

Through a biological approach, evaluating new techniques of phytochemicals demonstrating antimicrobial action from two important medicinal plants in Odisha-A review.

Manmath Kumar Rout*

Department of Biotechnology, North Orissa University, Odisha, India

Abstract

In recent years, the usage of and search for medications and dietary supplements produced from plants has increased. Ethno-pharmacologists, botanists, microbiologists, and natural-products chemists are scouring the globe for Phytochemicals and "leads" that could be used to cure infectious diseases. Infectious infections are a big issue in both developing and developed countries. Because of their high antibacterial activity and low cost, traditional medicinal plants are commonly utilized to treat microbial infections. Antimicrobial resistance is a major hazard to human health all around the world. The expense of bringing a novel antibiotic to market is considerable, with a minimal return on investment. Plants produce a variety of bioactive secondary metabolites that could be exploited to fuel the pipeline of future discovery. Secondary metabolites present in plants include tannins, terpenoids, alkaloids, and flavonoids, which have been shown to have antibacterial activities *in vitro*. Diverse solvents such as ethanol, methanol, chloroform, acetone, petroleum ether, alcohol, and ethyl acetate were used to extract different plant parts such as seed, fruit, root, bark, stem, leaf, and even the entire plant. Plant extracts have a high value as natural antimicrobials because they greatly alter pathogenic organisms' cell membrane hyperpolarization. As a result, the current study was developed to look into a phytochemical screening of two therapeutic plants.

Keywords: Antibacterial, Medicinal plants, Phytochemicals, Herbal drug.

Accepted October 07, 2021

Introduction

Higher plants are a rich source of new pharmacologically active chemicals that can be used as lead compounds in the creation of new medications. Several essential medications, such as antibacterial and anticancer compounds, have been developed as a result of the increasing interest in natural products over the previous few decades. Plants have been familiarised by man since the dawn of time, and they have been employed in several ways throughout history. We obtain a variety of natural products from plants, the majority of which are not only excellent and beneficial to our health, but also essential to our survival. As the hunt for therapeutically effective medications continues, the plant world has been employed as a treasure trove of possible drugs as natural antimicrobials and therapies in folk medicine to treat sickness caused by microorganisms [1]. Plant products are currently being regarded as important alternative sources of novel antimicrobial medications against antibiotic-resistant bacteria. As a result, the potential of regional flora becomes essential, given the trend in contemporary medicine to absorb and re-assimilate natural treatments in common practice, in various ways.

According to World Health Organisation (WHO) estimates from 2008, more than 80% of the world's population gets their main health care from traditional medicines. India is the world's eighth-largest country, with a high plant diversity of 47000 species, of which roughly 7500 are used as medicinal plants. Because natural medicine has excellent therapeutic components, it has been used as a remedy for human disorders for ages.

Plants with high therapeutic potential due to the presence of phytochemicals have been intensively researched as a source of medicinal compounds in recent years. Phytochemicals are plant-derived bioactive compounds [2].

They're classified as secondary metabolites since the plants that make them may not require them. They are naturally synthesized in all sections of the plant body, including the bark, leaves, stem, root, flower, fruits, seeds, and so on; in other words, active components can be found in any part of the plant body. Phytochemical screening is the process of subjecting plant parts to a variety of chemical tests to extract secondary plant elements. It also provides us with some basic information about the therapeutic value of the plant extract [3].

Secondary metabolites are created as a result of every chemical reaction that occurs in plants. The presence of various metabolites consisting of reducing compounds, free radicals, and other chemical constituents scavenging compounds such as gums, flavonoids, alkaloids, reducing sugars, terpenoids, saponins, coumarins, tannins, cardiac glycosides, anthraquinones, and phlobatinins, and other phenolic compounds such as gums, flavonoids, alkaloids, reducing sugars. The use of phytochemicals with good antibacterial activity for the treatment of microbial illnesses is expected. These phytochemical substances have played an important role in human health. With recent trends of significant percentages of microbe resistance to current antibiotics, the importance of medications made from plants cannot be overstated. Because of the widespread use of commercial antimicrobials to treat infectious disorders,

microorganism resistance and the development of strains with reduced susceptibility to antibiotics are on the rise.

