris buffer (pH 8.9) was added. Then 0.05 ml of freshly prepared Eliman's reagent 5,5-dithio bis-2-nitro benzoic

acid (CTNB) solution in absolute methanol was added and thoroughly vortexed. After addition of DTINE absorbance was measured at 412 nm after 2-3 min against a reagent blank. Standard reduced glutathione was used as standard. The content of GSH was expressed as µg/g of liver tissue.

c. Superoxide Dismutase (SOD):

In liver and kidney tissue sample SOD activity was measured according to the reported method. The total reaction mixture which contained 12 ml of sodium pyrophosphate buffer (0.025 M. pH 8.3), 0.1 ml of phenazine methosulphate (PMS) (186 µM), 0.3 ml of nitro-blue tetrazolium (NBT) (300 µM) admixed with 0.2 ml of the supernatant and distilled water and total assay volume was 2.8 ml. The reaction was started by the addition of 0.2 ml of NADH (780 µM) followed by incubation at 30°C for 30 seconds and the reaction was stopped by the addition of 1.0 ml of glacial acetic acid. The whole reaction mixture was then shaken with 4.0 ml of n-butanol, all the tubes were allowed to stand for 10 minutes and centrifuged. The absorbance of the butanol layer was measured at 560 nm against blankwithout sample. Enzyme activity was expressed as one unit of enzyme activity which is defined as the quantity of enzyme that gave 50% inhibition of NBT reduction in one minute. The value of SOD activity was expressed as unit of SOD/mg of liver tissue.

vi. Histopathological studies

Histopathological studies were carried out with the parts of pancreas which were isolated from the sacrificed rats. The tissues were washed with normal saline and immediately fixed in 10% formalin for 24 h, dehydrated with alcohol, embedded in paraffin, and then cut into 4-5-m-thick sections and stained with hematoxylin-eosin dye for photomicroscopic observations done at 40 (Madic et al.,2021).

vii. Statistical Analysis

All the values are expressed as Mean ± Standard error of mean (SEM) of theindicated number of experiments/animals. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by bonferroni comparisons to determine the level of significance. The *p* values less than 0.05 ($p<0.05$) were considered as statistically significant.

7. Results:

7.1. Pharmacognostic evaluation:

Morphological Characteristic:

Shape: Heart

Size:

Length:5-14 cm

Width: 2.5-7.5 cm

Colour: Light to dark green

Odour: Indistinct

Taste: Bitter and astringent

Type: Simple

Position of Leaves: Leaves in opposite pairs

Surface: Both surfaces rough, lower with appressed hairs

Margin: Entire or distinctly toothed

Apex: Acute

Base: Round to somewhat cuneate

Venation: Reticulate, lateral vein 3-6 pairs, more conspicuous on lower side.

Figure 5: Leaves of *Nyctanthes arbor-tristis* L.

7.2. Microscopical characteristics of the NAT leaf

The transverse section of leaf passing through the midrib convexly projects on lower side and slightly grooved with a shallow central elevation on upper side revealed the presence of different cells (Figure:6)and lower epidermis showed Stomata (Figure:7)

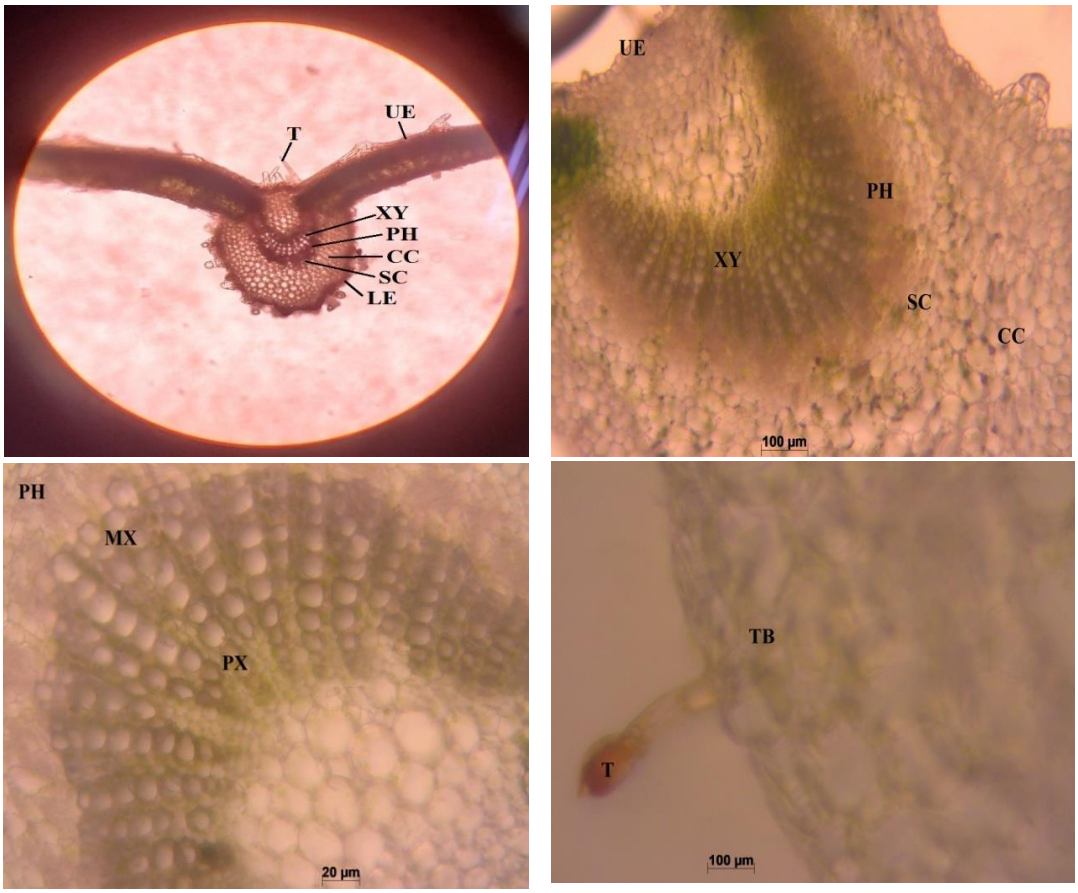


Figure 6:Transverse Section of leaf (CC = cortical cells; LE= lower epidermis; MX= meta xylem; PH= phloem; PX= proto xylem; SC= sclerenchymatous cells; T= trichome; TB= trichome base; UE= upper epidermis; XY= xylem)

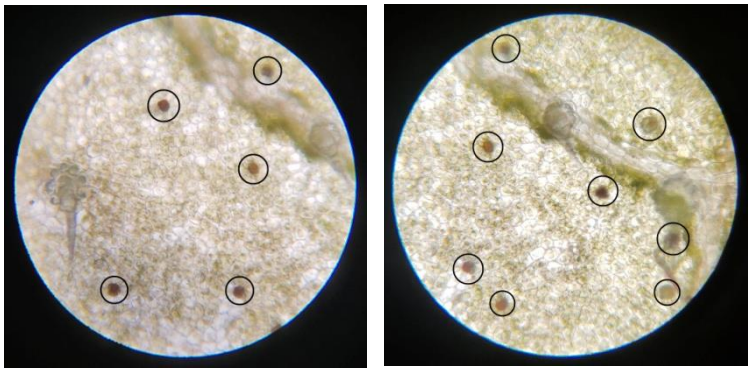
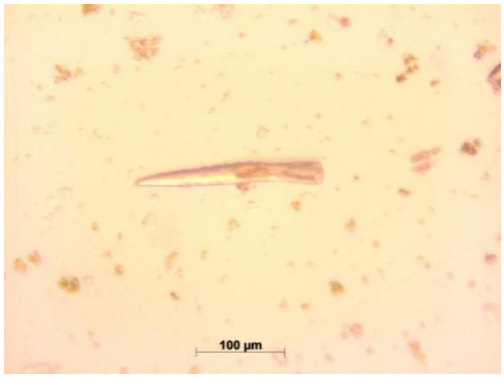


Figure 7: Stomata present in the lower epidermis

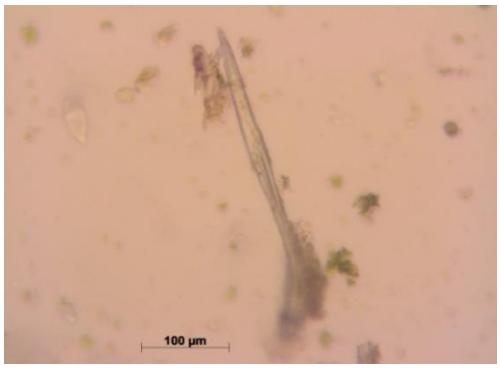
7.3. Powder Microscopy of NAT leaf

Powder microscopy exhibits the presence of simple fiber, simple septate fiber, fragment of bordered pitted xylem vessels, simple trichome, glandular trichome with spherical head, conical trichome etc.

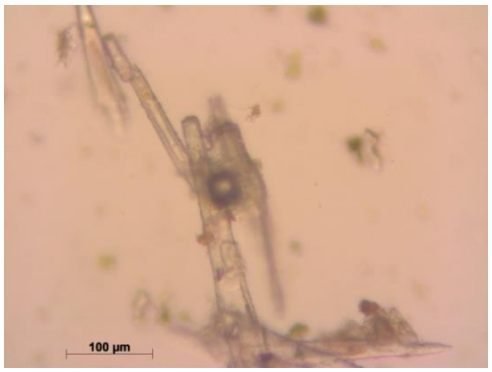


Trichome

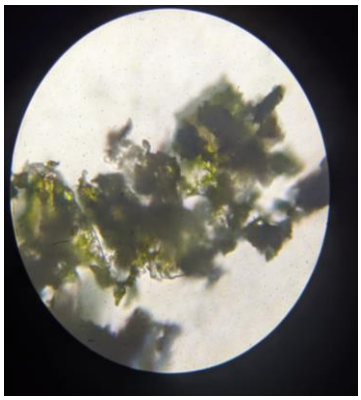
Conical trichome



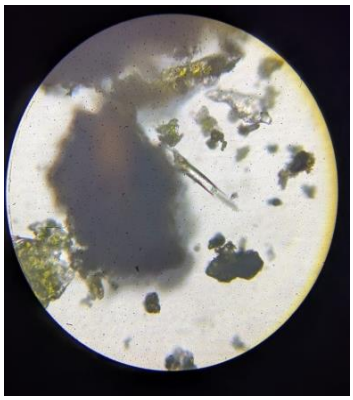
Glandular trichome



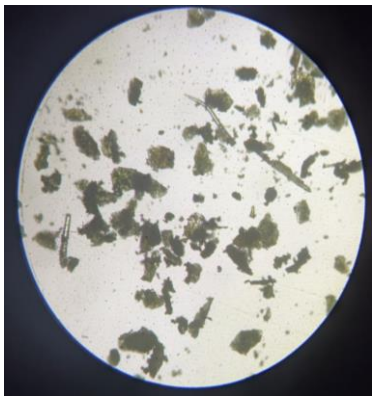
Glandular trichome with spherical head



Fragment of border pitted xylem vessel



Simple fiber



Simple septate fiber

Figure 8: Microscopic characteristics of leaves powder of NAT

7.4. Percentage yield of the extracts of NAT leaves.

Table 2: The yield of Petroleum ether and Methanolic extract of leaves are shown below.

