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Preliminary Phytochemical Analysis, Antimicrobial Activity of Nycthanthes Arbor-Tristis Linn Flowers (Stalk)

Tadikonda Sandeep[1], Valluru Hymavathi[2], Tadiboyina Likhitha Krishna Priya[3], Seggam Naga venkata sai prasanna[4], Pilli Anusha[5], Karumanchi Meghana[6], Mohammed Raheela Sulthana[7], Mudigarla Jyothsna[8]

[1]Assistant professor In Department of Pharmaceutical Analysis at Malineni Perumallu Pharmacy College affiliated with Jawaharlal Nehru Technological University Kakinada (JNTUK)

[2]Assistant professor In Department of Pharmaceutics at Malineni Perumallu Pharmacy College affiliated with Jawaharlal Nehru Technological University Kakinada (JNTUK)

[3,4,5,6,7,8]students Of 4 Th B. Pharmacy At Malineni Perumallu Pharmacy College affiliated with Jawaharlal Nehru Technological University Kakinada (JNTUK)

***Corresponding Author:**

Tadikonda Sandeep

Email ID: sandeeptadikonda1498@gmail.com

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ABSTRACT

Phytochemical Evaluation and Antibacterial Activity of Nyctanthes arbortristis L. Stalk Extracts. Nyctanthes arbortristis L., commonly known as the night-flowering jasmine, is recognized for its medicinal properties. Previous studies have reported the antioxidant activities of the plant, but antimicrobial activities, particularly in its flowers and stalks, remain underexplored. This study focuses on the extraction, phytochemical analysis, and antimicrobial evaluation of the hydroalcoholic extract and its successive fractions from the stalk of *Nyctanthes arbortristis L.* The hydroalcoholic extract of *Nyctanthes arbortristis* stalk was subjected to successive extraction and phytochemical analysis, which included screening for alkaloids, glycosides, flavonoids, and steroids. Chromatographic separation was employed to isolate the fractions, and antibacterial activity was evaluated against gram-positive and gram-negative bacterial strains using standard microbiological methods. Phytochemical analysis revealed the presence of alkaloids, glycosides, flavonoids, and steroids across all extracts. The chromatographic fractions displayed variability in their compound profiles. The hydroalcoholic extract did not show significant antibacterial activity, while the successive fractions exhibited marked inhibitory effects against both gram-positive and gram-negative bacteria. The fractions, which contained higher concentrations of bioactive metabolites, demonstrated enhanced antibacterial properties compared to the hydroalcoholic extract.

Keywords: *Nyctanthes arbortristis, phytochemical analysis, antibacterial activity, hydroalcoholic extract, chromatographic fractions, secondary metabolites, drug discovery.*

1. INTRODUCTION

Use of herbal medicines implies substantial historical use ,and this is certainly true for many products that are available as ‘traditional herbal medicines’ In many developing countries, a large proportion of the population relies traditional practitioners and their armamentarium of medicinal plants in order to meet healthcare needs. Although modern medicine may exhibit side-by-side with such traditional practice, herbal medicines have often maintained their popularity for historical and cultural reasons. Such products have become more widely available commercially, especially in developed countries. In this modern setting, ingredients are sometimes marketed for uses that were never contemplated in the traditional healing systems from which the yemerged. An example is the use of ephedra for weight loss or athletic performance enhancement1. While in some countries, herbal medicines are subject to rigorous manufacturing standards, this is not so everywhere. In Germany, for example, where herbal products are sold as ‘phytomedicines’, they are subject to the same criteria for efficacy, safety and quality as are other drug products. In the USA, by contrast, most herbal products in the marketplace are marketed

and regulated as dietary supplements, a product category that does not require pre-approval of products on the basis of any of these criteria. The use of medicinal plants to treat illness is "phototherapy". Plants are called as "Traditional healers "which provides health care to 80% of population i.e. over a billion people. Plants are used as medicine since time immemorial. The use of medicinal herbs as old as 1500 BC in India. Reviving this ancient, effective and rich medicinal heritage is the right way to improve the health status of our people. Plants from the aspic rawmaterial used for the preparation of drugs in the Indian system of medicine. From l990s, consumers began to view food not only as a means to satisfy hunger , prevent diet-deficiency diseases or to provide essential nutrition (e.g., water protein ,carbohydrate, fat, vitamins and minerals), but also as an important vehicle to keep us healthy[2]. We have begun to believe that a good diet could potentially help us reduce the

risk for a variety of chronic disease such as cancer, heart diseases, osteoporosis, arthritis and age-related macular degeneration[2, 3]. Many components have been evaluated as potential chemo preventive agents in diets. For examples, more than 4000 flavonoids in foods or plant origin were found to have a variety of biological effect, including serving as antioxidants, influencing drug detoxification mechanisms, and altering cell proliferation[4]. It has been reported that there has been an alarming increase in number of diseases caused by synthetic drugs promoting switch over to traditional herbal medicine. The increasing demand for complementary and alternative medicine over the past decade is a true grass-root phenomenon. The world health organization (WHO) estimates that about 80% of the world population currently uses herbs and other forms of traditional medicine to treat theirdiseases5.It has been estimated that only 10-15% of the 7, 50,000 existing species of higher plants have been surveyed for biologically active compounds. Natural products produced by plants, fungi, bacteria, insects and animals have been isolated as biologically active pharmacophores. It is estimated that about 80,000 plant species are utilized by the different system of Indian medicine. Even as we commence the new century with its exciting prospect of gene therapy herbal medicine remain one of the common forms of therapy available to most of world's population[6]. Some of the traditional medicinal practices may be seen strange and magical, others may appear rational and sensible but all of them are attempts to overcome illness and suffering and to enhance quality of life. The traditional approach makes use of material that has been found by trial error over many years in different cultures and systems of medicine[7]e.g. previously drugs like ephedrine, morphine and quinine were used as anti-malarial drugs but recently artemisinin and forskholin are used as anti-malarial drugs. The efficacy of herbal medicine has been confirmed by modern medicinal sciences. Estimates are that, even in America today most of the drugs prescribed by orthodox medical doctors are derived from or based upon natural plant derived materials. The importance of plant medicine popularly known as herbal medicine and their powers to diseases of human being as well documented in ancient literature .In the "Rig Veda "which is considered to be one of the oldest repository of human knowledge written between 4500 and 1600 BC, the medicinal uses of plants are emphasized in the "Atbara Veda "which is known as fourth Veda, the use of plants is documented in greater detail. In the Ayer Veda which is the Up Veda the Atbara Veda, definite properties of plant remedies and their uses are given in detail .In fact AyurVeda is the very foundation of the ancient medical science in India followed by monumental treaties of charka and sushruta[8].Some biologically active plant compounds have found application as drug entities or as model compounds for drug synthesis and semi-synthesis. A survey of current pharmaceutical use revealed that, of the total prescription drugs dispensed,25% are plant derived[9,10]. Plant compounds are highly varied in structure; many area romatic substances, most of which are phenols or their oxygen-substituted derivatives. However, there is an increased attention on extracts and biologically active compounds isolated from plant species used in herbal medicine, due to the side effects and the resistance that pathogenic micro-organisms build against the antibiotics[11]. New compounds inhibiting microorganisms such as benzoin and emetine have been isolated from plants. Of the various pharmaceuticals used in modern medicine, aspirin, atropine, ephedrine, digoxin, morphine, quinine, reserpine and tubocurarine serve as examples of drugs discovered through observations of indigenous medical practices[12]. The antimicrobial compounds from plants may inhibit bacteria by a different mechanism than the presently used antibiotics and may have clinical value in the treatment of resistant microbial strains[13].Plant constituents may be isolated and used directly as therapeutic agents or as starting materials for drug synthesis or they may serve as models for pharmacologically active compounds in drug synthesis. The general research methods includes proper selection of medicinal plants, preparation of crude extracts, biological screening, detailed chemo pharmacological investigations, toxicological and clinical studies, standardization and use of active moiety as the lead molecule for drug design[14]. Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms and are produced by a large variety of organisms including bacteria, fungi, plants, and animals. Many alkaloids are toxic and often have a pharmacological effect, which makes them to be used as medications and recreational drugs. Some alkaloids have a bitter taste[15].

Flavonoids

Flavonoids are derived from 2-phenylchromen-4-one (2-phenyl-1-4-benzopyrone) and are commonly known for their antioxidant activities. Flavonoids, which are widely distributed in plants, fulfill many functions including producing yellow, red or blue pigmentation in flowers and protection from attacks by microbes and insects. Compared to other active plant compounds, they are low in toxicity. Flavonoids are referred to as nature's biological response modifiers because of their inherent ability to modify the body's reaction to allergens, viruses and carcinogens. They show anti-allergic, anti-inflammatory, antimicrobial and anticancer activity[16,17,18].

Saponins

Saponins are the glycosides of 27 carbon atom steroids, or 30 carbon atom triterpenes in plants. They are found in various plant parts like roots, stems, leaves, bulbs, flowers and fruits. They are characterized by their bitter taste and their ability to haemolyze red blood cells. They are used medically as expectorant, emetic and for the treatment of excessive salivation, epilepsy, chlorosis and migraines. They are used in Ayurvedic medicine as a treatment for eczema, psoriasis and for removing freckles. Saponins are believed to be useful in the human diet for controlling cholesterol. Digitalis-type saponins strengthen the heart muscle causing the heart to pump more efficiently[19].Saponins also inhibit cancer tumor growth in animals, particularly, lung andblood cancers, without killing normal cells. Saponins are the plant's immune system acting as an antibiotic to protect the plant against microbes and fungus[20].

Anthraquinones

Anthraquinones are aromatic organic compounds and is a derivative of anthracene. It has the appearance of a yellow or light-gray to gray-green, solid, crystalline powder. It is fairly stable under normal conditions. Anthraquinones naturaly occur in some plants, fungi, lichen and insects, wherein they serve as a basic skeleton for their pigments. Anthraquinones are used in the production of dyes and are also used as a laxative[20,21].

Cardiac glycosides

Cardiac glycosides are drugs used in the treatment of congestive heart failure and cardiac arrhythmia. These glycosides are found as secondary metabolites in several

plants and in some animals. Some of these compounds are used as arrowhead poisons

in hunting.