Furthermore, popular synthetic antibiotics are usually associated with high costs and undesirable side effects (such as hypersensitivity, allergic responses, immunosuppression, and so on), which are major global challenges in the treatment of infectious infections. As a result, new infection-fighting tactics are needed to combat microbial (bacterial, fungal, and other) infections. In light of the limitations of conventional medicine, the use of natural products as an alternative to conventional treatment for a variety of ailments continues to be one of the best reservoirs of novel structural types of diseases [4].

They're employed as therapeutics, starting materials for synthesized pharmaceuticals, and models for pharmacologically active molecules. Antibiotic resistance is one of the most serious public health issues. This problem is a natural result of pathogenic bacteria adapting to antimicrobials used in a variety of settings, including medicine, food animals, crop production, and farm, hospital, and household disinfectants. Microorganisms have evolved resistance to all known antibiotics, resulting in a large economic cost associated with multidrug-resistant bacteria. Plants have been investigated as sources for the identification of new and effective antimicrobials to find novel antimicrobial agents with unique mechanisms of action. Antimicrobial activity against a variety of microorganisms is investigated using a large number of plant species. Phytochemicals can impede peptidoglycan formation, disrupt microbial membrane structures, and change the hydrophobicity of bacterial membrane surfaces.

Traditional applications

Around 80% of people in developed nations use traditional medicine, which is made up of compounds obtained from medicinal plants. As a result, such plants should research to discover novel antibacterial chemicals [5].

The Government of India has established a National Level Policy for the Growth, Promotion, and Development of the Traditional System of Indian Medicine to supplement the country's traditional medical system. Ayurveda, Yoga, and

Naturopathy, Unani, Siddha, and Homoeopathy, generally known as AYUSH, have their departments within the Ministry of AYUSH (Figure 1).

Coastal forests, particularly mangroves and their companions, contain a high degree of biological variety as well as a large store of potential medicinal plants, even though a large number of medicinal plants have been identified across several locations of India. Traditional remedies are especially important to marginalized individuals who cannot afford or access official health care. They are culturally familiar and technically simple. Traditional medicines are often inexpensive and effective. Traditional health systems should be promoted to address primary healthcare needs, and there should be popular interest in doing so [6]. As consumers become more concerned about the adverse effects of allopathic drugs, healthcare systems will become increasingly expensive. Small steps can always go a long way toward preventing the bulk of diseases. Traditional medical systems are gaining a lot of traction around the world as "green pharmaceuticals" that are healthier and safer than manufactured meds.

Purpose of this study

The goal of this study was to assess the effect of these antimicrobials on illness therapy using phytochemical extracts from two common medicinal plants: 1-Nyctanthes arbor-tristis Gangasiuli/sephali and 2-Gymnema sylvestre Gurmar because of their high value of the medicinal effect. As a result, for the development of innovative therapies from these herbal resources, a thorough identification, and analysis of the mechanism of antimicrobial principle from these plants is required.

Antimicrobials

Different types of antimicrobials have been produced to tackle the microorganisms that cause these diseases, which have plagued humanity from time immemorial and continue to do so now. Antimicrobials, which are substances that kill or inhibit the growth of microorganisms, can take the form of antibiotics, which are microorganisms' products or synthesized derivatives, antimicrobial peptides produced by complex organisms, and medicinal plants, which appear to be the focus of mainstream medicine today.

Types and sources of antimicrobials

Antibiotics, antiviral, antifungal, anti-protozoan, and other antimicrobials are available. Antibiotics are drugs that are used to treat bacterial infections and can be found in both natural and manufactured forms. Phenyl propanoids (chloramphenicol), polyketides (tetracycline), and aminoglycosides are examples of natural products (streptomycin, gentamycin). Sulphonamides, quinolones, and oxazolidinones are synthetic compounds. The majority of antibiotics work by inhibiting the bacterial cell wall or protein synthesis. The majority of antiviral, antifungal, anti-protozoa, and anti-cancer medications, on the other hand, are synthesized [7].

