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Extraction and Analysis of Medicinal Property in Secondary Metabolites Obtained from Selected Plant Species

Amita Chandrakant Kulkarni

Wockhardt Global School

5 Star MIDC Shendra

Aurangabad

Email address – kulkarniamita2828@gmail.com

Abstract:

Medical plants have been used from thousands of years to treat and prevent many diseases. Wockhardt global school Consist various plants variety and for this study three medicinal plants were selected and these plants are ***Tridax procumbiens*** (Tantani), ***Ficus racemosa*** (Umbar), ***Nyctanthes abortiristis*** (Parijat). The primary aim of this study was to extract and identify the presence of phytochemicals and to determine their medicinal uses for human beings. From Vedic era the traditional plant medicines has been considered as a primary healthcare for human beings. In the given study the presence of flavonoids, alkaloids, N-Containing chemicals were determined by using phytochemical extraction method. The chemicals were identified based upon various qualitative tests. The selected plants consist glycosides, alkaloids, saponins, flavonoids and carbohydrates. This phytochemicals are responsible for anti-inflammatory properties hence the selected medicinal plants can be used for inflammation, bone pain, joint pain, swelling, and common cough etc.

Keywords:

Medicinal plants, secondary metabolites, phytochemicals, flavonoids, alkaloids, leaf extract.

Introduction:

The plants are complete resource for the all living organisms especially for human beings from ancient era. Apart from food and shelter plants are significantly known for their medicinal properties and uses to cure or prevent many diseases. As India is known for its culture and tradition in this way from Vedic era the Indian people they used medicinal therapy for various diseases with the use of medicinal plants only, also known as the Ayurveda. Many plants are known as specific medicinal plants, but there are also some rarely known plants which are also consist some medicinal properties. Plant consist some chemicals known as phytochemicals, they are also known as the plant metabolites. There are 2 types of the metabolites present in the plants such as primary and secondary metabolites. They are also classified in different groups such as N- containing compounds, alkaloids, flavonoids etc. due to presence of such chemical compounds in the plants, they show various medicinal properties like anti-bacterial, anti-fungal, anti-inflammatory, anti-viral etc. plant are often used as a direct source of medicine because of its very rare side effects. The given study shows that, selection of 3 plant species, extraction and analysis of secondary metabolites and identifies their medicinal property.

Phytochemical found in plants have undergone extensive examination in past decades due to their implications for pharmaceutical, medicinal and functional foods. While numerous studies focuses on characterizing these phytochemicals in various plant parts. Most of the plants being neglected due to less popularity or unknown function. However some of these neglected plants have been traditionally use as a folk medicine. Phytochemicals are generally originated from plant sources. They are the organic chemicals produced by various parts of the plant based bioactive substances found in stem, leaf, fruit, grains, cereals etc.

These 2 kinds of metabolites are produced by some anabolic pathways. They are produced during growth phase of the plant. They are responsible for growth, development, respiration, photosynthesis etc. so generally primary metabolites perform some physiological function of plants hence also known as the central metabolites of the plant body. Examples of primary metabolites are amino acids, vitamins, hormones, and ethanol. Secondary metabolites are the chemicals with complex chemical structure and function like antibacterial, antifungal property as they have various phytochemicals.

To study the secondary metabolites from 3 different plant samples are as follows.

1) *Tridax procumbens*

This plant is belongs to Asteraceae family. It is commonly known as coatbuttons and widespread weed. This plant is known for various names across the different country as it is commonly found in tropical, subtropical and mild temperate region. In Marathi it is known as ‘’Tantani”. In india it is commonly used for wound healing and anticoagulant. After several scientific studies on animals it is proved that *T.procumbens* is an instant remedy used for inflammation, wound and insect repellent.

2) *Ficus racemosa*

This plant belongs to family Moraceae. Commonly known as cluster fig. In India this plant is widely used in ayurvedic medicines for its multiple uses on various disorder. It shows actions such as antidiabetic, anticancerous, antidiuretic, inflammatory actions. From *F. racemosa* wide range of phytochemicals were obtained from various parts of plants.

3) *Nyctanthes abortiristis*

This plant belongs from Oleaceae family. It is popularly known as ‘’Parijat’’ in India. Parijatha also famous in India for gods favourite flower. It is commonly known as night jasmine. In sanskrit it I known as shephalika and in Hind known as harsingar.

This plant is widely used in Ayurveda for medicinal purpose due to presence of some bioactive compounds. Traditionally this plant is used for joint pain and bone diseases. The leaf and flowers are source of oil and various phytochemicals. In arthritis and joint pain section of fresh leaf juice to be given internally. Person can get relief after consumption of this plant.