Antimicrobial activity of plants

Medicinal plants have always been considered as a source for healthy life for people. Therapeutical properties of medical plants are very useful in healing various diseases and the advantage of these medicinal plants are natural[22].In many parts of the world, medicinal plants have been used for its antibacterial, antifungal and antiviral activities for hundreds of years[23,24,25].Researchers are increasingly turning their attention to natural products and looking for new leads to develop better drugs against cancer, as well as viral and microbial infections[26,27,28] Several synthetic antibiotics are employed in the treatment of infections and communicable diseases. The harmful microorganisms can be controlled with drugs and this has resulted in the emergence of multiple drug resistant bacteria and it has created alarming clinical situations in the treatment of infections. In general, bacteria have the genetic ability to transmit and acquire resistance to synthetic drugs which are utilized as therapeutic agents[29,30,28,31].Therefore, actions must be taken to reduce this problem, such as to minimize the use of antibiotics, develop research of resistance among microorganism and to continue studies to develop new antibiotic and immune modulating compounds with diverse chemical structures and novel mechanisms of action, either synthetic or natural to control pathogenic microorganisms because there has also been an alarming increase in the incidence of new and re-emerging infectious diseases[32,33,34]. Antimicrobial studies have shown that Gram-negative bacteria show a higherresistance to plant extracts than Gram-positive bacteria. This may be due to the variation in the cell wall structures of Gram-positive and Gram-negative bacteria. More specifically, Gram-negative bacteria has an outer membrane that is composed of high density lipopoly saccharides that serves as a barrier to many environmental substances including antibiotics[35,36,37,38]. Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of have not been adequately evaluated[39,40]. The Indian flora offers great possibilities for the discovery of new compounds with important medicinal applications in combating infection and strengthening the immune system. The antimicrobial compounds found in plants may prevent bacterial infections by different mechanisms than the commercial antibiotics and therefore may have clinical value in treating resistant microorganism strains13. The indiscriminate use antibiotics has resulted in many bacterial pathogens rapidly becoming resistant to a number of originally discovered antimicrobial drugs25.There is, thus, a continuous search for new antibiotics, and medicinal plants may offer a new source of antibacterial agents. This is indeed very important because Candida albicans, Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli are some of the important human pathogens that have developed resistance to antimicrobials25. Role of Antibiotics in bacterial treatment Antibiotics are the mainstay of bacterial treatment[41].The goal of these drugs is to kill the invading bacteria without harming the host. Antibiotic effectiveness depends on mechanism of action, drug distribution, site of infection, immune status of the host, and resistance factors of bacteria[41,42]. Antibiotics work through several mechanisms. Some (such as vancomycin and penicillin) inhibit formation of bacterial cell walls. Erythromycin, tetracycline, and chloramphenicol interrupt protein synthesis. Still others inhibit bacterial metabolism(sulfa drugs) or interfere with DNA synthesis (ciprofloxacin, rifampin) and/or cell membrane permeability (polymyxin b)[43].When antibiotics were discovered in the 1940s, they were incredibly effective in bacterial infection treatment. Over time, many antibiotics have lost effectiveness against common bacterial infections because of increasing drug resistance[44,45,46,47]. Bacteria may be naturally resistant to different classes of antibiotics or may acquire resistance from other bacteria through exchange of resistant genes. Indiscriminate, in appropriate, and prolonged use of antibiotics have selected out the most antibiotic-resistantbacteria[48,49]. Antibiotic-resistant strains have emerged in

hospitals, long-term care facilities, and communities' worldwide[50]. Human Pathogenic Microbes Microorganisms are very diverse and even though their different cells look similar in morphology and produce similar colonies, it becomes necessary to identify the organisms by their biochemical characteristics that help to properly classify the organisms, causing diseases that kill people, animals and plants. Staphylococcus aureus is a common coloniser of human skin and mucosa. S.aureuscan cause disease, particularly, if there is an opportunity for the bacteria to enter the body. Prescott et al'(2005) states that S. aureusis the most important human staphylococcal pathogen and causes boils, abscesses, wound infections, pneumonia, toxic shock syndrome amongst other diseases. S. aureusis also a pathogen frequently reported to produce food poisoning, which leads to cramps and severe vomiting. Most strains of this bacterium are sensitive to many antibiotics, and infections can be effectively treated. Pseudomonas aeruginosa is an opportunistic pathogen and exploits some breakin the host defenses to initiate an infection. It is a common environmenta lmicroorganism present in water and soil and is notorious for its resistance to antibiotic sand is, therefore, a particularly dangerous and dreaded pathogen. The bacterium is naturally resistant to many antibiotics due to the impermeability characteristics of the outer membrane. Moreover, its tendency to colonize surfaces in a biofilm form makes the cells impervious to therapeutic concentrations of antibiotics. Bacillus subtilis, is a food-poisoning, Gram-positive, facultative, aerobic, sporulating bacteria normally found in soil. B. subtilisis normally considered as being non-pathogenic; but it has been linked to food-borne illnesses, causing diarrhoea, nausea, vomiting, and associated with rice dishes served in oriental restaurants and itsinfection is self-limiting. B. subtilis produces subtilism, which is an extracellular enzyme that catalyzes the breakdown of proteins into polypeptides, resembles trypsinin its action, and has been shown to be a potent occupational allergen .Escherichia coli are usually found in the gastro-intestinal tracts of warm blooded organisms. The most common cause of urinary tract infection in humans is E.coli, causing at least five types of gastro-intestinal diseases in humans. Pathogenicity is generally due to the presence of one or more virulence factors, including invasiveness factors, heat-liable and heat-stable enterotoxins, verotoxins and colonization factors. Pathogenic strains are usually identified by detection of specific virulence factors or of aserotype associated with a virulence factor.E. coli is an emerging cause of food-borne infection which leads to bloody diarrhoea and occasionally to kidney failure. Most cases of the illness have been associated with eating under-cooked, contaminated, ground beef. Person-to-person contact in families and child care centers is also an important mode of transmission if hygiene is inadequate. E. coli infection can also occur after drinking raw milk and after swimming or drinking contaminated water. Klebsiellapneumoniae is a Gram-negative, non-sporulating, facultative, aerobicshaped rod bacterium that is normally found in the human gastro-intestinal tract. An adhesion to a mucosal surface is often the first step in the development of an infection. A survey of the presence of Klebsiellain urban residents, hospital personnel, and newly admitted patients showed that 30-37% of individuals carried Klebsiella, including a 29-35% faecal carriage and a three-to-four-percent throat carriage. Strains of K.pneumoniae and K. Oxytoca which have not acquired any resistance are determined asnaturally resistant to ampicillin and carboxy penicillin but susceptible to other beta-lactam antibiotics. This is due to the production of a chromosomal penicillinase which is inhibited by clavulanicacid .Shigellasonnei is a non-motile, rod shaped nonspore-forming, facultative anaerobic Gram-negative and lactose-fermenting bacterium. S. sonneiis extremely fragile in experimental settings. Its natural habitat is a low pH environment such as the human gastrointestinal tract. Its optimal environmental temperature is 370 C, similar tothe temperature in the human body. Therefore, human's gastrointestinal tract appears to be the only found natural host of S. sonnei known so far. S. sonnei causes anentero bacterium disease called Shigellosis. S. sonneiis spread mostly by means of faecal-oral transmission. Other possible modes of transmission can be from ingestion of contaminated food or water, and subcutaneous contact with inanimate objects and,most rarely, sexual contact. Food prepared by the contaminated person may easily become contaminated with Shigellabacteria. Proteus vulgaris is a rod-shaped, Gram negative bacterium that inhabits the intestinal tracts of humans and animals. It can be found in soil, water and fecal matter. It is known to cause urinary tract infections and wound infections. Patients with recurrent infections, those with structural abnormalities of the urinary tract, those who have had urethral instrumentation, and those whose infections were acquired in the hospital have an increased frequency of infection caused by Proteus and other organisms (eg,Klebsiella, Enterobacter, Pseudomonas, Enterococci, Staphylococci) .Candida albicans is the most common organism implicated in fungal infections, which is found in the human digestive tract, mouth, and genital region[62]. Under normal circumstances, levels of Candida are controlled by beneficial bacteria. However, if the bacteria-fungus balance is upset, by the use of antibiotics or if the immune system is compromised, an overgrowth of Candida can occur, resulting in infection. Fungal overgrowth is encouraged by certain pH levels and the availability of sugar . People with the right conditions for fungal infection, such as a high sugar diet, are at higher risk. Also, Candida infections can be spread to vulnerable people with depressed immune systems who are in the hospital, where the fungus is commonly found on thehands of care givers .

Cryptococcus neo formans yeast lives in both plants and animals. C.neoformans grows as yeast (unicellular) and replicates by budding and does not exist in a hyphae or pseudohyphae form. C. Neoformans causes lung infection. However, fungal meningitis, occur as a secondary infection for AIDS patients. Infection with this fungus are rare in those with fully functioning immune systems.

2. PLANT DESCRIPTION

Geographical Source : Nyctanthes arbor-tristis Linn is native to India, distributed widely in sub-Himalayan regions and

southward to Godavari. It is also distributed in Bangladesh, Indo-Pak subcontinent and South-East Asia, tropical and sub-tropical South East

Asia. It grows in Indo-Malayan region and distributed across Terai tracts as well as

Burma and Ceylon. It tolerates moderate shade and is often found as undergrowth in

dry deciduous forests. Nyctanthes arbor-tristis Linn is a small sacred ornamental tree known across the country for its fragrant white flowers. Nyctanthes arbor-tristis Linn is commonly known as Night Jasmine or Parijata.

1: Flowers Figure of Nycthanthes Arbor Tristis Linn

Botanical description: Nyctanthes arbor-tristis Linn is a large shrub growing to 10 m tall, with flaky grey bark ,stiff whitish hair, young branches and rough leaves. The flowers are fragrant, with a five- to eight-lobed white corolla with an orangeredcentre; they are produced in clusters of two to seven together, with individual flowers opening at dusk. Calyx is 6-8mm long, narrowly campanulate, hairy outside, glaborous inside lobed ciliated. corolla glaborous and is more than 13 mm long ,tube is 6-8mm long orange colour about equalling the limbs lobes are white and unequally obcoradate and cuneate the leaves are opposite ,simple 6-12 cm long and 2-6.5cm broad, with an entire margin . the fruit is a flat brown heart shaped to round capsule 2cm diameter , with 2 sections each containing a single seed . They are long and broad obcordate or nearly orbicular, compressed, 2 celled .