General uses of medicinal plants

In most countries, medicinal plants (also known as herbs, herbal medicines, pharmacologically active plants, or phytomedicines) remain the most common kind of medication. Over a third of

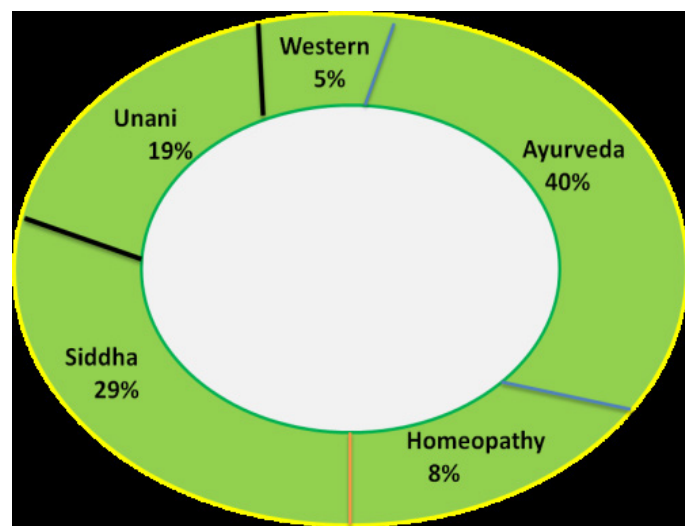


Figure 1. Depicts the percentage of herbal medications used in India by various medical systems.

the world's population relies on raw plant products to meet their daily health care requirements. The extract from the full plant or sections of it, such as leaves, roots, flowers, or fruit, is obtained using the majority of the plant materials acquired fresh. The bark, roots, and other components of woody forms are usually utilized.

In most countries, medicinal plants (also known as herbs, herbal medicines, pharmacologically active plants, or phytomedicines) remain the most common kind of medication. Over a third of the world's population relies on raw plant products to meet their daily health care requirements. The extract from the full plant or sections of it, such as leaves, roots, flowers, or fruit, is obtained using the majority of the plant materials acquired fresh. The bark, roots, and other components of woody forms are usually utilized.

Development of herbal drug and its challenges

Herbal medication is more effective, has fewer side effects, and is less expensive than allopathic medicine. Herbal medicines are made up of herbs, herbal materials, herbal preparations, and herbal products that contain active components such as plant parts or other plant materials. Herbal plants and their derivatives are widely known to play an important part in modern medicine development. Natural resources in the development of new medications are medicinal plants [8].

Relevance of this Research

The plants listed below have been used in the past. The determination of antimicrobial activity and the active components of *N. arbor-tristis* and *G. sylvestre* seeds, bark, and leaves, as well as the antimicrobial activity and active components of these plants, will provide baseline information on the potential use of extracts from these plants for the treatment of infectious diseases. The findings of this study could be used to produce medications derived from these plants or to synthesize pharmaceuticals that replicate the active components of these plants.

Nyctanthes arbor-tristis (Common name: In English (Night jasmine), In Odia (Gangasuli))

Nyctanthes arbor-tristis, also known as the "night flowering sad tree," belongs to the *Oleaceae* (*Nyctaginaceae*) family



Figure 2. *Nyctanthes arbor tristis*.

and is widely cultivated in tropical and subtropical regions around the world. It is well known in India and its neighbouring countries as one of the most versatile medicinal plants with a wide spectrum of biological activities. It is a terrestrial woody perennial with a 5 to 20-years life span. It's usually a shrub or a small tree with bright, fragrant blooms that bloom at night and fall off before daylight, leaving a beautiful blend of white and red on the ground beneath. As a result, the plant loses all of its brightness throughout the day, earning it the nickname "Tree of Sadness" (*Arbor-tristis*) (Figure 2).

Systemic position (Bentham and Hooker's)

Kingdom: Plantae

Division: Angiosperms

Class: Dicotyledonae

Subclass: Gamopetalae

Series: Bicarpetellatae

Order: Gentiales

Family: Oleaceae

Genus: *Nyctanthes*

Species: *arbor-tristis*

Immunostimulant, hepatoprotective, antileishmanial, antiviral, antifungal, antibacterial, and antioxidant properties have been found in extracts of the seeds, flowers, and leaves. The leaves have been used for sciatica, arthritis, fevers, and as a laxative in Ayurvedic and homeopathic treatment [9].