Sl. No.	Extract	% Yield
1.	Petroleum Ether	3.5%
2.	Methanol	11.97%

7.5. Physicochemical Characteristics of Leaves Powder

The leaves powder was used for the determination of different physicochemical parameters which helps in identification of the plants have been evaluated and they are represented in table: 3

Sl. No	Physicochemical parameters	Result
1.	Loss on drying	12.53%
2.	Total ash value	12.19%
	Water soluble	8.51%
	(b) Acid insoluble	3.92%
3.	(a) Water soluble extractive value	26.73%
	(b) Alcohol (ethanol) soluble extractive value	13.34%
4.	Determination of crude fiber	4.67%
5	Determination of Foaming Index	142.86
6.	Determination of Swelling Index	<100

7.	Determination of bitterness value	2.69

Table 3: Physicochemical properties of the leaves of *Nyctanthes arbor-tristis*.

7.6. Phytochemical screening of the extracts

The results of phytochemical screening of Petroleum ether and Methanolic extracts are shown in table: 4. Petroleum ether extract mainly revealed the presence of terpenoids, alkaloids,tannins and phenolics while the methanolic extract revealed the presence of carbohydrate, flavonoids, alkaloids, tannins and phenolics, saponins and glycosides.

Table 4: Phytochemical Screening of the petroleum ether and methanol extracts of the leaves of *Nyctanthes arbor-tristis*.

Sl. No.	Chemical Constituents	Chemical test	Petroleum ether extract	Methanolic extract
1.	Carbohydrate	Molisch Test	-	+
	Reducing Sugars	Fehling's Test	-	+
		Benedict's Test	-	-
	Monosaccharides	Barfoed's Test	-	-
2.	Proteins and amino acid	Biuret Test	-	-
		Ninhydrin Test	-	-
		Millon's Test	-	+
3.	Steroids	Salkowski test	-	-
		Libermann Buchard Test	-	-
4.	Terpenoids	Noller's Test	+	-
5.	Flavonoids	Shinoda Test	-	-
		Ferric Chloride Test	-	-
		Dilute ammonia+ conc. H2SO4	-	+

6.	Alkaloids	Mayer's test	+	+
		Dragondorff'sTest	+	-
		Hager'S Test	-	+
		Wagner's Test	-	+
7.	Tannins and Phenolics	5% FeCl3 Test	+	+
		10% NaOH Test	+	+
8.	Saponins	Foam Test	-	+
9.	Glycoside Test	Legal's Test	-	-
		Keller-killiani Test	-	+

7.7.TLC: Thin Layer Chromatography profiling

R_f values of the Petroleum ether and Methanolic extract in the selected mobile phase system are represented in table: 5

Table 5: TLC profile of extracts.

Petroleum ether extract	Methanolic extract
0.63	0.10
0.190	0.17
0.285	0.20
0.365	0.25
0.444	0.36
0.492	0.48
0.761	0.55
0.873	0.65
-	0.84
-	0.98

Petroleum ether extract **Methanolic extract**

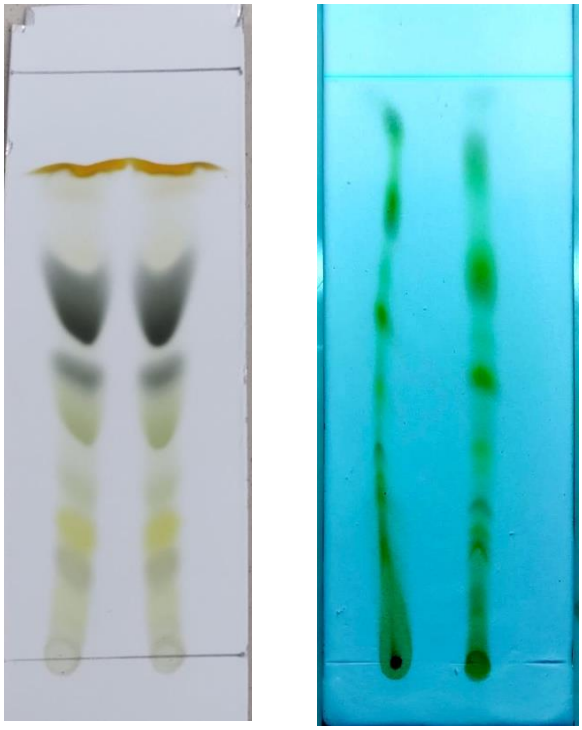


Figure 9: Illustrating distribution of compounds of different polarities after separation on a TLC plate

7.8. HPLC analysis of methanolic extract:

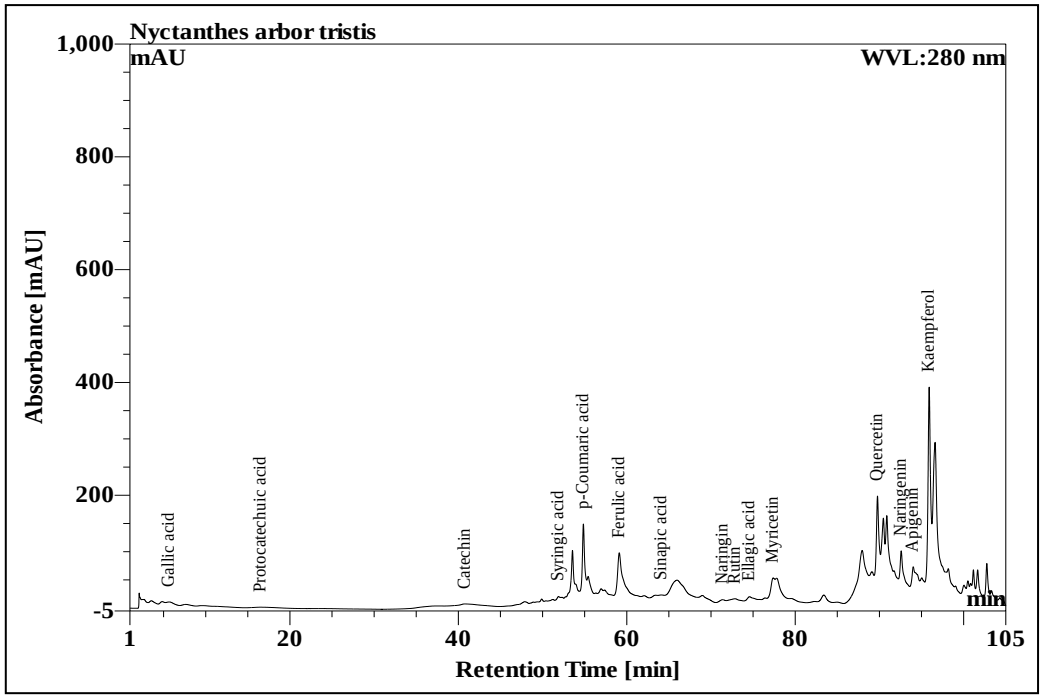
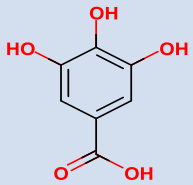
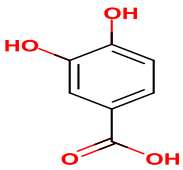
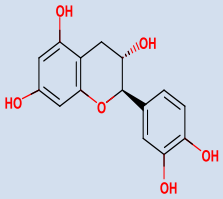
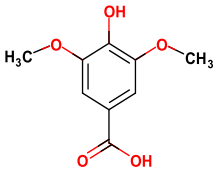
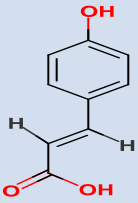
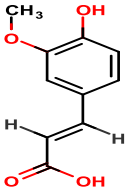
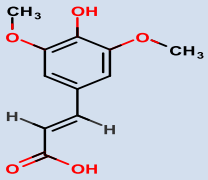
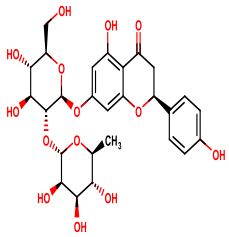
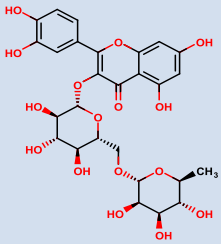
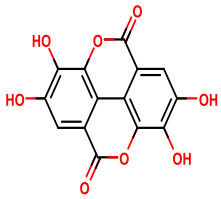
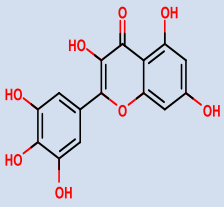


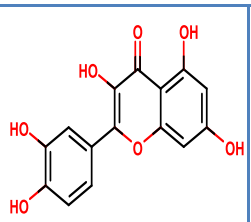
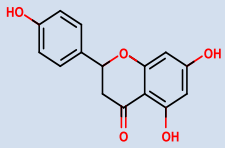
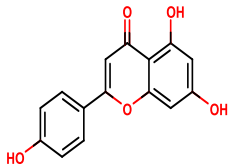
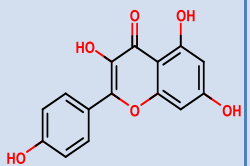
Figure 10: Typical chromatogram of the methanolic extract of *Nyctanthes arbor-tristis* L.

Table 6: Different compounds of *Nyctanthes arbor-tristis* found on HPLC analysis under UV light at wavelengths of 280 nm.

SL No.	Compounds found	IUPAC Name	Structure	Retention time (Minutes)	Peak height (mAU)	% Relative area	µg/ml
1	Gallic acid	3,4,5-trihydroxybenzoic acid		5.68	10.911	0.78	20.300

2	Protocatechuic acid	3,4-Dihydroxybenzoic acid		16.53	1.334	0.21	7.874
3	Catechin	(2R,3S)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol		40.82	5.967	1.80	138.497
4	Syringic acid	4-Hydroxy-3,5-dimethoxybenzoic acid		51.88	9.595	0.55	11.452
5	p-Coumaric acid	(2E)-3-(4-Hydroxyphenyl)prop-2-enoic acid		54.85	138.387	4.21	44.188
6	Ferulic acid	(2E)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoic acid		59.11	87.541	5.56	78.915
7	Sinapic acid	(2E)-3-(4-Hydroxy-3,5-dimethoxyphenyl)prop-2-enoic acid		64.10	0.780	0.02	0.064

8	Naringin	(2*S*)-4′,5-Dihydroxy-7-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyloxy]flavan-4-one		71.39	5.046	0.21	6.612
9	Rutin	3′,4′,5,7-Tetrahydroxy-3-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyloxy]flavone		72.87	6.706	0.56	24.007
10	Ellagic acid	2,3,7,8-Tetrahydroxy[1]benzopyrano[5,4,3-*cde*][1]benzopyran-5,10-dione		74.58	10.309	0.78	25.237
11	Myricetin	3,3′,4′,5,5′,7-Hexahydroxyflavone		77.39	43.903	3.77	104.292

12	Quercetin	2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one		89.77	191.270	6.40	201.457
13	Naringenin	(2*S*)-4′,5,7-Trihydroxyflavan-4-one		92.59	94.890	3.91	149.781
14	Apigenin	5,7-dihydroxy-2-(4-hydroxyphenyl)chromen-4-one		94.02	66.951	4.71	134.598
15	Kaempferol	3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one		95.91	385.878	8.50	194.033

7.9. Tincture Characterization:

Prepared tincture at different strengths of alcohol is represented in table. The study shows that greater alcohol concentration in the tincture increases acidity of the tincture while storage for longer period of time also slightly increases acidic property (Table 7). Solid content of the tincture also increases with time as seen from table 8, while greater alcohol concentration also imparts diminished solid content. Finally, as seen from table: 9, specific gravity the prepared tinctures do not vary substantially with time.