Traditional uses: The flowers are gathered for religious offerings and to make garlands. The orange heart is used for dyeing silk and cotton, a practice that started with Buddhist monks whose orange robes were given their colour by this flower. The Parijata is regarded in Hindu mythology as one of the five wish-granting trees of Devaloka. Different parts of *Nyctanthes arbor-tristis*Linn are known to possess various ailments by tribal people of India esp. Orissa and Bihar along with its use in Ayurveda, Sidha and Unani systems of medicines. It is used in several ailments including sciatica, rheumatism, gout and other joint diseases.

Flowers: The flowers are used as stomachic, carminative, astringent to bowel, antibilious, expectorant, hair tonic and in the treatment of piles and various skin diseasesand in the treatment of ophthalmic purposes. The bright orange corolla tubes of the flower contain a coloring substance nyctanthin, which is identical with ά-Crocetin ($C_{20}H_{24}O_4$) from Saffron. The corolla tubes were formerly used for dyeing silk, sometimes together with Safflower or turmeric.

Stem: Traditionally the powdered stem bark is given in rheumatic joint pain, in treatment of malaria and also used as an expectorant[49]. The bark is used for the treatment of snakebite and bronchitis. The stem bark pounded with *Zingiber officinale* and *Piper longum*is boiled in water and the resultant liquid is taken for two days for the treatment of malaria in Orissa. The resulting paste on mixing with Arjuna bark is rubbed on the body to treat internal injury and for joint broken bones.

Leaves: The leaves of *Nyctanthes arbor-tristis* Linn. are used extensively in Ayurvedic medicine for the treament of various diseases such as sciatica, chronic fever, rheumatism, and internal worm infections, and as a laxative, diaphoretic and diuretic. Leaves are used in cough. Leaf juice is mixed in honey and given thrice daily for the treatment of cough. Paste of leaves is

given with honey for the treatment of fever, high blood pressure and diabetes. Juice of the leaves is used as digestives, antidote to reptile venoms, mild bitter tonic, laxative, diaphoretic and diuretic. Leaves are also used in the enlargement of spleen. The leaf juice is used to treat loss of appetite, piles, liver disorders, biliary disorders, intestinal worms, chronic fever, obstinate sciatica, rheumatism and fever with rigors. The extracted juice of leaves acts as a cholagogue, laxative and mild bitter tonic. It is given with little sugar to children as a remedy for intestinal ailments. In several cases, it has been found to act efficaciously for malaria fever. The decoction of leaves is extensively used by Ayurvedic physicians for the treatment of arthritis, obstinate sciatica, malaria, intestinal worms and as a tonic, cholagogue and laxative. The expressed juice of leaves (10ml BD X 5days) is a native remedy for intermittent fever.

Seeds: The seeds are used as anthelmintics and in alopecia. It is antibilious and an expectorant, and is also useful in bilious fevers. The powdered seeds are used to cure scurfy affections of scalp, piles and skin diseases.The indigenous people of Chittoor district Andhra Pradesh (India) widely use the whole plant for treatment of cancer, root for fever, sciatica, anorexia; bark as expectorant.

Chemical constituents: Recent researches reported the olaistion of polysaccharides, iridoid glycosides, henylpropanoid glycoside, ß-sitosterol, ß-amyrin, hentri-acontane, benzoic acid, glycosides, nyctanthoside-a iridoid,nyctanthic acid, Friedelin and lupeol and oleanolic acid and 6ß-hydroxylonganin and iridoidglucosidesarborsides A, B and C, alkaloids, Phlobatanins, terpenoids and cardiac glycosides. Iridoidglucosides (arbortristosides-A, B, C) and 6ßhydroxyloganin has also been isolated from this plant.

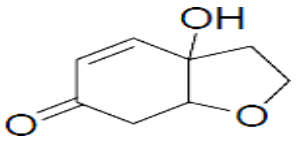

Figure .2: 4-hydroxy hexahydrobenzofuran-7-one

Khatune et al., in 2003 isolated 4-hydroxy hexahydrobenzofuran-7-one from the chloroform extract of flowers of *Nyctanthes arbor-tristis*. It was found to be antibacterial, larvicidalPhytochemical studies revealed the presence of tertiary alkaloids mainly 7- (alpha- anilino-p-nitrobenzyl)-8-quinolinol and quarternary alkaloids belonging to protoberberines and aporphines. Gadgoli et al., isolated carotenoid aglycone Ag- NY1 from the orange coloured tubular calyx of flowers of *Nyctanthes arbor-tristis*, which exhibited a good membrane stabilising activity as compared to the corresponding glycoside crocin.Three carotenoid glucosides were also isolated from the corolla tubes of the plant. A new phenylpropanoid glycoside (nyctoside A) has been isolated from the seeds and desrhamnosylverbascoside from the leaves. Isolation of a cyclohexylethanoid, rengyolone as an antimalarial principle. A new iridoidglucoside, 6-O-trans-cinnamoyl-7- O-acetyl-6β-hydroxyloganin and three known iridoidglucosides, arborside-C, 6β- hydroxyloganin and nyctanthoside were also isolated from the same plant.

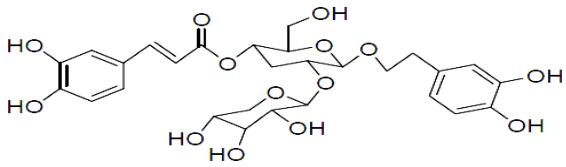

Figure .3: Nyctoside A51

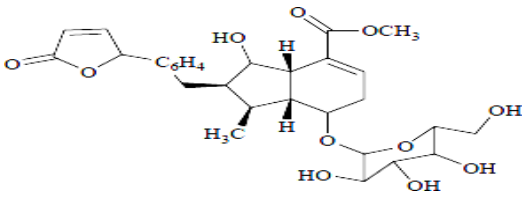

Figure .4: Arborside C51

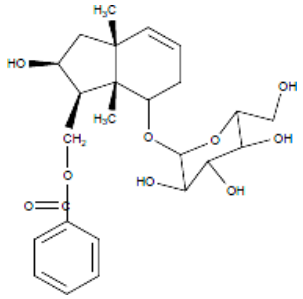

Figure .5: Arborside D51

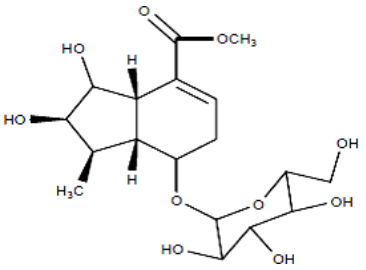

Figure .6: 6-_-hydroxylogan

Nyctantic acid, friedelin, beta-sitosterol and oleanolic acid isolated from leaves were used for antiviral activityThe leaves have been found to contain tannic acid, methyl salicylate, amorphous glucosides, mannitol, resin, ascorbic acid, carotene, andtraces of a volatile oil. Iridoidglucosides, arbortristosides-A, arbortristosides-B, arbortristosides-C and 6β-

hydroxyloganin isolated from *Nyctanthes arbor-tristis*.

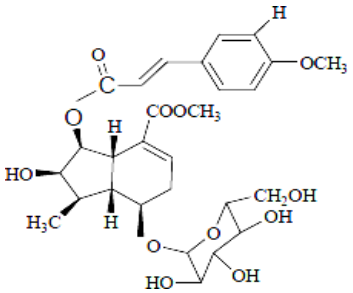

Figure .7 Arbortristoside-A51

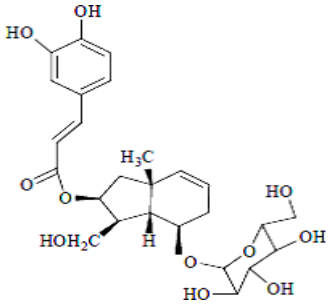

Figure .8: Arbortristoside-B51

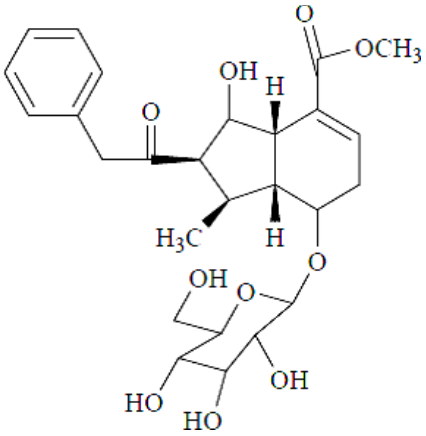

Figure .9: Arbortristoside-C51

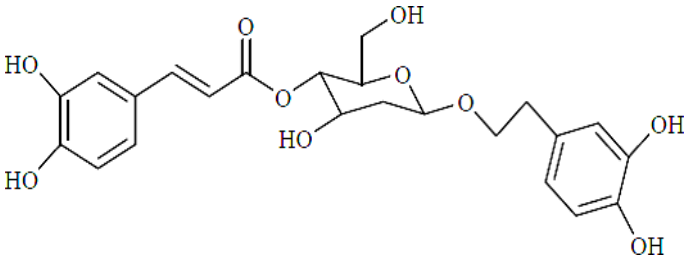

Figure .10: Desrhamnosylverbascoside51

Seed kernels yield 12-16% of the pale yellow brown fixed oil, consisting of fixed oil containing glucosides of linoleic, oleic, lignoceric, stearic, palmitic acid and b- sitosterol. On keeping the oil several weeks a tetracyclic triterpenoid acid named nyctanthic acid is deposited. Flowers contain essential oils, coloring matter (nyctanthin), m annitol, tannin and glucose. Its roots are composed of alkaloids, tannins and glucosides.

Toxicological Profile: Das et al., out acute toxicity studies carried on the water soluble fraction of ethanolic extract of different parts of *Nyctanthes arbor-tristis*Linn at doses of 400 mg/kg to 2000 mg/kg i.p. by staircase method *Nyctanthes arbor-tristis*(NAT) leaves has been shown to possess anti-arthritic properties. In addition, decoction of the leaves of NAT has been also shown to possess hepatoprotective, anti-leishmanial, anti-viral, and anti-fungal activities, as well as analgesic, antipyretic, and ulcerogenic activities.

Antispasmodic activity: Ethanolic extract of fresh flowers and dried leaves, stem and bark of *Nyctanthes arbor-tristis* Linn was tested for its antispasmodic activity using guinea pig ileum. It was found to inhibit contractile response of acetylcholine.