Morphology

Arbor-tristis Nyctanthes Linn is a huge shrub with quadrangular branches and flaky grey rough bark that can grow up to 10 meters tall. The leaves are rough, hairy, decussately opposite, simple, and 6 cm to 12 cm long with a 2 cm to 6.5 cm broad border. The blooms are commonly found in clusters of 2-7 clustered at the terminals of branches terminally or in the axils of leaves.

Pharmacological actions

Antihistaminic activity, CNS activities (hypnotic, tranquilizing, and anesthetics), analgesic, anti-inflammatory, antipyretic, antiulcer, amoebicidal, anthelmintic, antitrypanosomal, antidepressant, antiviral, and immunomodulatory properties have all been investigated in the plant.

Anti-microbial activity

The oil extracted from the leaves, seeds, and bark has a broad spectrum of antibacterial activity against both Gm -ve and Gm +ve bacteria. The bactericidal activity of aqueous and methanol extracts of mature leaves of *N. arbor-tristis* against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* was investigated. Except for *P. aeruginosa*, which was resistant to the aqueous extract, both extracts were effective against the bacterium. The leaves of *Nyctanthes arbor-tristis* Linn have antibacterial action. The methanol extract was more active than the aqueous extract in general. The chloroform and ethyl acetate extracts of *N. arbor-tristis* flowers inhibited gramme positive (*B. subtilis*, *B. cerues*,

B. megaterium, *S. aureus*, *Streptococcus* sp., *Sarcinia lutea*) and gram negative (*E. coli*, *Shigella dysenteriae*, *Shigella shiga*, *Shigella boydii*, *Shigella sonnei*).

Medicinal uses

Internal administration of leaf juice with honey is used to cure dry cough. The aqueous paste of leaves is applied externally to cure skin conditions, particularly ringworm. Skin disorders are treated with a special herbal oil made by boiling fresh leaves in mustard oil. Intestinal worms are treated with leaf juice mixed with honey and sugar and a pinch of salt, primarily in children. *Nyctanthes arbor-tristis* young leaves are used as a feminine tonic. Patients with gynecological issues should combine three fresh Night jasmine leaves with five black pepper seeds [10].

***Gymnema sylvestre* (Common name: In Hindi (gurmar), In Oriya (lakshmi, mendhasingia, nagapushpi), In English (gymnema))**

Gymnema sylvestre (Figure 3) is a medicinal plant of the *Asclepiadaceae* family. The plant's active ingredient is a group of acids known as gymnemic acid. For nearly two millennia, it has been used as a natural therapy for diabetes and is known as gudmar.

Gymnema sylvestre is a plant that is native to the tropical forests of central and southern India, but it has a far broader spread. *Gymnema* is a genus with 40 species found from western Africa to Australia. Uttar Pradesh, Madhya Pradesh, Maharashtra, Punjab, Haryana, Tamil Nadu, Kerala, Odisha, Bihar, and Bengal are among the Indian states where it can be found.

Systematic position

Kingdom: Plantae

Clade: Angiosperms

Clade: Eudicots

Clade: Asterids

Order: Gentianales

Family: Asclepiadaceae

Genus: *Gymnema*

Species: *G. sylvestre*

Various types of phytochemicals (active compounds) are found in *Gymnema*. These phytochemicals are thought to be responsible



Figure 3. *Gymnema Sylvestre*.

for gymnema's anti-diabetic, antipyretic, antimicrobial, anti-inflammatory, anti-cancer, and anti-allergic capabilities [11].

Morphology

Gymnema is a gregarious woody climber with many branches that sprawls across the summits of large trees. The pubescence of young stems and branches can be seen. Leaves are 3 cm-5 cm long and up to 3 cm wide, ovate-elliptic, acute or shortly acuminate, pubescent on both sides, with 6 mm-13 mm long pubescent petioles; base rounded or heart-shaped. Umbellate cyme inflorescences produce flowers.