Table 7 : pH of Tincture after maceration for different period of time.

Alcohol Strength	pH		
	7 Days	14 Days	21 Days
40%	6.37	6.24	6.08
50%	6.96	7.26	6.29
60%	6.42	6.35	6.25
70%	6.27	6.20	6.19
80%	6.25	6.02	5.97
90%	5.87	5.73	5.70
Absolute (100%)	4.71	5.51	5.50

Table 8 : Total solid content of tincture on different day.

Alcohol Strength	Solid Content (%)		
	7 Days	14 Days	21 Days
40%	17.19	18.35	22.61
50%	22.78	24.09	25.73
60%	20.95	21.87	22.41
70%	18.69	20.68	21.68
80%	13.96	15.39	16.72
90%	11.83	13.02	13.66
Absolute (100%)	5.93	7.19	10.34

Table 9 : Specific gravity of tincture.

Strength	Specific gravity(gm)		
	Day 7	**Day 14**	**Day 21**
40%	0.953	0.951	0.955
50%	0.936	0.939	0.941
60%	0.909	0.911	0.914
70%	0.885	0.889	0.892
80%	0.854	0.858	0.863
90%	0.831	0.837	0.840
Absolute	0.798	0.801	0.805

7.10. Fluorescence Characterization:

The fluorescence characteristic of leaves powder on treatment with various chemical and reagents on normal light and under both short(254 nm) and long (366 nm) are shown in table: 10

Table 10 : Fluorescence analysis of leaf powder after treatment with various chemical reagents.

Reagents	**(Colour) Visible light**	**Short wavelength(254 nm)**	**Long Wavelength (366 nm)**
Untreated	Olive Green	Olive green	Olive green

Powder+Picric acid	Army Green	Sacramento green	Sacramento green
Powder+HNO3	Bronze Orange	Hickory Orange	Kalley green
Powder+ HCl	Olive Green	Sacramento green	Forest green
Powder+H2SO4	Fire Orange	Lime Green	Lime Green
Powder+Glacial acetic acid	Lime Green	Oclre orange	Kalley green
Powder+NaOH(5N aq. solution)	Lime Green	Forest green	Kalley green
Powder+Iodine(aq. Solution)	LegunaYellow	Sacramento green	Kalley green
Powder+FeCl3(5% aq. Solution)	Olive Green	Anchor Gray	Hunter green
Powder+Antimony trichloride(alcoholic solution)	Olive Green	Bronze orange	Kalley green
Powder+Methanol	Lime Green	Trout grey	Light green
Powder+70% ethanol	Fern Green	Shadow grey	Mint green
Powder+Petroleum ether	Olive Green	Plum Violet	Fern green

Powder+50% H2SO4	Lime Green	Byzantine violet	Kalley green
Powder+1N NaOH(aq.solution)	Bumblebee Yellow	Forest green	Kalley green
Powder+1N NaOH(alc.solution)	Lime Green	Hunter Green	Kelly Green
Powder+ 50%HNO_3	Honey Orange	Kelly Green	Kelly Green
Powder+ 5% KOH	Lime Green	Forest Green	Kelly Green
Powder+ Ammonia	Lime Green	No change	No change
Powder+ Antimony trichloride(aq.solution)	Moss Green	Shadow Gray	Army Green

7.11. Anti oxidant assay

7.11.1. Total Phenolic content assay:

The absorbance of the standard and test solution were represented in table no.11 .The total phenolic content in terms of mg Gallic Acid Equivalent (GAE)of methanolic extract was found to be 19.457 mg/g . These results suggest that higher the presence of phenolic components was responsible for the levels of antioxidant activity. The Standard curve of Gallic acid was shown in figure 11.

Table 11 : Observation of absorbance in Total phenolic estimation.

Sl No.	Sample	Concentration(µg/ml)	Absorbance
1	Standard (Gallic Acid)	20	0.238
2		40	0.458
3		60	0.686
4		80	0.815
5		100	0.999
6	Methanolic extract	100	0.260

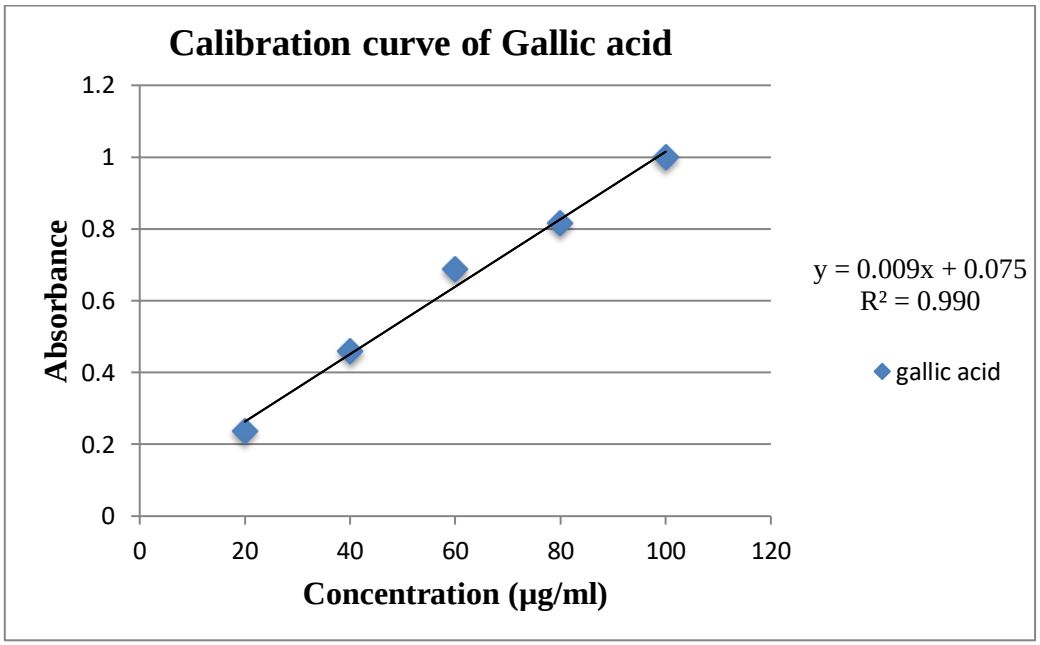


Figure 11: Calibration curve of Gallic acid.

7.11.2. Total Flavonoid content assay:

The absorbance of the standard and test solution were represented in table no.12.The total flavonoid content in terms of mg Quercetin Equivalent (QE)of methanolic extract was found to be 14.509 mg/g . These results suggest that higher the presence of flavonoid components was responsible for the levels of antioxidant activity. The Standard curve of Quercetin was shown in figure 12.

Table 12: Observation of absorbance in Total Flavonoid estimation.

Sl No.	Sample	Concentration(µg/ml)	Absorbance
1	**Standard (Quercetin)**	20	0.138
2		40	0.250
3		60	0.358
4		80	0.454
5		100	0.547
6	**Methanolic extract**	100	0.091

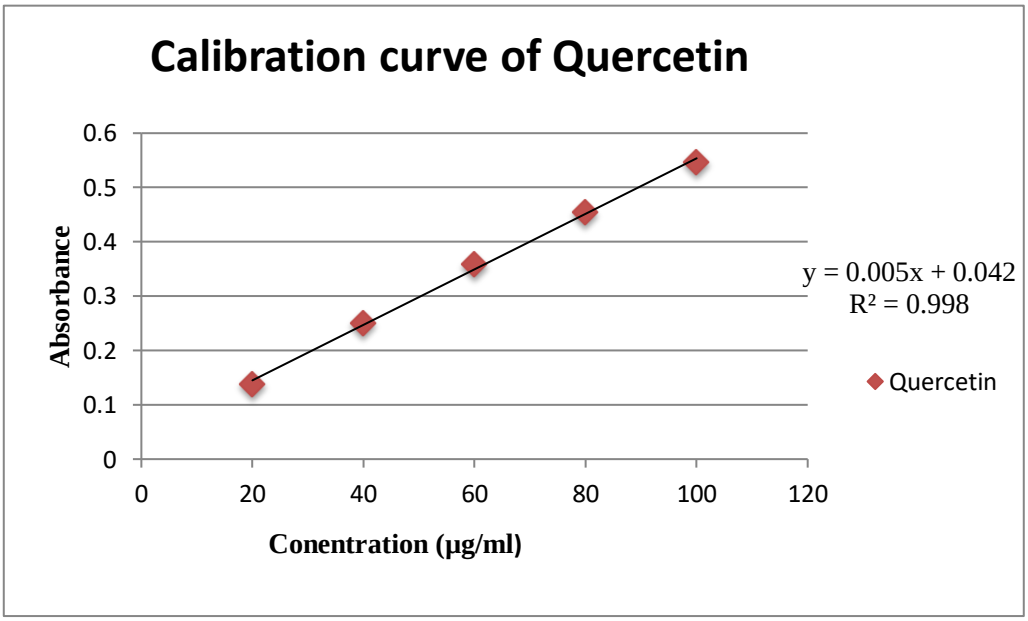


Figure 12: Calibration curve of Quercetin.

7.11.3. DPPH Radical Scavenging Assay:

The drop in DPPH's absorbance at 517 nm, which is brought on by antioxidants, was used to assess its capacity for reduction. The extract showed maximum hydrogen donating ability in the presence of DPPH free radicals at high concentrations. The extract showed antioxidant activity with an IC_{50} value of 165.27 µg/ml. However, the known antioxidant Ascorbic acid exhibited an IC_{50} value of 139.66 µg/ml on DPPH free radical. *Nyctanthes arbor-tristis* had significant scavenging effects with increasing concentration when compared with that of Ascorbic acid. The results of the DPPH scavenging activity was shown below in the table 13 represents DPPH radical scavenging assay of standard and test sample.

Table 13: DPPH Radical Scavenging Assay of Standard sample and Test Sample.