Antihelmintic activity: Ethanolic extract of fresh flowers and dried leaves, stem and bark of *Nyctanthes arbor-tristis* Linn was tested for its antihelmintic activity using piperazine citrate as a standard. The antihelmintic activity was studied on the basis of inhibition of contractile effect of acetylcholine by various dilutions of this extract. It was found that ethanolic extract of seeds and flowers possess more potent antihelmintic activity than that of bark and leaves but is less than that of piperazine citrate. Also, these extracts potentiated the antihelmintic activity of atropine, which might be due to the inhibition of motility by relaxing and depressing responsiveness to contractile action of acetylcholine·

3. CYTOTOXIC EVALUATION

That 4hydroxyhexahydrobenzofuran-7-one isolated from chloroform extract of flowers of Nyctanthes arbor-tristisLinn is not carcinogenic as it inhibits EAC cell growth only by43.27 %. Also it was found that this compound possesses no adverse effect on central nervous system.

Anti-inflammatory activity: The water soluble portion of the alcoholic extract of the leaves of *Nyctanthes arbor-tristis*Linn was screened for the presence of anti- inflammatory activity. NAT inhibited the acute inflammatory oedema produced by different phlogistic agents, viz. carrageenin, formalin, histamine, 5- hydroxytryptamine and hyaluronidase in the hindpaw of

rats. The acute inflammatory swelling in the knee joint of rats induced by turpentine oil was also significantly reduced. In subacute models, NATwas 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

· Water- soluble fraction of ethanolic extract of leaves of *Nyctanthes arbor- tristis*Linn has been screened for the anti-inflammatory activity. It was found to significantly inhibit acute inflammatory oedema produced by carragenan, formalin, histamine, 5-hydroxytryptamine and hyalouronidase in hindpaw of rats. It also reduced acute inflammatory swelling in the knee joint induced by turpentine oil.Das et al., in 2008 isolated arbortristoside-A isolated from the ethanolic extract of seeds of *Nyctanthes arbor-tristis*Linn. Arbortristoside-A was found to possess significant and dose-dependent anti-inflammatory and antinociceptive activity. It seems arbortristoside-A inhibited the histamine, serotonin and carrageenan-induced edema suggesting its inhibiting effect on carrageenan, arachidonic acid, histamine and serotonin-induced edema suggesting its anti-inflammatory activity may be due to the inhibiting effect of prostaglandin, histamine and serotonin. The analgesic activity of arbortristoside-A may be due to the inhibition of the action of prostaglandin

Immuno-stimulant: Oral administration of ethanolic extract of *Nyctanthes arbor- tristis*Linn at dose of 50, 100, 150, 200 mg/kg significantly enhanced circulating antibody titre when challenged with SRCs and heat-killed Salmonella antigens. The chronic administration significantly enhanced total WBC count and potentiated DTH reaction. It was concluded that the extract possesses immune-bioactivies.

Antidiabetic: The ethanol extract of stem bark of *Nyctanthes arbor-tristis*linn exhibited dose-dependent antidiabetic property. The levels of serum cholesterol and triglycerides were raised in diabetic rats but which were lowered significantly with the treatment of stem bark of *Nyctanthes arbor-tristis*linn. It indicates that the ethanol extract of stem bark of *Nyctanthes arbor-tristis*linn is more useful in the treatment of diabetes as it has hypolipidemic effect. Ethanolic extract of *Nyctanthes arbor-tristis*Linn significantly inhibited TBARS in liver. It was found to possess antioxidant and antidiabetic effect. Administration of *Nyctanthes arbor-tristis*Linn leaves and flower chloroform extracts (50, 100 and 200 mg/kg) orally for 27 days caused a significant reduction in LPO, SGPT, SGOT, AlkPhos, cholesterol and triglyceride levels on extracts treated STZ diabetic rats, compared to diabetic control rats. Further, *Nyctanthes arbor- tristis*Linn extract treated diabetic rats showed significant increase in SOD and CAT enzymatic antioxidant activity when compared to diabetic control rats. The administration of the extracts and glibenclamide (10 mg/kg) improved the activity of both enzymatic and non-enzymatic antioxidants and lipid profile in STZ-induced diabetic rats.

Hepatoprotective activity: Liver diseases have become a major stumbling block to twentieth century medicine. Capacity for regeneration of the liver is considerable and damage is usually extensive before it is evident. The effects of liver disease are seen when; regeneration of hepatocytes does not keep pace with damage leading to hepatocellular failure.Administration of alcoholic and aqueous extracts of the leaves of *Nyctanthes arbor-tristis*Linn protect the liver from toxic effects of carbontetrachloride by reducing the elevated levels of Serum glutamate pyruvate transaminase, Serum glutamate oxaloacetate transaminase and serum bilirubin(total and direct). Results revealed that both the alcoholic and aqueous extracts showed significant hepatoprotective activity by reducing the elevated levels of biochemical parameters at a dose of 500 mg/kg body weight.Methanolic extract of leaves of *Nycotanthes arbor-tristis*Linn exhibited significant hepatoregenerative potential in acetaminophen-induced hepatic damage. It acts by protecting against membrane fragility and by preventing decline in glutathione levels. The aqueous extracts of the leaves and seeds were proved to have antihepatotoxic activity against CCl4 inducedhepatotoxicity. The ethanolic and aqueous extract of leaves of *Nyctanthes arbor-tristis*Linn has been found to be hepatoprotective at a dose of 500mg/kg ·

Antibacterial: Infectious diseases are world's leading cause of premature death. Resistance to antimicrobial agents is emerging in a wide variety of pathogens and multiple drug resistance is becoming common in diverse organisms such as *Staphylococcus aureus, Staphylococcus epidermis, Salmonella typhi, Salmonella paratyphi A.* Methanolic extract of leaves of *Nyctanthes arbor-tristis*Linn exhibited significant antibacterial activity against *Staphylococcus aureus, Staphylococcus epidermis, Salmonella typhi, Salmonella paratyphi A* with MIC value ranging between 1-8 mg/ml.

Antimicrobial: The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immune compromised patients in developing countries and many infectious microorganisms are resistant to synthetic drugs. The stem bark extracts of the plant were tested for their in vitro antimicrobial activity by cup plate method. The test organisms were *Staphylococcus aureus, Micrococcus luteus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Candida albicans*and *Aspergillusniger.* The zone of inhibition and Minimum Inhibitory Concentration (MIC) of the extracts were determined and compared with the standard drugs ciprofloxacin and fluconazole. The chloroform extract was found to have both antibacterial and antifungal activity whereas thepetroleum ether and ethanol extracts possess only antibacterial activity.

Antileishmanial activity: Tandon et al., in 1991 reported that iridoidglucosides (arbortristosides A, B , C, and 6beta-hydroxy-loganin) isolated from *Nyctanthes arbor-tristis*Linn possess antileishmanial activity by assessing in vitro (against amastigotes in macrophage cultures) and in vivo (in hamsters) test systems

Antiviral activity: The ethanolic extracts, various fractions and two pure compounds isolated from *Nyctanthes arbor-tristis*Linn were tested against *Encephalomyocarditis Virus* (EMCV) and *Semliki Forest Virus* (SFV). There was pronounced in-vitro virus inhibitory activity with the ethanolic and n-butanol fractions as well as with the pure compounds arbortristoside A and arbortristoside C. In addition, ethanolic extracts and n-butanol fraction protected EMCV infected mice to the extent of 40 and 60% respectively against SFV at a daily dose of 125 mg/kg body weight.

Prevention of lung injury: Liver injury was induced in Swiss mice through inhalation exposure to silica particles (< 5 mu) using a Flow Past Nose Only Inhalation Chamber at the rate of -10 mg/m3 respirable mass for 5 h. Inhalation of silica increased the level of tumor necrosis factor-alpha (TNF-alpha), and of the 66 and 63 kDa peptides in the BAL fluid in comparison to sham-treated control. Pre- treatment of silica exposed mice with *Nyctanthes arbor-tristis*Linn leaf extract significantly prevented the accumulation of TNF-alpha in the BAL fluid, but the 66 and 63 kDa peptides remained unchanged. The extract was also found to be effectivein the prevention of silica-induced early fibrogenic reactions like congestion, edema and infiltration of nucleated cells in the interstitial alveolar spaces, and thickening of alveolar septa in mice lungs.

CNS depressant action: The leaves, flowers, seeds and barks (600 mg/kg) of *Nyctanthes arbor-tristis*Linn showed significant and dose-dependent prolongation of onset and duration of sleep and was found to cause decrease in dopamine and increase serotonin level. From which it can be concluded that the CNS depressant activity of the ethanol extracts of seeds, leaves and flowers may be due to the decrease in dopamine and increase in serotonin level.

PROCEDURE:

4. MATERIAL AND METHODS

Plant material: Fresh and healthy Flowers of Nycthanthes arbor-tristis Linn were obtained from local growers of Guntur. The sample specimen was identified based on the taxonomical characteristics. The Flowers were dried under shade at 28^0C for 48 h, powdered to 100- 120 mesh in an apex grinder [Apex Constructions, London] and used for extraction.

CHEMICALS

Solvents *viz*., ethanol, hexane, benzene, ethylacetate, chloroform, methanol was of AR grade from Merck (Mumbai, India), distilled water from reverse osmosis (R.O) system.