Pharmacological action

Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH), a critical enzyme in the glycolysis cycle, has been identified to interact with gymnemic acid. The studies also revealed that the acylmoieties found in gymnemic acids are critical for GA-induced smearing of GAPDH and G3PDH, as well as for GA derivatives' antihyperglycemic effect. GS has its chemical makeup and contains a vast number of compounds, the majority of which have their own obvious and well-known therapeutic function, while some are secondary and some are inert [12].

Antimicrobial property

Different extracts of *G. sylvestre* were tested for antibiotic and antibacterial activities. *S. aureus*, *E. coli*, and *B. subtilis* were found to have activity against gram-positive bacteria, but no activity was found against *S. aureus*, *E. coli*, or *B. subtilis* and *G. sylvestre* leaf extracts showed promise as an antibiotic herbal cure and were efficient in treating microbe-related infections as an herbal composition [13].

Medicinal uses

Sugar consumption is reduced when *Gymnema sylvestre* extracts are administered as lozenges, mouthwash, or tea. This reduces sweet food consumption and overall calorie intake. *Gymnema sylvestre* extracts (in the form of a mint lozenge) were found to diminish the craving for high-sugar foods while also enhancing the pleasant flavor of candy. *Gymnema sylvestre* extracts have also been shown to lower sugar cravings in studies. Participants who got a gymnemic acid lozenge declined confectionery more often than those who received a placebo in double-blind research. Diabetic: Early study reveals that when a specific *Gymnema* extract is taken orally along with insulin or diabetes medicines, blood sugar levels in persons with type 1 or type 2 diabetes are reduced more effectively.

Material and Methods

Selection of plant

There are thousands of medicinal plants in the Indian subcontinent. Odisha state has a great potential to produce a large quantity of medicinal products as it has a wide range of eco-climatic regions. Although quite a good number of medicinal plants have been wiped away from the state due to the operation of various biotic factors coupled with other abiotic reasons, still Odisha is a grand repository of many indigenous medicinal plants.

Among these plants, it was not easy to select a few plants for the



Figure 4. Soxhlet apparatus using phytochemical extraction.

research purpose. Plant secondary metabolites often accumulate in specific plant parts. Thus, unless it is already known which parts contain the highest level of the compounds of interest, it is important to collect multiple plant parts or the whole plant to ensure the extracts prepared are representative of the range of secondary metabolites. For drug discovery from plants, the sample may be selected using a number following criteria by which the research work will run smoothly. From the literature review, it is seen that there is a lot of work on the plant *Nyctanthes arbor-tristis* and *Gymnema sylvestre* about the pharmacological activity of the plant. But there is a least of work has been found about the chemical investigation of this plant, especially about the membrane pore-forming mechanism of leaves extract of these plants. So I got a chance to select the leaves of *Nyctanthes arbor-tristis* and *Gymnema sylvestre* for my project work to see whether the leaves have a pore-forming activity or not.

Plant collection

After the selection of the plant, it is must to collect the plant parts for the research purpose. Throughout Odisha the plants *Nyctanthes arbor-tristis* Linn and *Gymnema sylvestre* is available. From the similar biosphere, Mayurbhanj both plant leaves were collected.

Extraction method

Extraction is the separation of medicinally active portions of plant tissues using selective solvents through standard procedure. There are different types of extraction methods are available but in this research, extraction is done by soxhlet method, shown in (Figure 4).

Soxhlet extraction method

Extraction is the separation of medicinally active portions of the plant using selective solvents through standard procedures. The purpose of all extraction is to separate the soluble plant metabolites, leaving behind the insoluble cellular marc (residue). The initial crude extracts using these methods contain a complex mixture of many plant metabolites, such as alkaloids, glycosides, phenolic, terpenoids, and flavonoids.