Review of Literature:

The researcher studied in detail about phytochemicals in medicinal plants. Mainly alkaloid, phenols, terpenoids and their uses in medicinal plants. Medicinal plants have a long history in the treatment of several kinds of diseases. (Sayantani and Chandra, 2019).Another researcher focuses on, medicinal plant exploring phytochemical composition such as alkaloids, flavonoids, terpeoids. (RNS Yadav, munin Agrawal). Also the use of many medicinal plants for humankind has explained by the researcher. There use for treatment of various diseases has been practiced by man from many years and even today.(Kokwaro, 1993). They mainly focus on plants like Terminalia chebula (Hirda), syzigum cumini (jambhul), salvadora persica (Miswak) (Twinkle Bansode & Dr. Salarkar, 2015). Another study done by researcher is investigation of anti-inflammatory, anti-oxidant and anti-microbial effect. Recent research explores the sustainable civilization of medicinal plants. Researcher gave importance to understand the medicinal properties of selected species and to practice such conservation and cultivation. The researcher studied about the medicinal plants which are used in the treatment of asthma. They also focused on how humans were exploring and using various plants and plants products to cure the deadly diseases. (Kuldip, Sandip, jalal 2015). Next researcher explained about how medicines were prepared from plants from many years. The study shown by researcher that how plant were using from vedic era. They explained about properties of 21 medicinal plants by using various parts of the plants.(poonam verma, Neelam Bamola, Chandranandani negi, 2017). In another article researcher explained about traditional medicinal plants need to be conserved and promote explained by them. Their study shows the traditional African medicines and focused on what are the challenges and opportunities in developing traditional medicines. (karunamoorthi, Jgjeevanram, Embialle, 2015).

Another researcher specifically explained medicinal property of common herb. That is Oscimum sanctum (Tulsi). They extracted all the phytochemicals from Oscimum sanctum by using various tests. They explained the role of aromatic property of herbs in foods, oral and dental medicines and also for many diseases. Phytochemical screening of Oscimum sanctum was done by them (Panchal and Parvez, 2019). Various general techniques for phytochemical analysis is also explained in detail by them. In that they explained in detail about sample collection, processes for drying sample and powdering the sample and methods of extraction. For their analysis they used the methods such as plant tissue homogenization, serial exhaustive extraction, soxhlet extraction, maceration, decotation, infusion, sonication etc. they also perform various qualitative and quantitative methods such as, test for alkaloids (Mayer’s test, Wagner’s test), amino acid test, for fixed oils and fats legals test, saponification test etc. qualitative and quantitative analysis done using gas chromatography, mass spectroscopy (GCMS), HPLC, IR-NMR, H-NMR for detection of phytochemicals. (Sahira banu and Catherin, 2015)

Objectives:

Nowadays many medicines are plant based due to its unlimited therapeutic properties and less side effects on human body. The research on medicinal properties of plant will help to identify bioactive compounds from various plants and it will help to cure many diseases or physiological conditions.

Following are some objectives for analysis of selected medicinal plants.

1) To identify the presence of metabolites like phenolic, terpenoids and N-containing compounds in *Tridax procumbens, Ficus racemosa, Nyctanthes arbor-tristis*

2) To study medicinal properties of *Tridax procumbens, Ficus racemosa, Nyctanthes arbor-tristis.*

3) Analysis of unpopular medicinal plant as a home remedy for various physiological conditions which can be used as a substitute for allopathic medicines as they have instant results with less side effects.

Hypothesis:

Effects of extract obtained from selected plants on some physiological condition.

Hypothesis 1

There is significant medicinal effect of phytochemicals alkaloids, glycosides, flavonoids and terpenoids present in ***Tridax procumbens*** plant on inflammation and wound healing.

Hypothesis 2

There is significant effect of phytochemical flavonoids, alkaloids, glycosides, saponines Present in ***Ficus racemosa*** plant on wound healing. (As astringent)

Hypothesis 3

There is significant effect of phytochemical alkaloids, flavonoids, glycosides and saponins present in ***Nyctanthes arbor-tristis*** on joint pain.

Research Methodology:

The given study shows the significance of medicinal properties present in selected plants. Such as, *Tridax procumbens(Tantani), Ficus racemosa(Umbar), Nyctanthes arbor-tristis (Parijat).* The presence of secondary metabolite with medicinal properties shows multiple therapeutic applications. The given study is to identify the presence of phytochemicals in the selected medicinal plants and their therapeutic uses. Plants are richest source of drugs of traditional system of medicine, and the products obtained from plant known as phytochemicals. Phytochemicals are also known as secondary metabolites as they contain various types of bioactive organic compounds. In the given study the test will perform to check the presence and significance of some commonly available phytochemicals such as, alkaloids, flavonoids, phenolics, tannin, saponins, lignin, terpenoids, proteins, carbohydrates etc.