Extraction Procedure

A Soxhlet extractor is a piece of laboratory apparatus invented in 1879 by Franz von Soxhlet

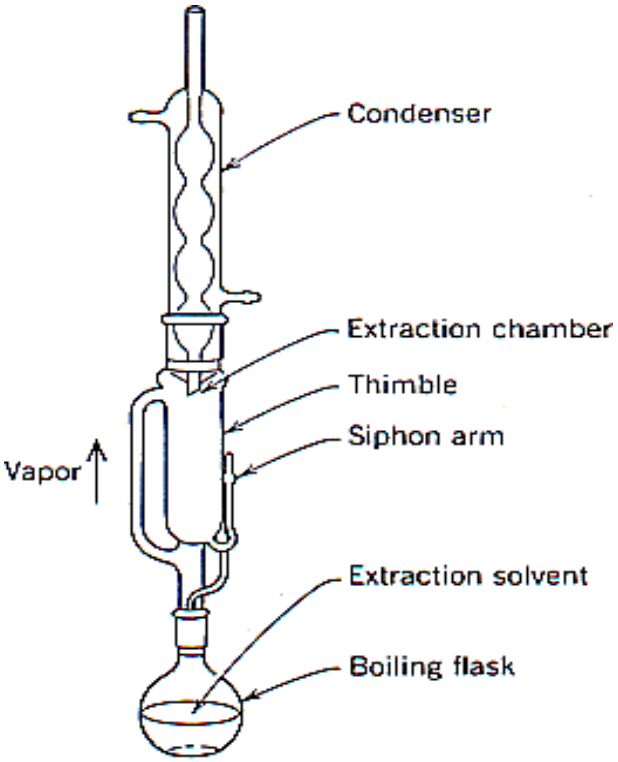

Figure .11: Assembly of Soxhlet apparatus

Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent Normally a solid material containing some of the desired compound is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor is placed onto a flask containing the extraction solvent. The Soxhlet is then equipped with a condenser. The solvent is heated to reflux. The solvent vapour travels up a distillation arm and floods into the chamber housing the thimble of solid. The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound will then dissolve in the warm solvent. The process is maintained for 48 hours for each solvent. After extraction the solvent is removed, typically by means of a rotary evaporator, yielding the extracted compound. Hydroalcoholic was used as solvent in this method of extraction etc.

5. PREPARATION OF HYDRO ALCOHOLIC EXTRACT

Extraction was carried out according to the method of Okigbo *et al*. (2005). Fifty grams each of the Fresh flowers and powdered material of stalk and petals were kept in the thimble and extracted using 250 ml of hydro-alcoholic solvents in soxhlet apparatus for 8 – 10 hours at 45 ± 2^0C. The extract was filtered with sterile Whatman No. 1 filter paper to remove all unextractable matter, including cellular materials and other constitutions that are insoluble in the extraction solvent into a clean conical flask. The entire extract was concentrated to dryness using rotary flash evaporator under reduced pressure [Heidolph] for the evaporation of the solvents. The evaporated extracts were preserved at 4^0C in airtight bottles until further use. The dried extract was re-dissolved in ethanol to yield solutions containing 50, 100, 200 and 300mg of extract per ml solvent

6. PARTITIONING OF HYDRO ALCOHOLIC EXTRACT

After solvent removal, the extract was successively partitioned between Hexane, Chloroform, Ethylacetate and water. The fractions were concentrated following the same procedure using rotavapor (Natrori *et al*., 1981). These fractions along with the crude methanol part were sterilized by passing through 0.22µM - syringe filter before studying their biological activity After the extraction and concentration of plant material by different solvents like hexane, chloroform, ethylacetate, ethanol, methanol and water for several days.

Isolation of phytoconstituents:

From each plant, the extracts that produced significant anti diabetic, activities were subjected to column chromatography for isolation of bioactive molecules which may be responsible for the activity as mentioned in aimed to carry out

7. CHROMATOGRAPHIC TECHNIQUES

The Column chromatography was done by standard procedure silica gel was used as adsorbent. The column was eluted with n-hexane, benzene, chloroform, ethyl acetate, ethanol, methanol and finally with water.

Isolation: The ethyl acetate extract was fractionated initially with n-hexane, and then chromatographed over silica gel column. The column was eluted with increasing polarity using different solvents. The resulting dark brownish extract was subjected to column chromatography. Approximately 5g of crude extract was placed in gravity column for isolation of chemical constituents. 5g of residue was dissolved in hexane and adsorbed on 15g of silica gel (100-200 mesh) contained in a column chromatography. Weight of extract= 5g, Weight of silica gel= 100g, Volume of each fraction= 100ml

Column length= 50.5cm, Column diameter= 5cm

Phytochemical evaluation

Table No 1: Phytochemical Evaluation of Flowers of Nycthanthes Arbor-Tristis of hydro-alcoholic extract

S.no	Experiment	Observation
I	**TEST FOR STEROIDS:**	
1	**Liebermann Burchard Test:** To the extract add glacial acetic acid, acetic anhydride &two drops of conc. H_2SO_4	The solution becomes red, then blue and finally bluish green.
2	To the sample add 2ml of chloroform& 1ml of conc. H2SO4	The solution becomes red colour
II	**TEST FOR FLAVONOIDS:**	
Shinoda Test:		

1	To the extract, a few magnesium turnings and 1-2 drops of conc. HCl were added	formation of red colour
2	To the extract, 10% sodium hydroxide or ammonia was added,	dark yellow color
3	To small quantity of extract add lead acetate solution,	yellow color
III	**TEST FOR GLYCOSIDES:**	
1	**Legal's test:** To the extract add 1ml of pyridine, 1ml of sodium nitroprusside	pink colour was not observed
2	**Keller-killani test:** To the extract add 2ml glacial acetic acid,1drop of 5%ferric chloride and 1 drop sulphuric acid	Reddish brown colour at the junction indicates
IV	**TEST FOR PHENOLS:**	
1	**Ferric chloride test**: To the extract, few drops of 10 %aqueous ferric chloride were added.	A blue or green color is observed
2	**Test with dil. nitric acid**: To the extract, few drops of dil.nitric acid were added.	reddish to yellow colour is observed
3	**Test with acetic acid:** To the extract, few drops of acetic acid were added.	A white colour precipitate is observed
V	**TEST FOR SAPONINS:**	
1	**To 1 ml of the extract, 5 ml of water was added and the tube was shaken vigorously.**	Copious lather formation indicates
VI	**TEST FOR ALKALOIDS:**	
1	**Dragendroff's Test: In a test tube containing 1ml of extract, few drops ofdragendroff's reagent was added**	orange color not Appeared
2	**Wagner's Test: To the extract, 2ml of wagner's reagent was added**	the reddish brown precipitate not formed indicates

Screening of Antimicrobial Activity

Preparation of stock solution

The calculated amount of each dried stalk extract obtained from different solvents were dissolved in a calculated volume of 1:1 ethanol/ DMSO solvent (used as control) in the ratio of 1mg/ml to make stock solution and were used for determination of Zone of inhibition against different bacteria.

Culture media

Culture medium used for growth of bacteria was Nutrient broth, Nutrient Agar, Mueller Hinton Agar purchased from Hi Media.

Bacterial strains

Bacterial standard strains of gram-positive, gram negative bacteria *Bacillus subtilis*, and *Escherichia coli* were used during the investigation.

Preparation of stock culture

The stock culture of each organism was prepared by taking two nutrient agar slants and sub culturing each confirmed test organism aseptically. One set slant was kept as stock culture and another as working set. The cultures of bacteria in their

appropriate agar slants were stored at 4°C and used as stock cultures. One counter glycerol stock was also maintained at -20oC temperature.

Inoculums' preparation

The test microorganisms were maintained at 4°C on nutrient agar slants. Active cultures for each bacterial were prepared by transferring a loopful of cells from the stock cultures to test tubes of nutrient broth. The inoculated tubes were incubated without agitation for 24 h at 37^0C and 25^0C respectively. The cultures were diluted with fresh nutrient broth to achieve optical densities corresponding to 10 -5 colony forming units per milliliter (cfu/mL)

Agar well diffusion Assay

The agar well diffusion method was used to test the anti-microbial effect hydroalcoholic extract, successive extract and isolated fractions of *Nyctanthes arbortristis* stalk extracts in different stages of germination. All media plates (9 cm in diameter) wereprepared with nutrient agar. After agar solidification, the well (7 mm in diameter) was cut from the agar to produce a total of two wells per each agar plate. For test, a dose of each extract (500 µg/well) were prepared using 99.5% analytical Dimethyl Sulphoxide (DMSO) as an organic solvent. One hundred µL of each diluted microbial suspension were inoculated on nutrient agar plates using sterile cotton swab. The inoculums were allowed to dry for 5 min. Then, 100 µL of each extract solution, blank (DMSO) and positive control was added separately to each well of agar plate and allowed to diffuse at room temperature for 15-20 min. After incubation at 370C/250C for 24 h, all plates were examined for any zones of growth inhibition and the diameter of these zones was measured. The assay was repeated three times for each extract. The anti-microbial effect was recorded as the mean diameter of the resulting inhibition zones of growth in millimetre.

8. RESULTS AND DISCUSSIONS

The preliminary phytochemical analysis of the hydroalcoholic extract of N*ycthanthes arbortristis* stalk and successive extraction of N*ycthanthes arbortristis* stalk extract revealed the presence of alkaloids, tannins, glycosides, esters, sugars, terpenoids, acids and resins as presented .The tests were conducted for the stalk of *Nycthanthes arbor-tristis* Linn belongs to family oleaceae. The tests were shown for extracts of stalk and successive extraction of stalk in various solvents. The stalk was shown positive tests for Cardiac glycosides, Flavonoids, Alkaloids and Steroids in *Nycthanthes arbor-tristis* Linn. Tannins, terpenoids and Anthraquinones was absent in stalk of *Nycthanthes arbor-tristis* Linn. The tests were conducted for successive fractions of *Nycthanthes arbor-tristis* were and were shown positive tests for hexane, chloroform, ethylacetate for alkaloids, cardiac glycosides, flavonoids and steroids and water shown positive test for cardiac glycosides. The successive extracted fractions were isolated by using column chromatography. The compounds were eluted for fractions in column chromatography by increasing polarity of solvents and these fractions were depicted

These fractions were shown waxy and precipitate: The tests was conducted for successive fractions were isolated by column chromatography in various solvents of Nycthanthes arbor-tristis stalk Successive fractioned ethylacetate solvent by column chromatography fractions were depicted hexane, hexane: chloroform, chloroform: ethylacetate (50:50), ethylacetate, ethylacetate: ethanol (50:50), were shown positive tests for alkaloids, cardiac glycosides, flavonoids and steroids. Ethanol was shown positive test for cardiac glycosides, flavonoids and water shown positive test for cardiac glycosides. Successive fractioned chloroform solvent by column chromatography fractions were depicted hexane, hexane : chloroform (50:50), chloroform, chloroform : ethylacetate (50:50) were shown positive tests for alkaloids, cardiac glycosides, flavonoids and steroids and water shown positive test for cardiac glycosides

Table. 2: Preliminary Phytochemical constituents of Hydroalcoholic extract of Nyctanthes arbor-tristis Stalk and Successive Extracted Solvents of Nyctanthes arbor-tristis Stalk