Principle

The choice of extraction procedure depends on the nature of the

plant material and the components to be isolated. The principle of solid-liquid extraction is that when a solid material comes in contact with a solvent, the soluble components in the solid material move to the solvent. Thus, solvent extraction of plant material results in the mass transfer of soluble active principle (medicinal ingredient) to the solvent, and this takes place in a concentration gradient. The rate of mass transfer decreases as the concentration of active principle in the solvent increases until equilibrium is reached, i.e. the concentrations of active principle in the solid material and the solvent are the same. Thereafter, there will no longer be a mass transfer of the active principle from plant material to solvent. Since the mass transfer of the active principle also depends on its solubility in the solvent, heating the solvent can enhance the mass transfer. Moreover, if the solvent in equilibrium with the plant material is replaced with fresh solvent, the concentration gradient is changed.

Procedure

The process of extraction by drying, size reduction, extraction, filtration, concentration, drying and reconstitution quality of an extract is influenced by several factors such as plant parts used as starting material, the solvent used for extraction, extraction procedure, and plant material: solvent ratio, etc. From laboratory scale to pilot scale all the parameters are optimized and controlled during extraction. Extraction techniques separate the soluble plant metabolites through the selective use of solvents.

30 grams of powdered leaves were weighed and packed in soxhlet. The solvent used for soxhletion was petroleum ether, chloroform, and methanol for *Nyctanthes arbor-tristis* and petroleum ether, chloroform, ethyl acetate, and methanol for *Gymnema sylvestre*. Extraction was continued at the temperature of 45°C till clear solvent was observed in a thimble. The extract was concentrated at room temperature. The concentrated extract was weighed and packed in an air-tight container.

Drying of plant sample

After the collection of the sample, it needs to be dried to make the sample extract. In general, the plant material should be dried at room temperature in a shaded area, to avoid the decomposition of thermolabile compounds. So sun drying can be very effective but the drawback is sometimes water molecules are absorbed by the sample and hence microbial growth can affect the phytochemical study.

Grinding of dried sample

Grinding is a simple mechanical technique to prepare the powder of the plant leaves, whether it should be properly dried. Grinding improves the efficiency of extraction by increasing surface area. It also decreases the amount of solvent required for the extraction. The dried samples were ground to a coarse powder and powdered samples were kept in clean closed glass containers pending extraction. During the grinding of the sample, the grinder was thoroughly cleaned to avoid contamination with any remnant of previously ground material or other foreign matter deposited on the grinder.

Collection of extract

In this study, I have not used to dry the extract by rotary evaporator. The collected extract from different solvents was

dried at room temperature. After completely dry, the extract was scraped with sterilized knife and stored in a clean dry vial for further analysis.

Chemicals and other reagent

Sulfuric acid, Folin reagent, proleuinamino acid (protein), glacial acetic acid, Ninhydrine solution, Acetone, Phosphate buffer, Ttrichloro Acetic Acid (TCA), Ferric chloride, Sodium carbonate, Sodium nitrite, Sodium hydroxide, Wagner's reagent, Hydrochloric acid, Ammonia, Sodium potassium tartrate, potassium iodide, phenol, nitric acid, picric acid, tannic acid, 99% alcohol, mercury chloride.

Types of equipment and other necessary tools

In the case of the extraction procedure and for various phytochemical tests many types of equipment and materials were used. Some of them are analytical balance, beaker (in various sizes), pipette, micro-pipette, hot air oven, dryer, storage cabinet, spatula, test tube, volumetric flask, conical flask, test tube holder, test tube rack, aluminium foil paper, refrigerator, water bath, electronic shaker, ultra-violet lamp, mask, gloves, lab coat, sprayer, reagent bottle, capillary tube, mortar and pestle, laminar airflow cabinet, loop, burner, micropipette tip, Petri dishes, glass rod, cotton, filter paper, funnel, hot plate, centrifugal machine, autoclave, glassware washers, stirrer, UV spectroscopy, knife, ephedrine tube, Whitman's filter paper, incubator, vortex machine, PH meter.

Solvents for experiments

Dimethyl Sulfoxide (DMSO), Acetone, Chloroform, Distilled

water, Ethanol, Methanol, petroleum benzene, Ethyl acetate

Preliminary phytochemical investigation of secondary metabolites

Principle

The secondary metabolites in the plant sample are the main concern of research work. There are many tests available for this purpose. In the following Table 1, these tests are shown.