Sl No.	Concentration(µg/ml)	Percentage Inhibition	
		Test Sample (Methanolic extract)	Standard(Ascorbic acid)
1	20	12.12	16.92
2	40	15.01	18.01
3	60	21.98	28.46
4	80	27.78	30.54
5	100	32.45	39.9
IC_{50}		165.27µg/ml	139.66µg/ml

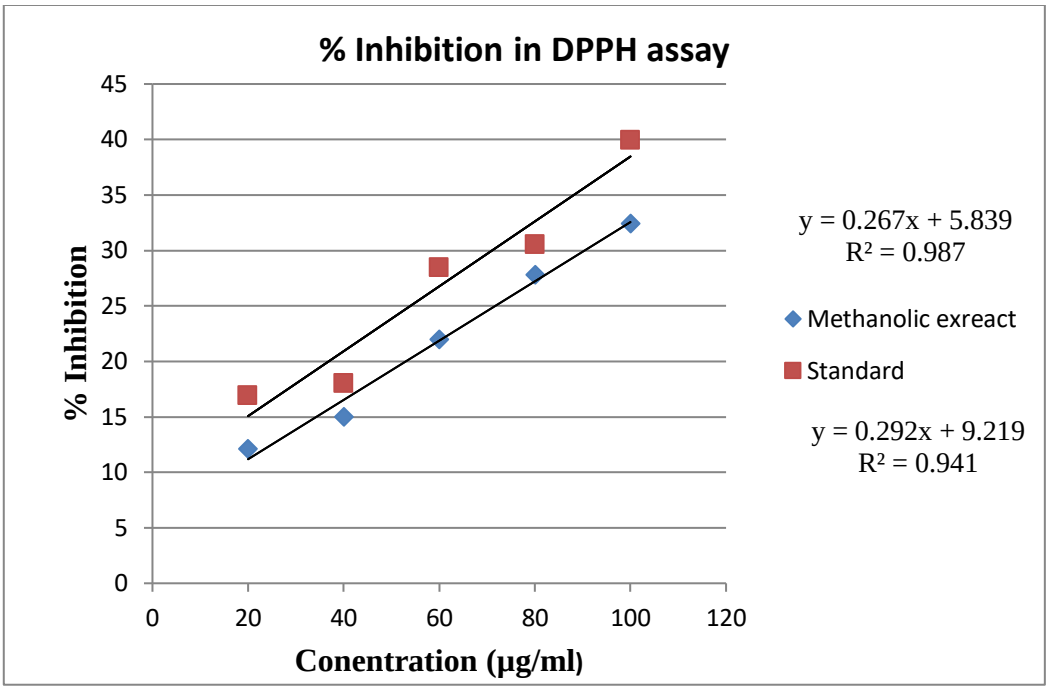


Figure 13: Percent inhibition of extract in DPPH antioxidant assay.

7.12. Determination of *in-vitro* Antidiabetic Activity

Table 14: Alpha amylase inhibition activity of Extract and Standard.

Sl. No.	Concentration(µg/ml)	% Inhibition of Acarbose	% Inhibition of methanolic extract
1	20	13.2	6.030151
2	40	25.12	15.07538
3	60	30.75	28.1407
4	80	39.38	43.21608
5	100	55.64	49.74874
IC_{50}		94.66 µg/ml	97.45µg/ml

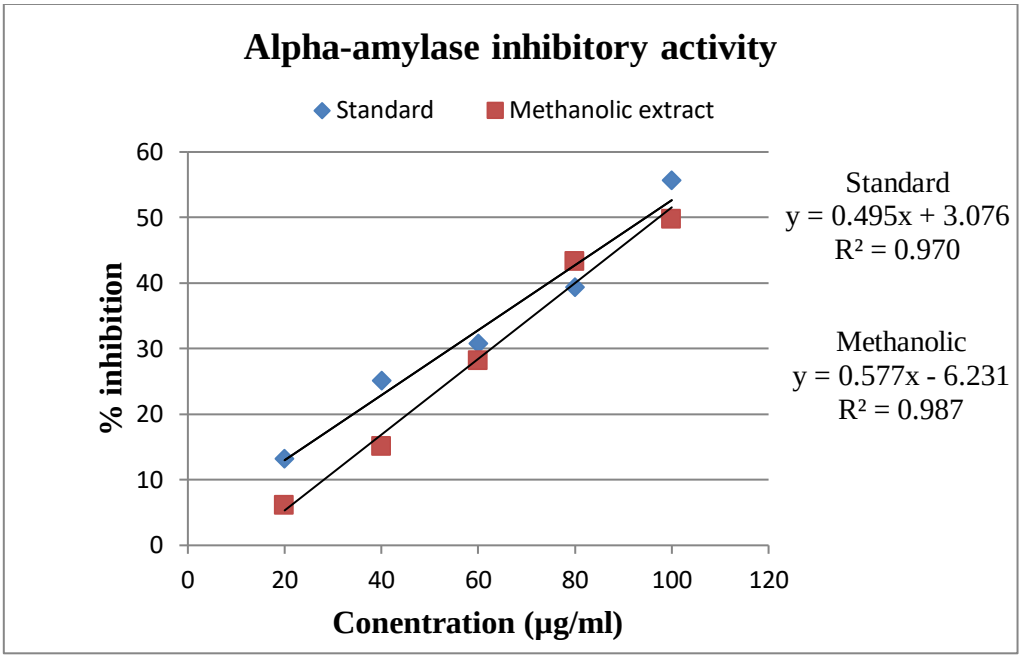


Figure 14: α-amylase inhibition activity of Extract and Standard

7.13. In vivo anti diabetic assay:

The level of FBG in HFD-STZ induced diabetic rats was found to be considerably ($p<$ 0.05) higher than in the normal control group. When diabetic rats were given NATME at doses of 200 and 400 mg/kg for 28 days, the FBG level was found to be significantly lower (P<0.05) than in the diabetic control group. [Table 15]

Table 15: Effects of *Nyctanthes arbor-tristis* methanolic extract on Fasting Blood glucose level (mg/dl) of different groups of rat.

Day	Normal control	STZ (35mg/kg)	STZ+ Metformin(50 mg/kg)	STZ+ NATME (200 mg/kg)	STZ+ NATME (400 mg/kg)
0	80.333±2.028	401.33±6.936 *	416±18.009 *	421±3.454 *	405.67±6.741 *
7	82.333±1.764	407±7.937 *	289.31±5.812 *#	371.22±9.713 *#@	317.33±5.207 *#@
14	81.333±2.404	422.33±4.333 *	169.50±8.963 *#	319.43±7.234 *#@	225.67±6.173 *#@
21	82.000±3.000	442±5.292 *	115.11±1.732 *#	224.69±8.686 *#@	168.56±4.096 *#@
28	82.667±1.856	450.67±7.839 *	102.33±1.453 #	151.93±4.041 *#@	114.67±3.18 *#

Each values are expressed as **Mean ± SEM (n=6)**,
Values are analysed at 5% level of significance,***p*<0.05** considered as statistically significant.
* All other groups versus **normal control group**,
#All treated group versus **diabetic control group**,
@Both extract group versus **Standard drug group**.
STZ: Streptozotocin, **NATME**: *Nyctanthes arbor-tristis* methanolic extract

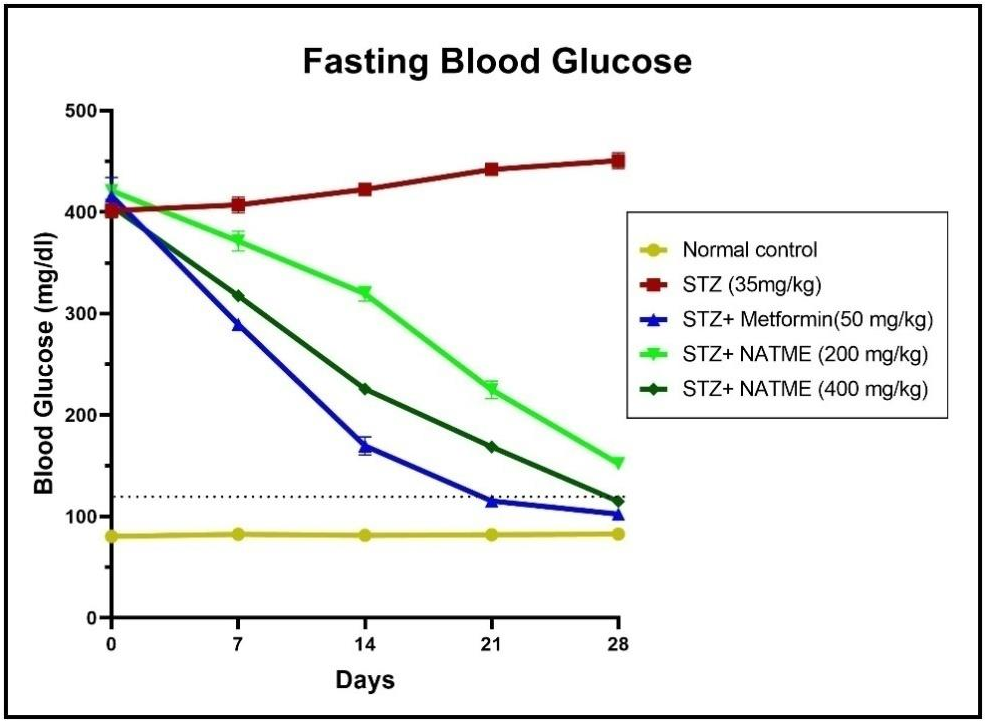


Figure15: Illustrative representation of changes in Blood Glucose level from Table no. 15

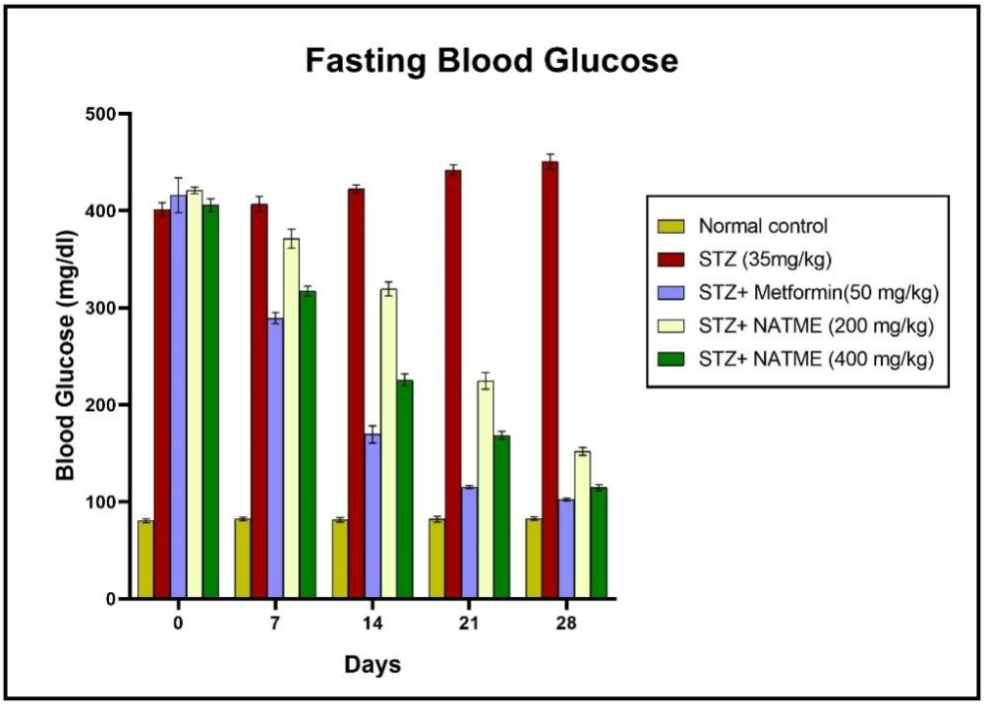


Figure 16: Illustrative representation of changes in Blood Glucose level from Table no. 15

Body weight

The diabetes control group's end body weights were statistically substantially (p<0.05) lower than the normal control group's. When compared to the diabetic control group, NATME administration at doses of 200 and 400 mg/kg significantly improved body weight (P<0.05) which has been shown in Table 16.