Phytoconstituents	Name of the Test	Result				
		Hydroalcoholic Crude stalk extract	Hexane	Chloroform	Ethylacetate	Water
Alkaloids	Hager's Test	+++	+++	+++	+++	---
Anthraquinones	Chloroform Layer Test	---	---	---	---	---
Cardiac Glycosides	Killer-Killani's Test	++	++	++	++	++

Flavonoids	**Ammonia Test(Modified)**	++	++	++	++	---
Reducing Sugars	**Fehling's Test**	---	---	---	---	---
Saponins	**Frothing Test**	---	---	---	---	---
Steroids	**Salkowski Test**	+++	+++	+++	+++	---
Tannins	**Ferric Chloride Test**	---	---	---	---	---
Terpenoids	**Salkowski Test (Modifed)**	---	---	---	---	---

Key: +++ = Highly present; ++ = Moderately present; + = Slightly Present; --- = Absent

Table no.3: Isolated compounds of Ethylacetate extract of Nyctanthes arbor- tristis

Sl. No	Fraction No	Eluent composition	Colour of the compound	Compounds isolated
1	1	Benzene	Light Yellow	Waxy substance
2	2	Hexane	Nil	Nil
3	3	Hexane: Chloroform	Greenish Brown	Waxy substance
4	4	Chloroform	Nil	Nil
5	5	Chloroform : Ethylacetate (50:50)	Brown	Waxy substance
6	6	Ethylacetate	Nil	Nil
7	7	Ethylacetate: Ethanol (50:50)	Dark brown	Waxy substance
8	8	Ethanol	Nil	Nil
9	9	Water	yellow	precipitate

Table no.4: Isolated compounds of Chloroform extract of Nyctanthes arbor-tristis

Sl. No	Fraction No	Eluent composition	Colour of the compound	Compounds isolated
1	1	Hexane	Yellowish Brown	Waxy substance
2	2	Hexane: Chloroform (50:50)	Yellowish	Waxy substance
3	3	Chloroform	Brownish yellow	Waxy substance
4	4-8	Chloroform : Ethylacetate (50:50)	Yellowish	Waxy substance
5	9-11	Ethylacetate	brownish	Waxy substance

6	12-14	Ethylacetate: Methanol (50:50)	Yellowish brown	Waxy substance
7	15-16	Methanol	Very light yellow	Precipitate
8	17	Methanol: Water (50:50)	----	----
9	18	Water	Yellow	Waxy substance

Table no.5: Isolated compounds of Hexane extract of Nyctanthes arbor-tristis

Sl. No	Fraction No	Eluent composition	Colour of the compound	Compounds isolated
1	1	Benzene	No Colour	Not obtained
2	2	Benzene: Hexane (50:50)	No Colour	Not obtained
3	3	Hexane	No Colour	Not obtained
4	4	Hexane: Chloroform (50:50)	Yellowish Brown	Waxy Substance
5	5-7	Chloroform	Light yellow	Semisolid
6	8-9	Chloroform : Ethylacetate (50:50)	Yellowish Brown	Waxy Substance
7	10	Ethylacetate	brown	Semisolid waxy
8	11-12	Ethylacetate: Methanol (50:50)	Yellowish Brown	Waxy substance
9	13-14	Methanol	Yellowish Brown	Semisolid
10	15	Methanol: Water (50:50)	Light yellow	Waxy Substance
11	16	Water	Yellow	Waxy substance

Table. 6: Preliminary Phytochemical constituents of Ethylacetate extract of Nyctanthes arbor-tristis

Fractions	IR Interpretation								
	-NH	-ROR-	C=0	-OH	CYCLIC KETONE	PHENOLIC COMPOUND	-CH ALIPHATIC	-C≡N	-COOH
Crude Drug	3346	1046	1642	----	----	----	----	----	----
Hexane Fraction	----	1711							2902
Ethylacetate Fraction				3675	1711				
Hexane									2923
Hexane: Chloroform		1730						1214	2925.13

Chloroform									
Chlorofor: Ethylacetate (50:50)		1733				3000			2987
Ethylacetate		1733				1715.3			
Ethylacetat: Methanol (50:50)	3387, 3354	1043			1710		3000		2975
Water	3392	1065			1711		2924		

Hexane, hexane: chloroform (50:50), were shown positive tests for alkaloids, cardiac glycosides, flavonoids and steroids. Chloroform, chloroform: ethyl acetate (50:50), ethyl acetate, ethyl acetate: ethanol was shown positive tests for flavonoids and water shown positive test for cardiac glycosides.Previous studies have been reported that Nycthanthes arbor-tristis plant contains various types of medicinal compounds. The plant leaves have potential phytochemical like nyctantic acid, friedelin, beta-sitosterol and oleanolic acid and responsible for antiviral activity[156]. Plant of Nycthanthes arbor-tristis L contain D-mannitol, β-sitosterole, Flavanol glycosides-Astragaline, Nicotiflorin, Oleanolicacid, Nyctanthic acid, tannic acid, ascorbic acid, methyl salicylate, an amorphous glycoside, an amorphous resin, trace of volatile oil, carotene, friedeline, lupeol, mannitol, Glucose, fructose, iridoid glycosides, benzoic acid derivative of kaempferol and carotene

Here we also found that various types of phytochemical compounds are present in the hydroalcoholic extract of the stalk of *Nycthanthes Arbor-Tristis* found in Guntur. We also found that there is a possibility of the presence of several types of flavonoids in the flowers of the plants

Table .7: Antimicrobial activities of Hydroalcoholic extract, Successive Fraction and Isolated Fractions of Nyctanthes arbor- tristis

Sl. No	**Fractions**	**Ethylacetate Fraction**		**Chloroform Fraction**		**Hexane Fraction**	
		B.S	**E.C**	**B.S**	**E.C**	**B.S**	**E.C**
	Organism	**----**	**----**	**----**	**----**	**----**	**----**
1	Benzene	----	----	----	----	----	----
2	Hexane	**----**	**12±0**	**----**	**2±0.0**	**----**	**----**
3	Hexane: Chloroform	**4.25±0.35**	**5±0.0**	**4±0.0**	**5±0.0**	**10±2.82**	**12±1.41**
4	Chloroform	**----**	**----**	**----**	**----**	**17.5±2.12**	**10±0.0**
5	Chloroform: Ethylacetate (50:50)	**17.5±2.12**	**10±0.0**	**11.5±0.70**	**11.5±2.12**	**5±0.0**	**5±0.0**
6	Ethylacetate	**22±2.82**	**21.5±0.70**	**----**	**----**	**----**	**----**
7	Ethylacetate: Ethanol (50:50)	**----**	**----**	**17.5±2.12**	**10±0.0**	**10±1.41**	**16.25±0.35**
8	Ethylacetate: Methanol (50:50)	**----**	**----**	**----**	**----**	**----**	**----**
9	Ethanol	**----**	**----**	**19±1.4**	**15.5±0.70**	**7±0.0**	**5±0.0**

10	Methanol	**5±0**	**----**	**5.5±0.70**	**0±0**	**3±1.41**	**----**
11	Water						

FT-IR studies: FT-IR spectroscopy was employed to obtain conformational information in the extract, successive fractions and compounds isolated from successive fractions. Interpretation of FT-IR spectra of extract, successive fractions and isolated compounds from successive

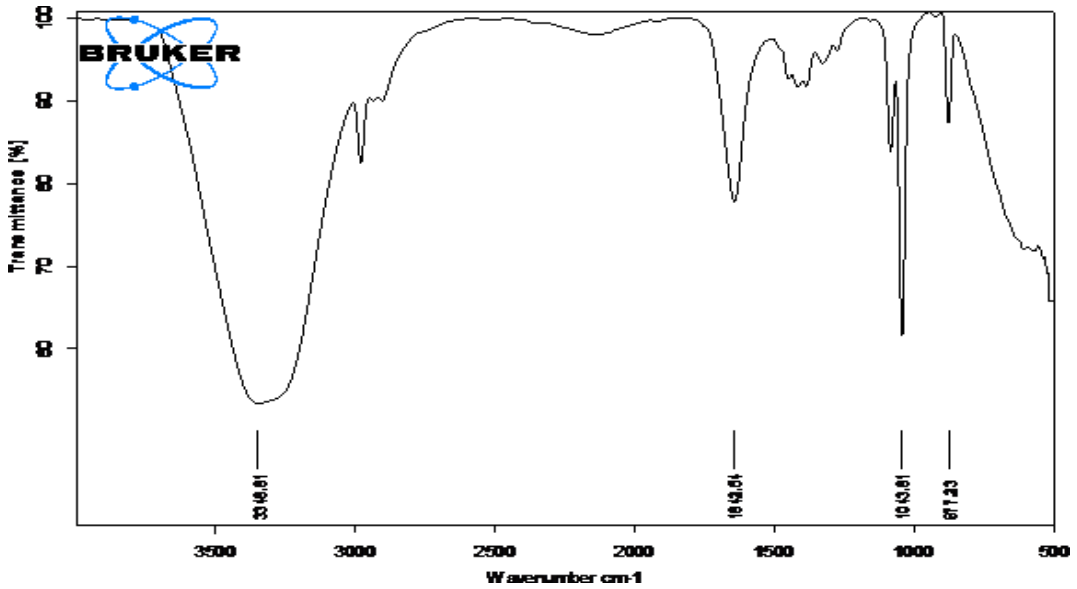

Figure.12: IR spectrum of hydroalcholic extract of Nyctanthus arbortristis STALK

fractions was done; the functional peaks are tabulated in Table 6 the presence of characteristic peaks of fractions was presented in Figure 6.1 to Figure 6.5 respectively. The extract, successive fractions and isolated compounds from successive fractions of absorption peaks of fractions were appeared and denoting stretching vibration of –NH, C=0, ether, -OH, aliphatic C-H Stretch, -C≡N, -COOH, NO2Stretch, showed peaks at ranges from 3346 - 3392, 1642, 1046 - 1733, 3675, 3000-2924, 1214, 2987-2975 and 1238-1372 showed characteristic peaks respectively