Result and Discussion

Qualitative estimation of phytochemicals

The phytochemical analysis is very useful in the evaluation of some active biological components of medicinal plants. The phytochemical screening carried on the leaves extract of *Nyctanthes arbor-tristis* (Table 1) and *Gymnema sylvestre* revealed the presence of some active ingredients such as alkaloids, glycosides, tannins, saponins, anthraquinones, phenols, and flavonoids (Table 2).

This analysis determines the biologically active compounds that contribute to the flavor, color, and other characteristics of leaves. The phytochemical screening on a qualitative level showed that the leaves of the plant *Gymnema sylvestre* were *Nyctanthes arbor-tristis* rich in alkaloids, flavonoids, tannins, and saponins. They were known to show medicinal activity as well as exhibiting physiological activity (Table 3).

Table 1. Preliminary phytochemical investigations of secondary metabolites [14].

Test Compounds	Test Name	Procedure	Remarks
Alkaloids	Mayers Reagent	1 ml of test solutions few drops of Mayer's reagent add and mix properly	Cream precipitate indicates the presence of alkaloids
	Wagners Reagent	1ml of test solutions to an equal volume of Wagner's reagent added	Reddish precipitate indicates the presence of alkaloids
	Hagers Reagent	2 ml of test solutions and a few drops hagers reagents	Bright Yellow precipitate indicates the presence of alkaloids
	Tannic acid test	The extract was treated with 10% tannic acid (Dissolve 10 g of tannic acid in 10 ml of alcohol and dilute with water to 100 ml.)	Pale yellow precipitate indicates the presence of alkaloids (buff color precipitate)
Glycosides	Keller kiliani test	To 1 ml test solution and 1ml of glacial acetic acid was added, dissolved, and then cool. after cooling 2-3 drops of ferric chloride were added then carefully 2ml of conc. H ₂ SO ₄ was added to the wall of the test tube	Redish Brown obtained at the junction of two layers indicates the presence of glycosides

Phytochemical screening of nyctanthes arbor-tristis

Table 2. Phytochemical screening of *Nyctanthes arbor-tristis*.

Test compounds	Test Name	Petroleum benzine extracts	Chloroform extracts	Methanol extracts
Alkaloid	(Mayer's test, Wagner's test, Tannic acid test)	++	++	—
Glycoside	(Keller killiani test, Molisch's test, Conc. H ₂ SO ₄ test)	+	++	—
Quinone	(Conc.H ₂ SO ₄ , Conc. HCl)	—	—	+
Carbohydrates	(Fehling's test, Molisch's test)	+	++	++
Tannin	(FeCl ₃ test , Alkaline reagent test)	—	+	+++
Protein	(Biuret test , Ninhydrin test)	—	—	—
Phenol		—	++	+
Terpenoid		++	—	+
Resin		+	+	—

Phytochemical screening for *Gymnema sylvestre*

Table 3. Phytochemical screening of *Gymnema sylvestre*.

Test compounds	Test Name	Petroleum benzene extracts	Chloroform extracts	Methanol extracts	Ethyl acetate extracts
Alkaloid	(Mayer's test, Wagner's test, Tannic acid test)	–	+	+	–
Glycoside	(Keller killiani test, Molisch's test, Conc. H ₂ SO ₄ test)	+	+	–	–
Quinone	(Conc.H ₂ SO ₄ , Conc. HCl)	+	+	+	+
Carbohydrates	(Fehling's test, Molisch's test)	–	+	–	–
Tannin	(FeCl ₃ test, Alkaline reagent test)	–	+	+	+
Protein	(Biuret test, Ninhydrin test)	–	–	–	–
Saponin	–	–	–	–	–
Phenol	–	–	–	–	–
Flavonoid	(Jones test)	–	–	+	+
Coumarin	(NaOH test)	–	–	+	+
Steroid	–	–	–	–	–
Terpenoid	(Salkowski test)	–	–	+	+