Table 16: Effects of *Nyctanthes arbor-tristis* methanolic extract on body weight (g) of different groups of rat.

Day	Normal control	STZ (35mg/kg)	STZ+ Metformin (50 mg/kg)	STZ+ NATME (200 mg/kg)	STZ+ NATME (400 mg/kg)
0	199.33±4.66	274.67±7.53*	271.67±7.26*	307.67±7.311*#@	268.33±11.66*
7	204±5.85	273.67±8.353*	267.67±8.192*	304±9.53*#@	262.33±12.66*
14	199.33±2.603	267.67±7.53*	262.67±7.26*	296.33±6.33*@	256.33±11.69*
21	204±2.64	262.67±4.48*	257.67±4.8*	289.67±4.66*@	252.33±10.41*
28	208.33±5.207	256.67±4.48*	252.67±5.54*	282.33±5.81*	247.67±8.96*

Each values are expressed as **Mean ± SEM (n=6)**,
Values are analysed at 5% level of significance, ***p*<0.05** considered as statistically significant.
* All other groups versus **normal control group**,
# All treated group versus **diabetic control group**,
@ Both extract group versus **Standard drug group**.
STZ: Streptozotocin, **NATME**: *Nyctanthes arbor-tristis* methanolic extract

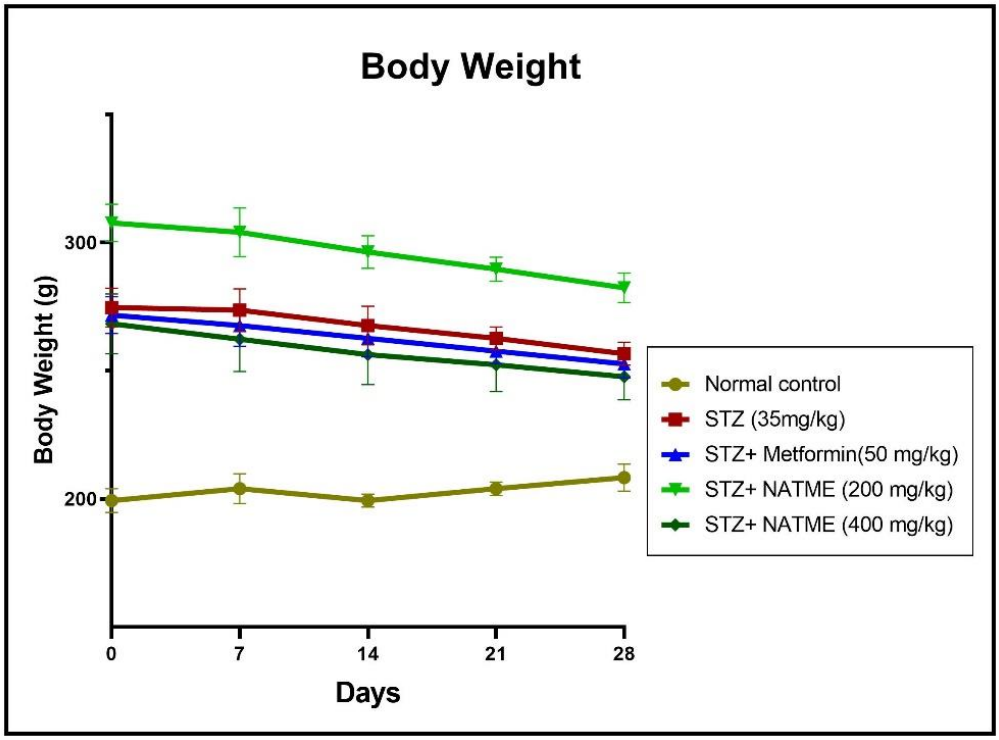


Figure 17: Illustrative representation of changes in Body Weight from Table no.16

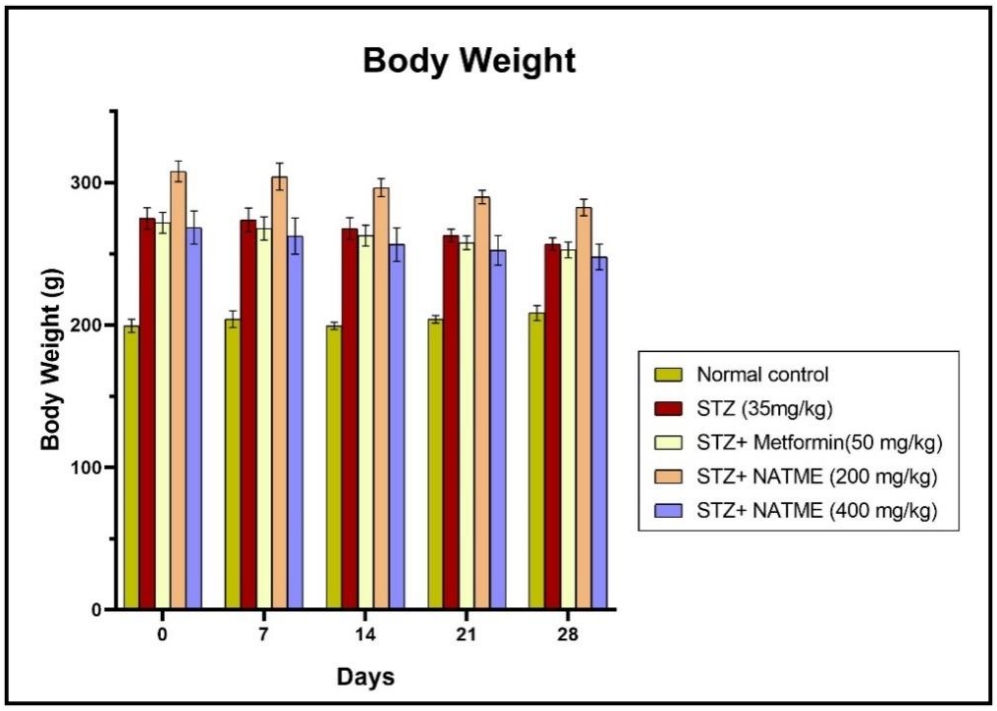


Figure 18: Illustrative representation of changes in Body Weight from Table no. 16

Estimation of Glycosylated Haemoglobin (HbA1c):

Glycosylated haemoglobin level in STZ-induced diabetic rats was significantly ($p < 0.001$) increased compared to normal control group. Treatment with NATME at the doses of 200 and 400 mg/kg significantly ($p< 0.05$) reduced the HbA1c level when compared to the diabetic control group (Table 17).

Table 17: Effects of NATME on HbA1c

Groups	HbA1c(%)
Normal Control	5.639±0.0269
STZ Control	9.669±0.0276*
STZ+ Metformin (50 mg/kg)	6.102±0.0447*#
STZ+ NATME(200mg/kg)	7.312±0.0587*#@
STZ+ NATME(400mg/kg)	6.358±0.0313*#@

Each values are expressed as **Mean ± SEM (n=6)**,
Values are analysed at 5% level of significance, ***p*<0.05** considered as statistically significant.
* All other groups versus **normal control group**,
# All treated group versus **diabetic control group**,
@ Both extract group versus **Standard drug group**.
STZ: Streptozotocin, **NATME**: *Nyctanthes arbor-tristis* methanolic extract

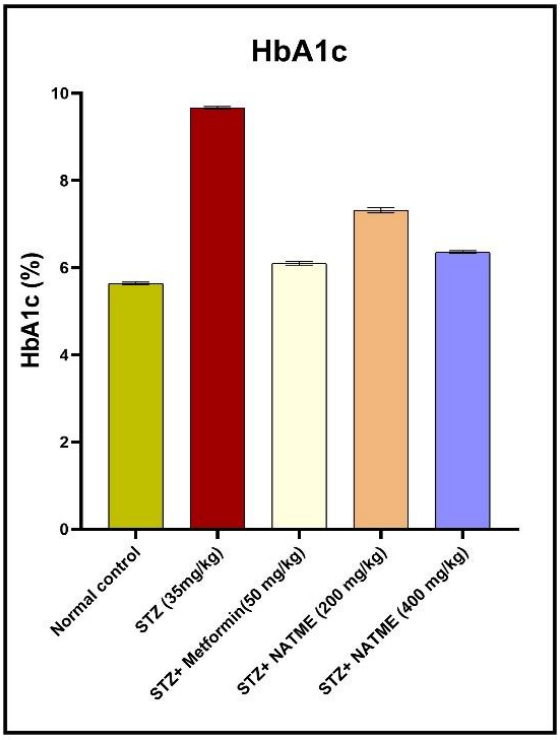


Figure 19: Illustrative representation of HbA1c from Table no 17

Estimation of Total protein:

Total protein content was significantly ($p< 0.05$) decreased in STZ induced diabetic rat compared to the normal control group.Treatment with NATME at the doses of 200 and 400 mg/kg significantly ($p < 0.05$) increased the total protein level as compared to the diabetic control group (Table 18)

Table 18: Total Protein of Serum liver

Groups	Total protein (g/dl)
Normal Control	9.204±0.694
STZ Control	4.834±0.573*
STZ+ Metformin (50 mg/kg)	8.448±0.382$^{\#}$
STZ+ NATME (200mg/kg)	6.779±0.457*
STZ+ NATME (400mg/kg)	7.833±0.375$^{\#}$

Each values are expressed as **Mean ± SEM (n=6)**,
Values are analysed at 5% level of significance, ***p*<0.05** considered as statistically significant.
* All other groups versus **normal control group**,
[#] All treated group versus **diabetic control group**,
[@] Both extract group versus **Standard drug group**.
STZ: Streptozotocin, **NATME**: *Nyctanthes arbor-tristis* methanolic extract

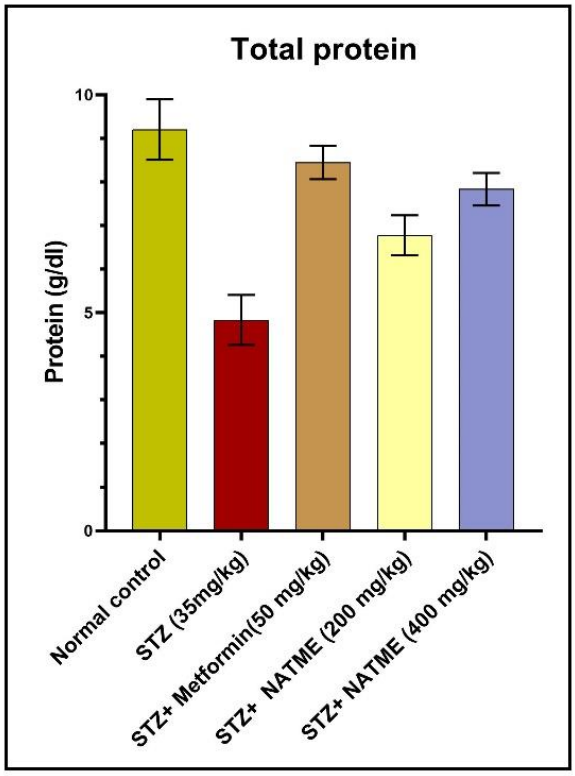


Figure 20 : Illustrative representation of Total Protein from Table no. 18

Estimation of serum Lipid profile:

Serum lipid profiles like total cholesterol, triglyceride in STZ-induced diabetic rats were significantly ($p< 0.05$) elevated compared to normal control group. Treatment with NATME at the doses of 200 and 400 mg/kg significantly ($p< 0.05$) reduced the total cholesterol, triglyceride level and when compared to the diabetic control group (Table 19).