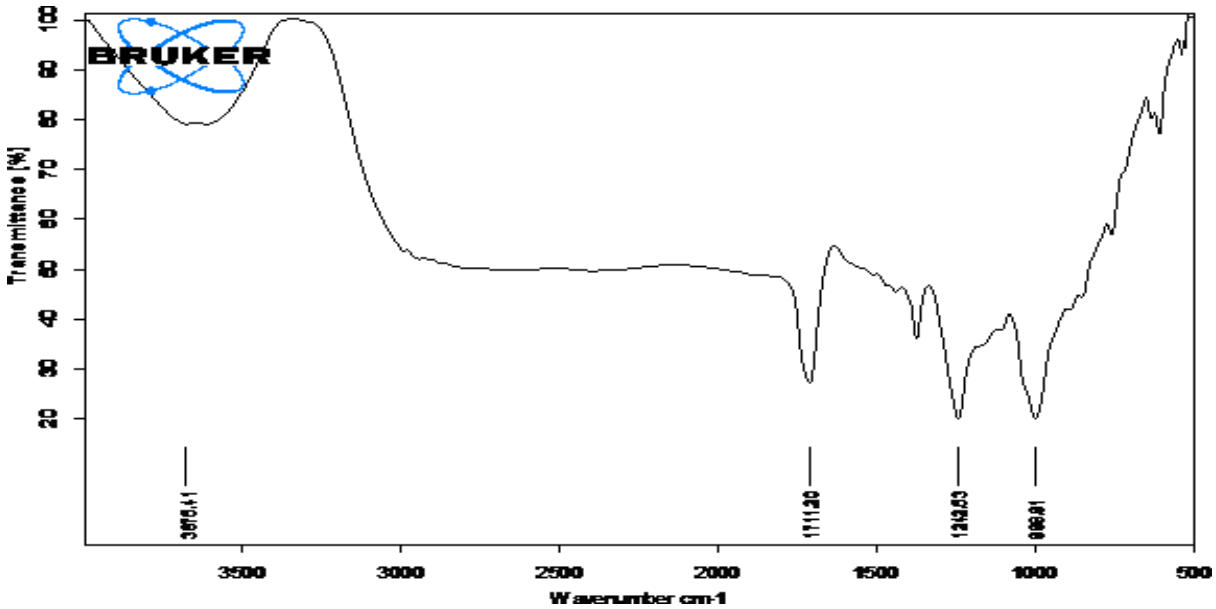

Figure .13: IR Spectrum of Ethyl Acetate Fraction (Column 1)

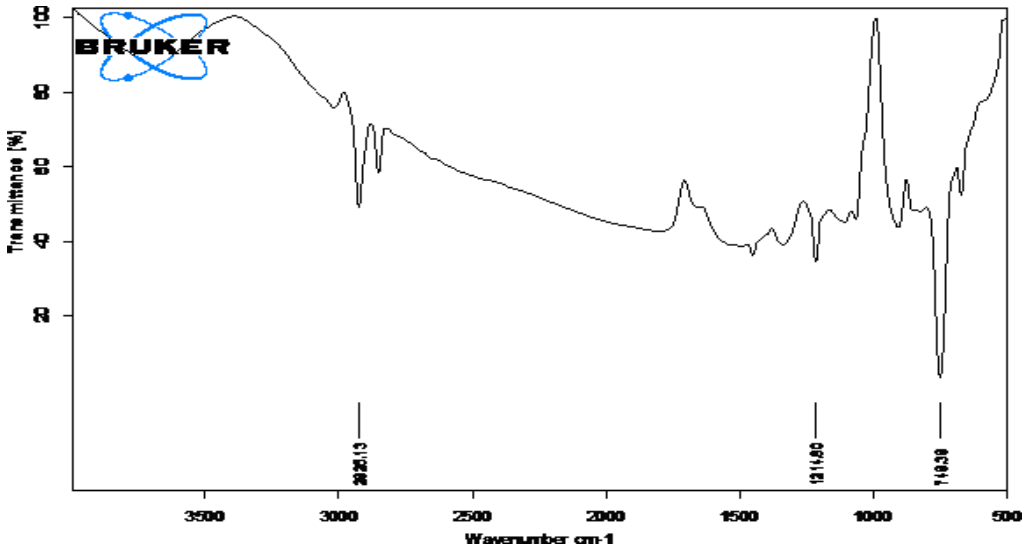

Figure. 14: IR Spectrum of Hexane + Chloroform Fraction (Column 1)

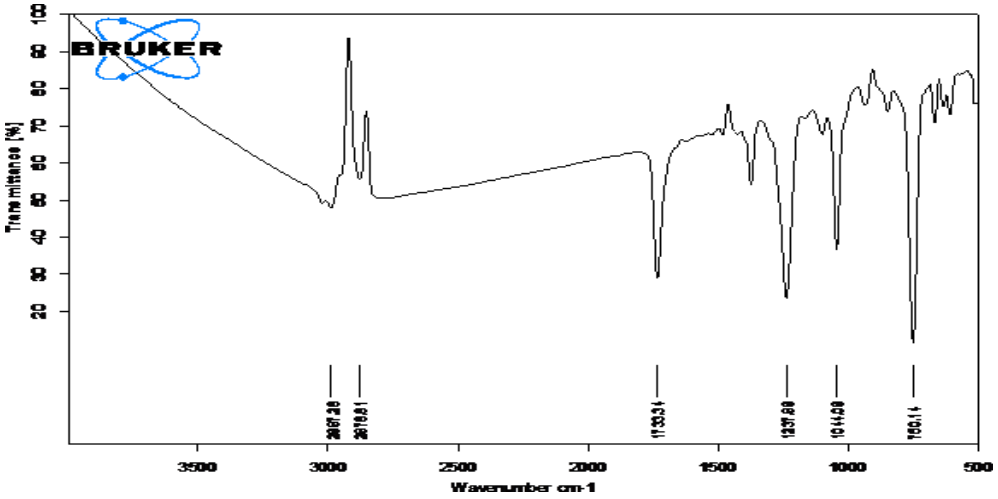

Figure .15: IR Spectrum of Chloroform + Ethyl Acetate Fraction (Column 1)

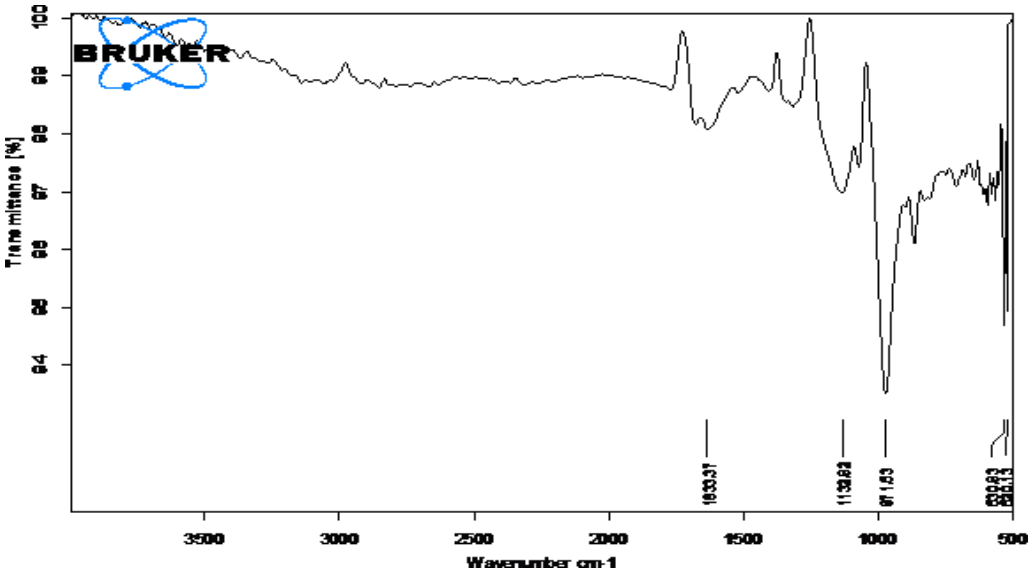

Figure .16: IR Spectrum of Ethyl Acetate + Ethanol Fraction (Column 1)

Antimicrobial Studies: The antimicrobial activities of the various solvent extracts of *Nyctanthes arbortristis* shows significant variation as shown in The hydro alcoholic and successive fractions of the stalk showed antimicrobial activity on gram positive (*Bacillus subtilis*) and gram negative bacteria (*Escherichia coli*). The successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Traditional healers use primarily water as the solvent but in present studies the plant extracts in organic solvent provided more consistent antimicrobial activity compared to those extracted in water. These observations can be rationalized in terms of the polarity of the compounds being extracted by each solvent and in addition to their intrinsic bioactivity, by their ability to dissolve or diffuse in the different media used in the assay. It is also quite possible that stalk of the test plant material that were effective and possess antibiotic, antimicrobial properties, in sufficient concentrations. It is also possible that the active chemical constituents in drying process may have caused conformational changes to occur in some of the chemical constituents found in the test plants material.The results obtained from the disc diffusion assay showed that there has been an increasing effect on bacterial growth inhibition with different solvent extract. The extract showed good inhibitory activity on almost gram positive and gram negative bacteria tested. It has been found that among all the tested organisms, the Gram positive bacterial strain, *Bacillus subtilis*, was found to be more susceptible to the plant extract by showing inhibition zone ranging from 3±1.41 – 22 ± 2.82 mm and the gram positive strain *Escherichia coli* was less susceptible with the inhibition zone ranging from 2 ± 0.0– 16.25±0.35 mm. The antimicrobial activity in terms of zone of inhibition was presented **.**Antibacterial activity assay of all the five solvent extracts were tabulated in Ethyl acetate and chloroform extracts showed significant activity against both phytopathogenic and human pathogenic bacteria. Other solvent extracts viz., hexane shown significant zone of inhibition on gram negative bacteria but not in gram positive bacteria. Hydroalcoholic extract did not shown any activity on both gram positive and gram negativebacteria. Pathogenic bacteria Ethyl acetate extract showed moderately significant antibacterial activity when compared with Streptomycin. Antibacterial activity assay of ethyl acetate isolated fractions Ethyl acetate, ethyl acetate: ethanol and chloroform extracts showed significant activity against both phytopathogenic and human pathogenic bacteria. Other solvent extracts like hexane: chloroform shown significant zone of inhibition on gram negative bacteria but not in gram positive bacteria. Water extracts show inhibition on gram positive bacteria but not in gram negative bacteria. Benzene, hexane, chloroform: ethyl acetate, ethanol extract did not shown any activity on both gram positive and gram negative bacteria. Ethyl acetate: ethanol extract showed highest significant antibacterial activity on both microbial strains when compared with Streptomycin.Antibacterial activity assay of chloroform isolated fractions were tabulated in Ethyl acetate, ethyl acetate: methanol, methanol, and chloroform, showed significant activity against both phytopathogenic and human pathogenic bacteria. Ethyl acetate: ethanol extract showed highest significant antibacterial activity on both microbial strains when compared with Streptomycin.Antibacterial activity assay of chloroform isolated fractions were tabulated in Ethyl acetate, ethyl acetate: methanol, methanol, and chloroform, showed significant activity against both phytopathogenic and human pathogenic bacteria. Other solvent extracts like hexane: chloroform shown significant zone of inhibition on gram negative bacteria but not in gram positive bacteria. Water extracts show inhibition on gram positive bacteria but not in gram negative bacteria.Ethyl acetate: methanol and methanol extract showed highest significant antibacterial activity on both microbial strains when compared with Streptomycin.Antibacterial activity assay of hexane isolated fractions Ethyl acetate, ethyl acetate: methanol, methanol, chloroform, and chloroform: ethyl acetate showed significant activity against both phytopathogenic and human pathogenic bacteria. Water extracts show inhibition on gram positive bacteria but not in gram negative bacteria. Other solvent extracts like hexane, hexane: chloroform, benzene: hexane and benzene did not show any activity on both gram positive and gram negative bacteria. Ethyl acetate: methanol and ethyl acetate: chloroform extract showed significant antibacterial activity on both microbial strains when compared with Streptomycin.