Conclusion

Work on medicinal plants brings up a wide range of research opportunities for plant physiologists, and plant physiological investigations would play an important role in this developing discipline. Many commonly used medicinal plants have not obtained the same level of plant physiological characterization as food crops or model plant systems, with a few exceptions. Although active phytochemicals have been identified, numerous mechanisms for the biosynthesis of specific therapeutic compounds, as well as the biotic and abiotic variables that regulate their formation, are still unknown. The maintenance of consistent medicinal quality in plant medicines is currently a key challenge with the use of phytomedicines. Another big problem in the medical field is the antibiotic resistance of certain dangerous microorganisms. Plant materials, therefore, can be potential sources of chemically fascinating and biologically important drug entrants, since the idea dates back to the Vedic era. Traditional uses for distinct soxhlet extracts of *N. arbor-tristis* and *G. sylvestre* leaves from the *Oleaceae* and *Asclepiadaceae* families, respectively, include numerous illness situations. There are numerous published study papers on this product's phytochemical and pharmacological qualities. There are still plenty of opportunities to develop a range of qualities that are extremely valuable to humanity.

Future Prospective

As per our research, we are trying to develop a liposomal cell-based therapy treatment. It is a novel work in the future. So this study of research is only to find phytochemicals and how to extract the secondary metabolites.

References

- Ahmad N, Fazal H, Abbasi BH, et al. Biological role of *Piper nigrum* L. (Black pepper): A review. Asian Pac J Trop Biomed. 2012; 2(3): S1945-53.
- Khatune NA, Mosaddik MA, Haque ME. Antibacterial activity and cytotoxicity of *Nyctanthes arbor-tristis* flowers. Fitoterapia. 2001;72(4):412-4.
- Barrett B, Kieffer D. Medicinal plants, science, and health care. J Herbs Spices Med plants. 2001;8(2-3):1-36

- Chukwujekwu JC, CA Lategan, PJ Smith, et al. Antiplasmodial and cytotoxic activity of isolated sesquiterpene lactones from the acetone leaf extract of *Vernonia colorata*. S Afr J Bot. 2009; 75, 176-9.
- De Feo V, Aquino R, Menghini A, et al. Traditional phytotherapy in the peninsula sorrentina, Campania, southern Italy. J Ethnopharmacol. 1992; 36(2):113-25.
- Heinrich M. Ethnobotany and its role in drug development. Phytotherapy Research. Int J Devoted Pharmacol Toxicol Eval Nat Prod Deriv. 2000;14(7):479-88.
- Kiew R, Baas P. *Nyctanthes* is a member of Oleaceae. Proc Indian Acad Sci Plant Sc. 1984; 93(3):349-58.
- Malik JK, Manvi FV, Nanjware BR, et al. Anti-arthritis activity of leaves of *Gymnema sylvestre* R. Br. leaves in rats. Pharmacia Lettre. 2010; 2(1):336-41.
- Rates SMK. Plants as source of drugs. Toxicon. 2001; 39(5), 603-13.
- Saxena RS, Gupta B, Lata S. Tranquilizing, antihistaminic and purgative activity of *Nyctanthes arbor tristis* leaf extract. J Ethnopharmacol. 2002; 81(3):321-5.
- Ushie OA, Neji PA, Nsor, G.E. Phytochemical screening and antimicrobial activities of *phyllanthus amarus* stem bark extracts. Int J Mod Biol Med. 2013; 3(3): 101-12
- Sharma V. Part based HPLC-PDA quantification of podophyllotoxin in populations of podophyllum hexandrum royal indian mayapple from higher altitude himalayas. J Med Plants Stud. 2013; 1(3):176-83.
- Yadav M, Chatterji S, Gupta SK, et al. Preliminary phytochemical screening of six medicinal plants used in traditional medicine. Int J Pharm Pharm Sci. 2014;6(5):539-42.
- De Castro ML, Garcia-Ayuso LE. Soxhlet extraction of solid materials: an outdated technique with a promising innovative future. Analytica Chimica Acta. 1998;369(2):1.

*Correspondence to:

Manmath Kumar Rout
Department of Biotechnology
North Orissa University
Odisha, India
E-mail: manmathkumarrou5@gmail.com