Table 19: Effects of NATME on Lipid Profiles.

Groups	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)
Normal control	48.16±1.276	63.9±1.544
STZ (35mg/kg)	128.28±1.605*	156.13±1.831*
STZ+ Metformin (50 mg/kg)	51.59±1.815$^{\#}$	78.563±1.105$^{\#}$
STZ+ NATME (200 mg/kg)	73.16±1.18*$^{\#@}$	98.023±1.616*$^{@}$
STZ+ NATME (400 mg/kg)	56.02±1.901*$^{\#}$	87.003±2.04*$^{\#}$

Each values are expressed as **Mean ± SEM (n=6)**,
Values are analysed at 5% level of significance, ***p*<0.05** considered as statistically significant.
* All other groups versus **normal control group**,
$^{\#}$ All treated group versus **diabetic control group**,
$^{@}$ Both extract group versus **Standard drug group**.
STZ: Streptozotocin, **NATME**: *Nyctanthes arbor-tristis* methanolic extract

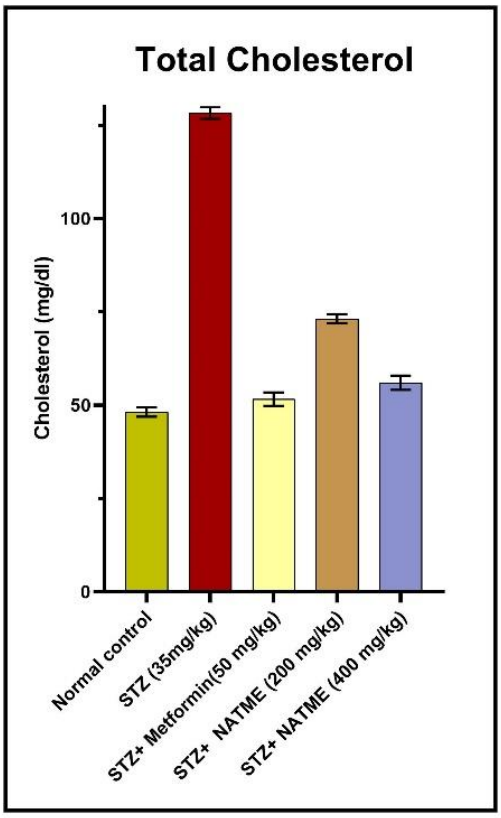


Figure 21: Illustrative representation of Total Cholesterol from Table no. 19

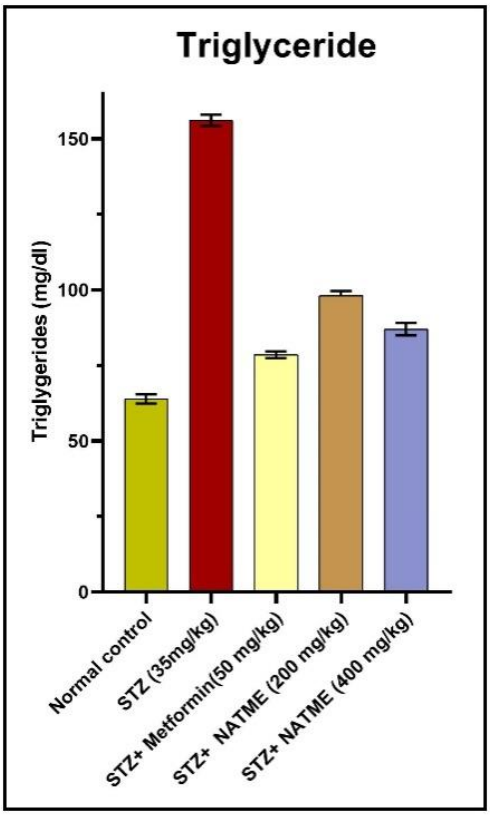


Figure 22:Illustrative representation of Triglyceride from Table no.: 19

Estimation of Tissue antioxidant parameter:

Lipid peroxidation (LPO) results in the formation of ROS species and subsequently elevates the level of malondialdehyde (MDA) in liver and kidney tissue of STZ-induced diabetic rats. In the present study the MDA level was significantly ($p< 0.05$) increased in HFD/STZ-induced diabetic rats compared to normal control group. Interestingly, treatment with BCME at the doses of 200 and 400 mg/kg significantly ($p< 0.05$) reduced the MDA levels compared to diabetic control group (Table 20) The levels of reduced GSH and SOD were significantly ($p<0.05$) decreased in HFD/STZ-induced diabetic rats compared to normal control group. Administration of BCME at the doses of 200 and 400 mg/kg significantly ($p< 0.05$) increased GSH, SOD antioxidant enzyme levels in the liver of HFD/STZ-induced diabetic rats compared to the diabetic control group (Table 21, 22).

Table 20: Effects of NATME on LPO

Groups	LPO (µM/100g Tissue Homogenate) Liver	LPO (µM/100g Tissue Homogenate) Kidney
Normal control	1.409±0.065	1.428±0.032
STZ (35mg/kg)	3.457±0.232*	3.046±0.046*
STZ+ Metformin(50 mg/kg)	1.791±0.197$^{\#}$	1.713±0.097$^{\#}$
STZ+ NATME (200 mg/kg)	2.773±0.163*$^{@}$	2.304±0.073*$^{\#@}$
STZ+ NATME (400 mg/kg)	2.076±0.324$^{\#}$	1.849±0.081*$^{\#}$

Each values are expressed as **Mean ± SEM (n=6)**,

Values are analysed at 5% level of significance, ***p*<0.05** considered as statistically significant.

* All other groups versus **normal control group**,

$^{\#}$ All treated group versus **diabetic control group**,

$^{@}$ Both extract group versus **Standard drug group**.

STZ: Streptozotocin, **NATME**: *Nyctanthes arbor-tristis* methanolic extract

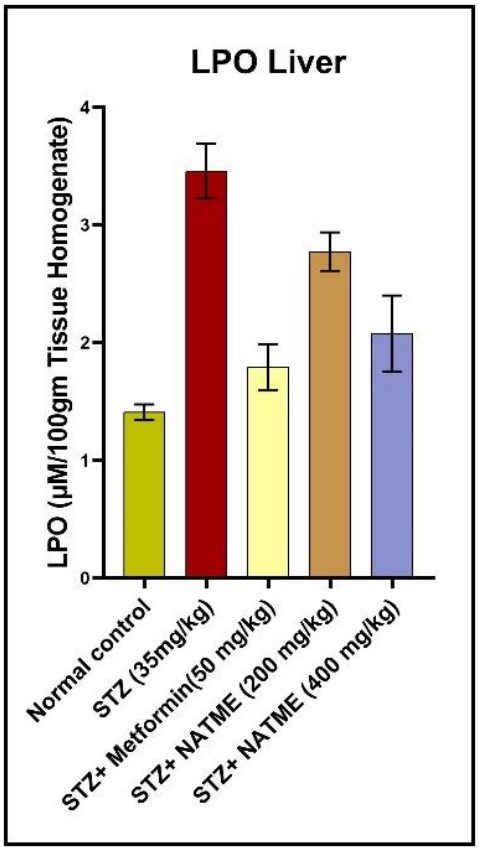


Figure 23: Illustrative representation of LPO Liver from Table no. 20

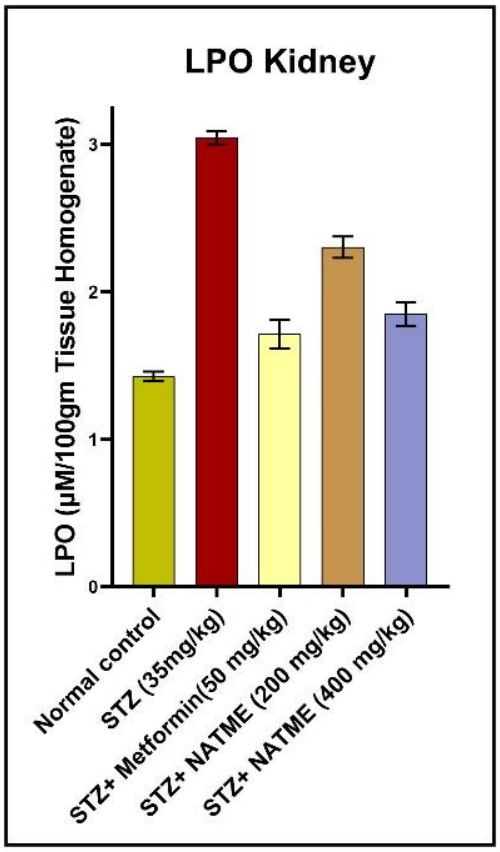


Figure 24: Illustrative representation of LPO Kidney from Table no. 20

Table 21: Effects of NATME on SOD

Groups	SOD Liver (U/mg)	SOD Kidney (U/mg)
Normal control	3.249±0.032	3.537±0.045
STZ (35mg/kg)	0.947±0.049*	1.045±0.032*
STZ+ Metformin(50 mg/kg)	2.953±0.019*#	3.213±0.097#
STZ+ NATME (200 mg/kg)	2.531±0.083*#@	2.603±0.143*#@
STZ+ NATME (400 mg/kg)	2.824±0.107*#	2.997±0.154*#

Each values are expressed as **Mean ± SEM (n=6)**,

Values are analysed at 5% level of significance, **p<0.05** considered as statistically significant.

* All other groups versus **normal control group**,

All treated group versus **diabetic control group**,

@ Both extract group versus **Standard drug group**.