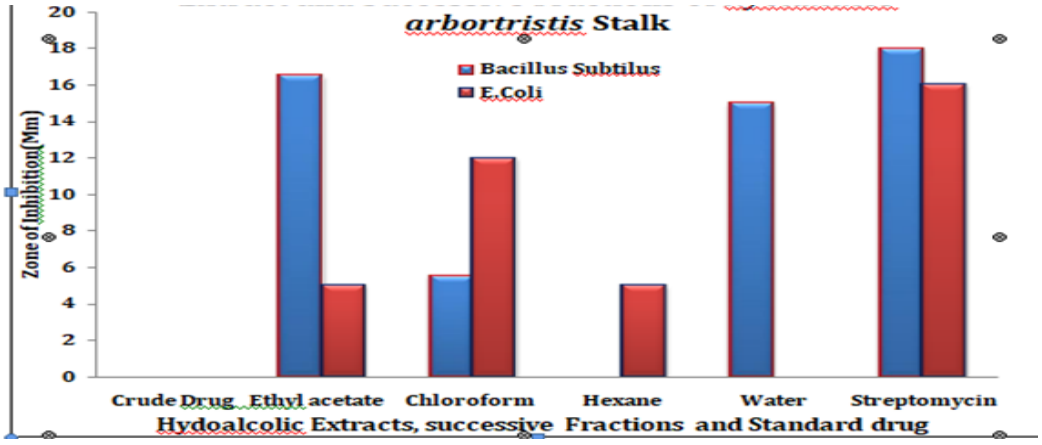

Figure .17:Antimicrobial activity of hyroalcoholic extract and successive fractions of Nyctanthes arbortristis Stalk

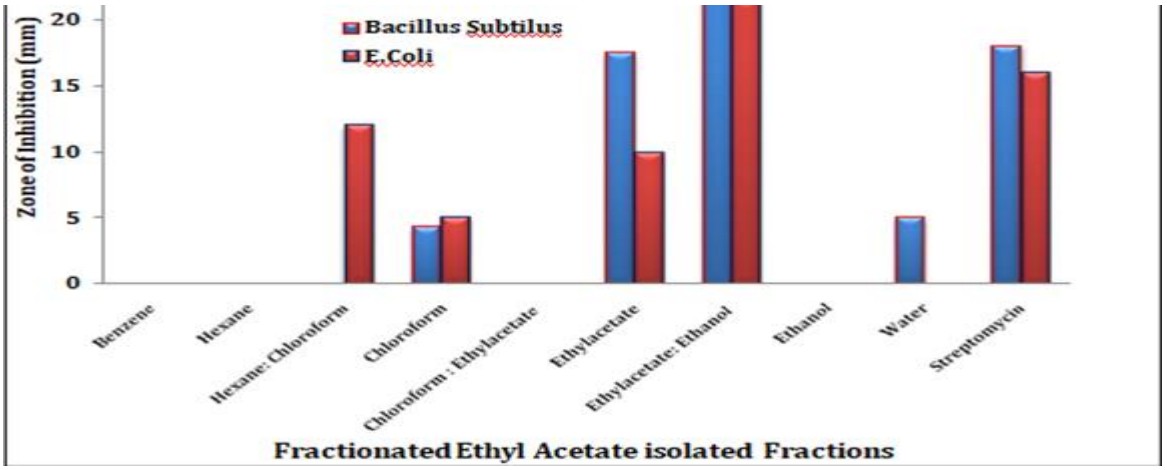

Figure .18: Antimicrobial activity studies of ethyl acetate isolated fractions

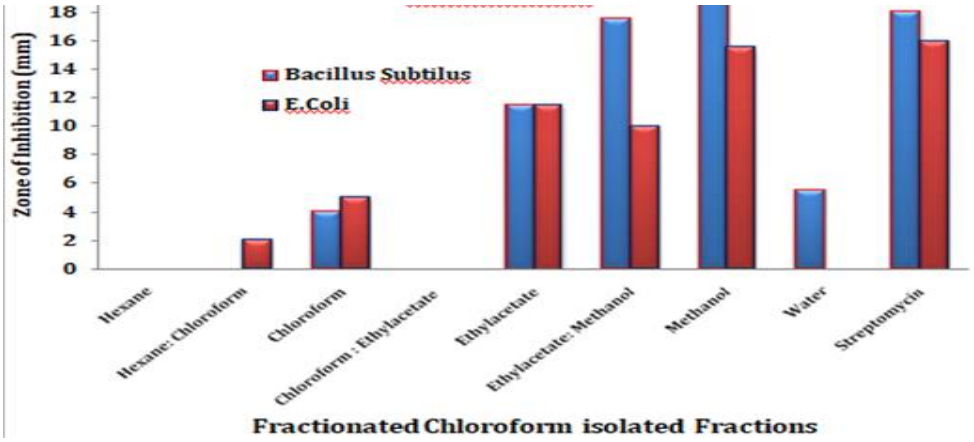

Figure .19: Antimicrobial activity of fractionated chloform isolated fractions of Nyctanthes arbortristis stalk

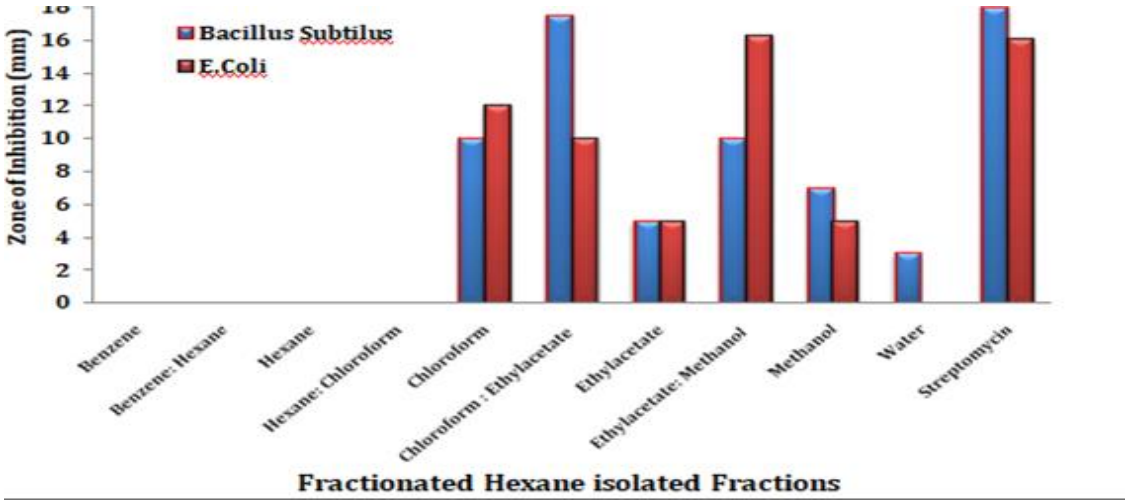

Figure .20: Antimicrobial activity of fractionated Hexane isolated fractions of Nyctanthes arbortristis stalk

One of the most common biological properties of alkaloids is its toxicity against cells of foreign organism. Steroidal extracts from some medicinal plants which exhibited antibacterial activities on some bacterial isolates. Antibiotics provide the main basis for the therapy of bacterial infections. However, the high genetic variability of bacteria enables them to rapidly evade the action of antibiotics by developing antibiotic resistance. Thus, there has been a continuing search for new and more potent

antibiotics. According to World Health Report of Infectious diseases 2000, overcoming antibiotic resistance is the major issue of the WHO for the next millennium. Hence the last decade witnessed an increase in the investigation of plants as a source of human disease management.

9. CONCLUSION

The preliminary phytochemical analysis of the hydroalcoholic extract of Nycthanthes arbortristis stalk and successive extraction of Nycthanthes arbortristis stalk extract revealed the presence of alkaloids, glycosides, flavonoids and steroids in all extracts. The fractions showed difference of compounds in the phytochemical tests. Because the the fractions isolated by chromatography are separated and shown few tests. The hydroalcoholic extract, successive fractions and isolated compounds of extracts of Nycthanthes arbortristis L flower (stalk) showed the broadest antibacterial activity by inhibiting the growth gram positive and gram negative bacterial strains which might be mainly due to sensitive metabolites responsible for their antibacterial activity. Thus, the potential of the plant extract can be simply observed from overall result that may play significant role as the antibacterial.The tested bacteria exhibited growth inhibition against all the extracts of Nyctanthes arbortristis L flower (stalk). The hydroalcoholic extract did not show any inhibition on gram positive and gram negative but the fractions shown significant inhibition. So, we can say that overall antimicrobial profile of some extract were better which may be due to the enhanced synthesis of the secondary metabolites responsible for the antibacterial due to the presence of some derivatives of the metabolites which is more inhibitive or due to the environmental factors. Nyctanthes arbortristis showed maximum antibacterial activity and so this plant can be used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals that address hither unmet therapeutic needs. Such screening of various natural organic compounds and identifying active agents is the need of the hour, because successful prediction of lead molecule and drug like properties at the onset of drug discovery will pay off later in drug development.

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