STZ: Streptozotocin, **NATME**: *Nyctanthes arbor-tristis* methanolic extract

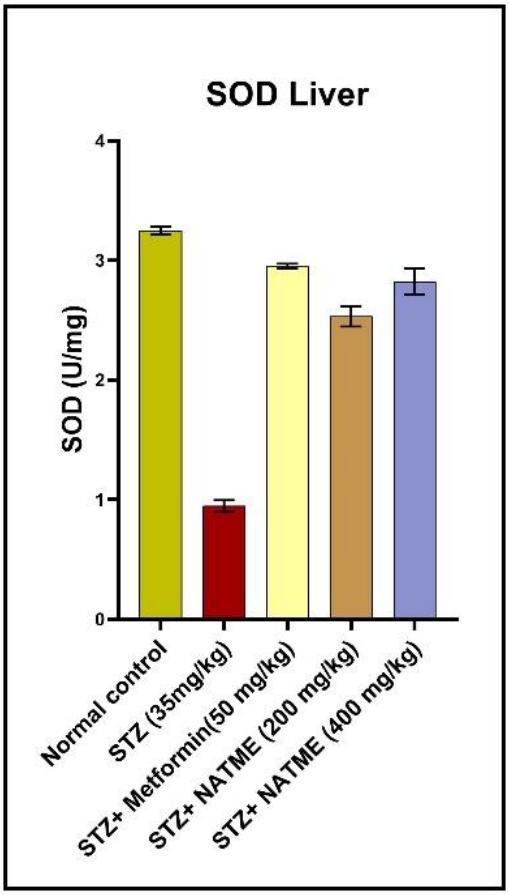


Figure 25: Illustrative representation of SOD Liver from Table no. 21

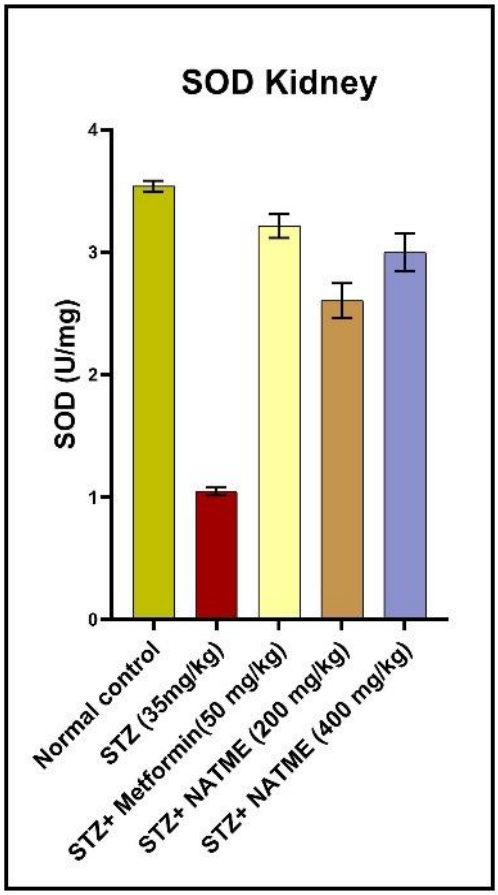


Figure 26: Illustrative representation of SOD Liver from Table no. 21

Table 22: Effects of NATME on GSH

Groups	GSH Liver (µg/mg tissue)	GSH Kidney (µg/mg tissue)
Normal control	3.678±0.092	3.401±0.062
STZ (35mg/kg)	1.769±0.103*	1.809±0.046*
STZ+ Metformin(50 mg/kg)	3.361±0.069$^{\#}$	3.109±0.023*$^{\#}$
STZ+ NATME (200 mg/kg)	2.082±0.049*$^{@}$	2.693±0.073*$^{\#@}$
STZ+ NATME (400 mg/kg)	3.271±0.037*$^{\#}$	3.020±0.081*$^{\#}$

Each values are expressed as **Mean ± SEM (n=6)**,

Values are analysed at 5% level of significance, **p<0.05** considered as statistically significant.

* All other groups versus **normal control group**,

$^{\#}$ All treated group versus **diabetic control group**,

$^{@}$ Both extract group versus **Standard drug group**.

STZ: Streptozotocin, **NATME**: *Nyctanthes arbor-tristis* methanolic extract

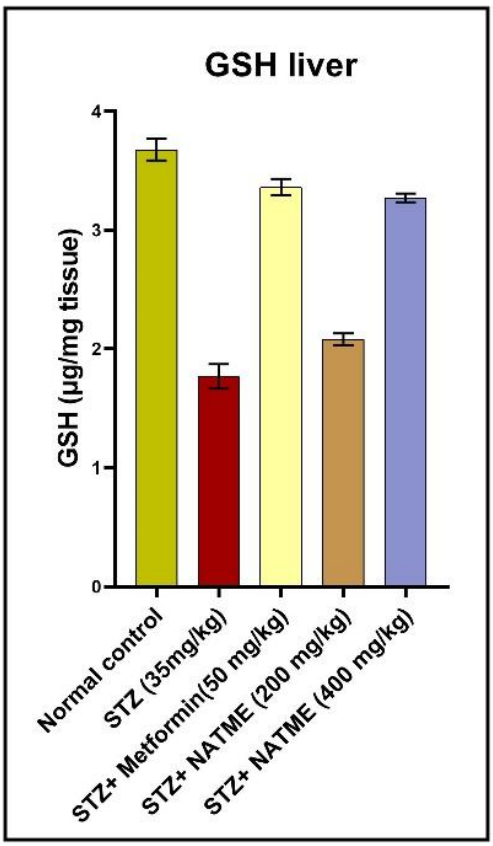


Figure 27: Illustrative representation of GSH Liver from Table no. 22

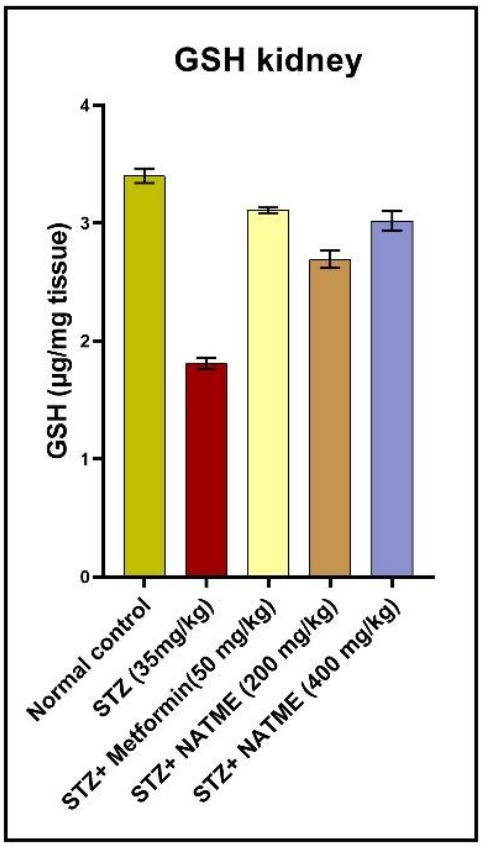
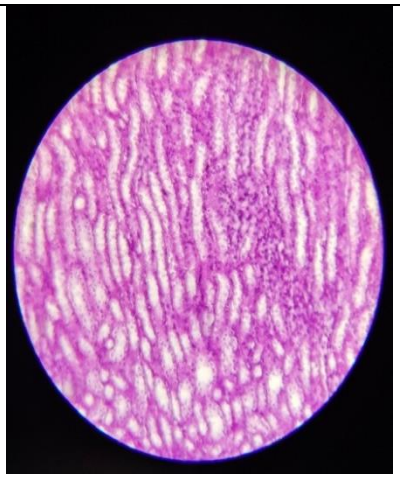
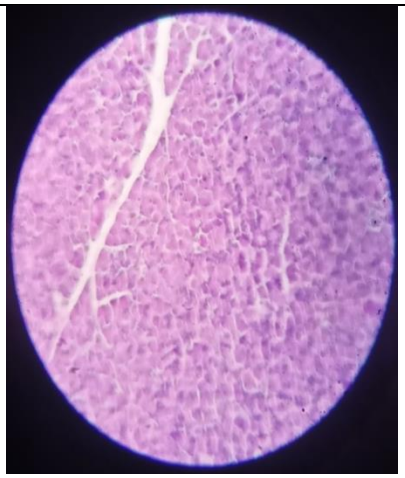


Figure 28: Illustrative representation of GSH Kidney from Table no. 22

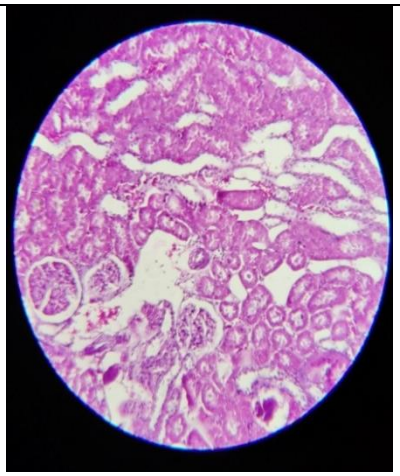
Histopathology:



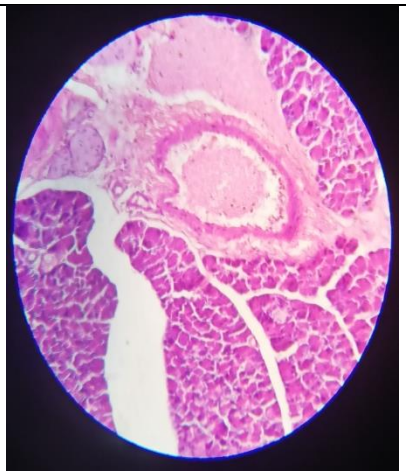
A1- Normal control Kidney



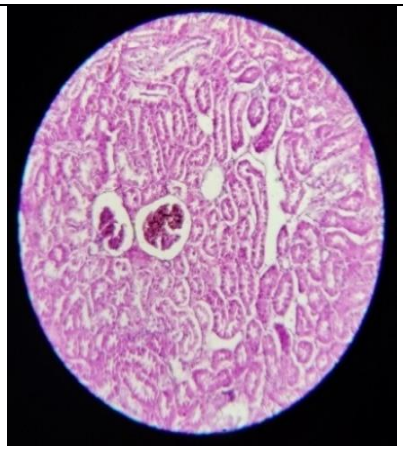
B1- Normal control Pancreas



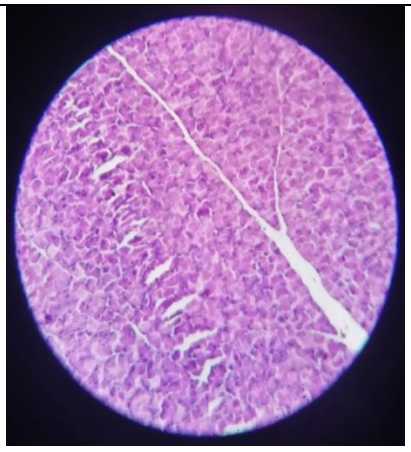
A2- Disease control Kidney



B2- Disease control Pancreas



A3- Standard drug Kidney



B3- Standard drug Pancreas

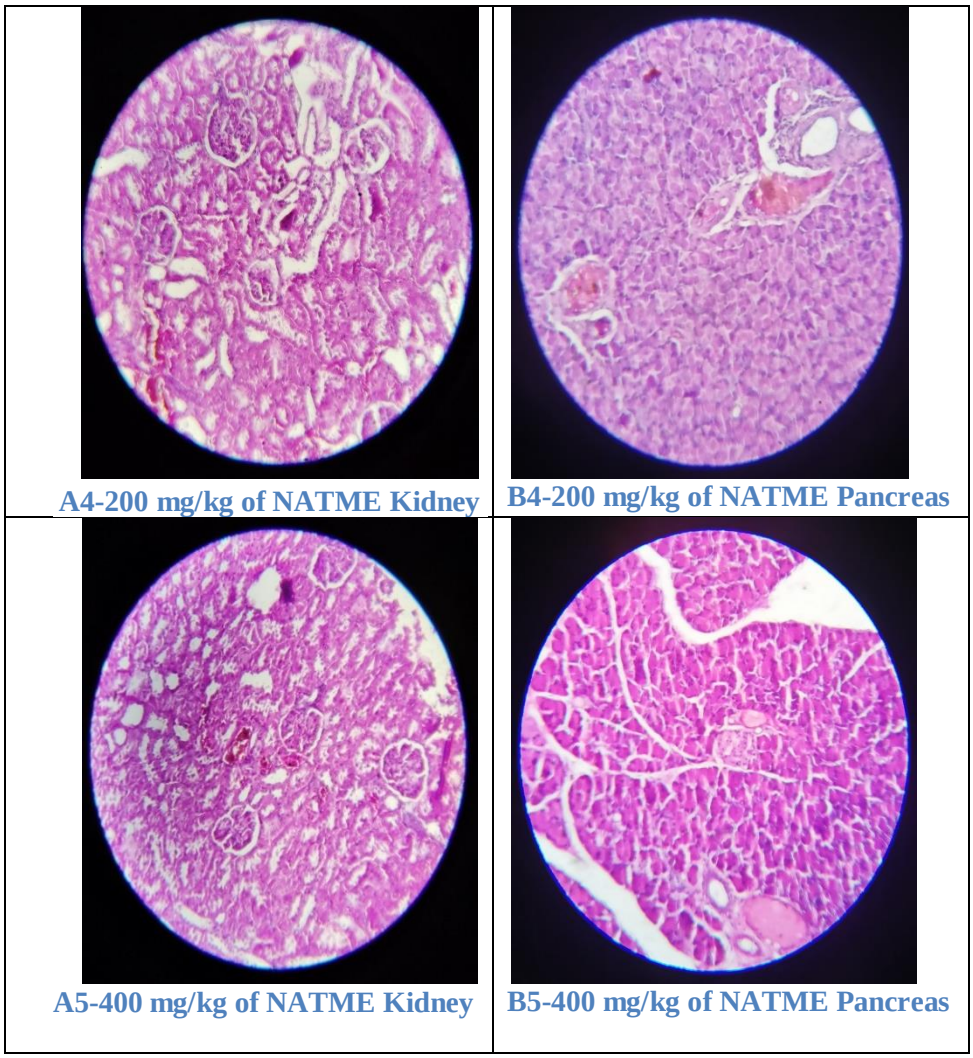


Figure 29: Histopathological sections of the Kidney & pancreas of different group of rat with H&E (Hematoxylin &Eosin) Stains

Histopathology of kidney and pancreas was shown in the figure 29, where thickening of Glomerular membrane is seen in disease control (A2) with respect to normal control (A1). But thickness of glomerular membrane is decreasing in a dose gradient manner in 200 mg/kg (A4), 400 mg/kg (A5) treatment of NATME and Standard drug (A3) as compare to disease control.

B1-β cell in normal control rats, B2- Total destruction of βcells In STZ control rats, B3- regeneration of βcells in standard drug treated rats, B4- remnants of beta cell in NATME (200 mg/kg) treated rats. B5- Gradual regeneration of β cells in NATME (400 mg/kg) treated rats.

8. Discussion:

The present study evidence the antioxidant and antidiabetic activities of the methanolic extract of *Nyctanthes arbor-tristis* L. (NAT) leaves in STZ induced HFD diabetic rats. Type 2 diabetes is commonly treated with a combination of diet restriction, exercise, and oral hypoglycemics. These drugs can control hyperglycemia and glycosuria, but they also raise cardiovascular difficulties and cannot avoid the macrovascular and microvascular complications that come with them in the long term [Chakraborty et al., 2018]. Medicinal plants and their natural compounds have always been important in drug development, serving as a pivot for early treatments [Biswas et al., 2018]. This has become a landmark in the search for alternate treatments to conventional or folk medicine for this ailment. *Nyctanthes arbor-tristis* methanolic extract (NATME) was proven to be safe orally at a dose of 2000 mg/kg in an acute toxicity investigation. The digestive endoenzymes alpha amylase and alpha glucosidase are responsible for the hydrolysis of starch and disaccharides to glucose and the breakdown of long-chain carbohydrates. These enzyme inhibitors are being investigated as potential targets in the treatment of diabetes mellitus [Tiwari et al., 2014]. NATME has in vitro alpha amylase inhibitory activity, and the results imply that NATME can limit monosaccharide absorption, which helps manage blood sugar levels. When rats were given NATME at oral doses of 200 mg/kg and 400 mg/kg b.w. each day for 28 days, the FBG level was considerably lower than in diabetic control rats, indicating antidiabetic efficacy. The concentration of HbA1c in the blood is an important diagnostic measure for determining diabetic status and glycemia. HbA1c level of 6.5 percent is considered a high-risk diabetic state, and it is proportional to FBG content. In diabetic rats, *Nyctanthes arbor-tristis* methanolic extract (NATME) dramatically lowered HbA1c levels. The level of free fatty acidsin the systemic circulation is higher in diabetes patients. The circulating freefatty acids have deleterious effect on the endothelial functions by various pathways and mechanisms which include free radical generation and protein kinase C activation, and thus aggravate dyslipidemia (Goldberg, 2001). Dyslipidemia is defined as a high plasma level of triglyceridesand a low plasma level of cholesterol. Due to little or no scavenging of these extra free radicals in diabetes patients, the generation of free

radicals (reactive oxygen and nitrogen species) increases, resulting in increased oxidative stress [Matough et al., 2012]. Several micro and macrovascular problems of diabetes are caused by oxidative stress (as stated in introduction). When compared to diabetes management, NATME has a considerable potential to lower lipids (triglycerides and total cholesterol).

Endogenous antioxidant mechanisms become less effective in diabetics to scavenge overproduced free radicals, resulting in a variety of pathophysiological alterations in various organs. Because the liver and kidney are key organs for metabolite storage, detoxification, metabolism, and excretion, they are particularly prone to oxidative injury. Oxidative stress, which involves the secretion of cytokines such as tumour necrosis factor, interleukin 1, and interferon c, is the cause of liver and kidney tissue deterioration [Aboonabi et al., 2014].

The beginning of tissue LPO is caused by free radicals such as superoxide (0_2) and hydroxyl (OH). The development of TBA reactive material, i.e. MDA, is commonly used to test LPO. (Biswas et al., 2011) Increased hepatic and renal MDA levels in diabetic rats suggested that endogenous antioxidant mechanisms were impaired. MDA levels in NATME-treated groups were considerably lower than in diabetic control groups, indicating that *Nyctanthes arbor-tristis* methanolic extract (NATME) has a protective effect against oxidative damage at the cellular level. SOD is an important endogenous enzymatic antioxidant that protects organs from free radical oxidative damage. GSH is a nonenzymatic endogenous antioxidant that works with glutathione peroxidase to detoxify hydrogen peroxide radicals and prevent LPO [Forman,2016]. Due to oxidative damage and decreased GSH synthesis in diabetics, SOD and GSH were reduced in the diabetic control group. Hepatic and renal SOD activities, as well as GSH levels, were considerably higher in NATME-treated diabetic rats than in diabetic control rats, indicating a strong presence of endogenous ant oxidative defence at the tissue level.

In comparison to diabetic control rats, histopathological analysis of the pancreas of NATME-treated diabetic rats revealed a dose-dependent increase in cell density, indicating that NATME treatment promotes cellular development. NATME revealed significant antidiabetic effect in albino rats in a dose-dependent manner in the current investigation. *Nyctanthes arbor-tristis* methanolic extract contains flavonoids and alkaloids, according to preliminary phytochemical investigations. Flavonoids are natural antioxidants that have been studied extensively. (Chatterjee, et al., 2012; Bhattacharya, et al., 2018)

9. Summary & Conclusion:

Diabetes is adisorder of carbohydrate, lipid, and protein metabolism ailment caused by decreased insulin production or increased insulin resistance. Herbal diabetes treatments have been used in patients with insulin- dependent and non-dependent diabetes, diabetic retinopathy, diabetic peripheral neuropathy and other diabetes related conditions. The usefulness of botanicals in lowing sugar levels has been validated using many Indian plant species. This thesis is a pioneer of scientific investigations on methanolic extract of the leaves of *Nyctanthes arbor-tristis* in the research field. The introductory part of the thesis is designated by the concept of diabetes. The mature leaves of NAT were collected from Cooch Behar, West Bengal, India. After collection, leaves were cleaned, Shade dried and grind into a coarse powder. The powdered plant material was extracted usingPetroleum ether followed by methanol by Soxhlet extraction. The extract was filtered and evaporated to dryness to get the dry extract that was used in the study. Qualitative phytochemical analysis of Petroleum ether extract revealed the presence of terpenoids, alkaloids,tannins and phenolics compounds whilethe methanolic extract revealed the presence of carbohydrate, flavonoids, alkaloids, tannins phenolics, saponins andglycosides.

According to the HPLC analysis of the methanolic extract of NAT has been found to possess the Kaempferol, Quercetin, Naringenin, Myricetin, Apigenin, Catechin, Ferulic acid and 8 other major flavonoid and phenolic compounds. Though a thoroughly investigations by various high through put instruments like HPTLC, MS, LC-MS/MS are required, in future, to confirm the existence of such Phytochemicals. Science HPLC analysis have shown several picks of unknown compounds it is necessary to isolate such phytochemicals through various isolation techniques which include preparative Column chromatograph preparative thin layer chromatography and characterizing them by the help of mass spectrometry, NMR spectrometry.

The study shows that greater alcohol concentration in the tincture increases acidity of the tincture while storage for longer period of time also slightly increases acidic property. Solid content of the tincture also increases with time while greater alcohol

concentration also imparts diminished solid content. It also shows that specific gravity of the prepared tinctures does not vary substantially with time.

In vitro antioxidant and antidiabetic studies of *Nyctanthes arbor-tristis* methanolic extract (NATME) showed significant Free radical scavenging and alpha-amylase inhibitory activity. Total Phenolic and Flavonoid content of the extract was found 19.457 mg/g and 14.509 mg/g respectively. The extract showed antioxidant activity with an IC_{50} value of 165.27 µg/ml.

The in vivo antidiabetic activity of NATME was assessed by using high fat diet and low dose streptozotocin induced diabetic rat model. NATME were administered orally in a dose of 200 mg/kg and 400 mg/kg, respectively for consecutive 28 days. After 28 days of study rats were sacrificed and all biochemical parameters, tissue antioxidant parameters, immune histopathology were performed at the end of the study.

NATME was showing significant blood glucose lowering effect in fasting blood glucose. NATME also exhibited its antidiabetic effect such as glycaemic control, total protein level, where increased dose convey more effect on these biochemical parameters. *Nyctanthes arbor-tristis* methanolic extract also exhibited significant tissue antioxidant activity with increased dose and of pancreas reveals that NATME have beta cell protective property during diabetic condition. From the present study it can be concluded that NATME have in-vitro and in vivo antioxidant and antidiabetic activity against STZ- induced high fat diet diabetic rat model.

10. Clinical Implications and Future Directions

The findings related to the antioxidant and antidiabetic activities of *Nyctanthes arbor-tristis* leaves hold promise for the development of complementary and alternative therapies for diabetes management. However, further comprehensive research is essential to establish the mechanisms of action, optimal dosages, and potential side effects. Clinical trials involving human subjects are necessary to validate the therapeutic potential of these leaves and their derivatives.

In conclusion, *Nyctanthes arbor-tristis* leaves exhibit noteworthy antioxidant and antidiabetic activities attributed to their rich content of bioactive compounds. These properties offer a multifaceted approach to tackling the intertwined challenges of oxidative stress and diabetes. As scientific exploration advances, the integration of traditional knowledge with modern research could lead to innovative treatments and preventive strategies for diabetes and its associated complications.

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