Secondary Metabolites

Terpenes	Phenolics	N-Containing compounds
Carotenoids		
Alkaloids	Flavonoids	
Sterols	Lignan	
Glycosinolates	Tannin	Proteins
Cardiac glycosides		
Plan Volatiles	Lignin	Amino acid

Above mentioned are 3 main groups of secondary metabolites. Phytochemicals are secondary metabolites of plants and they are classified based upon their structure, their property, chemical composition(presence of Nitrogen, sulphur or not), having ring etc. investigative study of secondary metabolites demonstrated that many plant contain secondary metabolites and can be divided into 3 main groups such as, alkaloids, terpenes and phenolics. That could be potential source for several medicines.

In the present work phytochemical extraction and analysis were carried out in 3 plants. Such as ***Tridax procumbens, Ficus racemosa, Nyctanthes arbor-tristis.***

1) ***Tridax Procumbens***
2) ***Ficus racemosa***
3) ***Nyctanthes arbor-tristis***.

Materials and Methods:

1) Steps involved in a plant collection.
2) Methods of extraction.
3) Qualitative and quantitative analysis of phytochemicals.

- **Collection of plant Material:**
  The required plant samples selected for extraction and analysis of phytochemicals from the campus of wockhardt Global school shendra, MIDC Aurangabad, Maharashtra. The school comprises diverse variety of plants including flowering, medicinal and ornamental species. The fresh part of the plant (leaves) of selected plant species, such as ***Tridax procumbens, Ficus racemosa, Nyctanthes arbor-tristis*** were collected from the wockhadt school campus.

Methods of Extraction:

Ideally fresh plant tissue should be used for phytochemical analysis and the material should be plunged into boiling alcohol after collection. In the present study ‘’plant tissue homogenization’’ method is used for extraction process. In this method dried or wet, fresh plant parts are grinded in blender or (mortar and pestle) to fine particle. Put in certain quantity of solvent and shaken vigorously for 5-10 min or 24 hours after which the extract is filtered. The filtrate then may be dried and re-dissolved in the solvent to determine the concentration and centrifuge the filtrate for the classification of the extract.

Preparation of filtration:

10 gram of fresh leaves of selected plant were taken and crushed by using mortar and pestle, Then soaked it in water, chloroform and ethanol separately. These are the solvents used to make plant extract. Using muslin cloth

they were filtered and subjected to centrifugation for 10 min. then make up the final volume up to 100 ml using water, ethanol and chloroform to prepare aqueous extract, chloroform extract and ethanolic extract to check the presence of phytochemicals in each extract individually, because all the phytochemicals are not soluble in water or not only in organic solvents. Hence different solvent extract were used to check the presence of various phytochemical.

Phytochemical analysis:

Preliminary qualitative screening for phytochemicals of all these plants extract, such as ***Tridax procumbens, Ficus racemosa, Nyctanthes arbor-tristis*** carried out by following methods.

1) Test for presence of alkaloids.
2) Test for presence of flavonoids.
3) Test for presence of tannins.
4) Test for presence of phenolic compounds.
5) Test for presence of terpenoids.
6) Test for presence of Carbohydrates and reducing sugars.
7) Test for presence of saponins.
8) Test for presence of glycosides.
9) Test for proteins and amino acids.

Qualitative Analysis

Phytochemical	Test	Reagent	End Point.(positive result)
Alkaloids	Mayers test, HCl Test few drops of dil. HCl was added in each extract. Or 4-5 drps of mayer's regent added in each extract.	Mayer's reagent 1% HCl	Prominent yellow ppt. Turbid extract is obtained.
Flavonoids	Ammonia test. Sodium hydroxide test. Extracts were treated with few drops of sodium hydroxide solution. Addition of dil. HCl was done.	1% NH_3 20% NaOH, HCl	Yellow colour. Yellow colour turns to colourless.
Tannins	Ferric chloride test. Extracts were treated with few drops of $fecl_3$ solution.	5% $Fecl_3$	Blue-black or blue-green colouration.
Phenolic Compounds	Gelatine Test	1% gelatine solution containing 10% NaCl	White ppt.

Terpenoids	Salkowaski test.	0.5 ml chloroform, 1ml conc. H_2SO_4	Reddish brown colour at the interface.
Carbohydrates Reducing sugar	Molish test Fehlings test Bnedicts test.	Molish reagent, conc. HCl Conc. HCl and Mg turnings. Benedicts reagent	Violet ring Yellow and brick red ppt. Brick red ppt.
Saponins	Lead aceate test. Foam test.	10% lead acetate solution 20 ml distilled water(mixed vigorously for 15 minutes)	Bulky white ppt. Presence of froth
Glycosides	Keller killani test.	Glacial acetic acid, 5% $FeCl_3$ solution.	Reddish brown and bluish green colour.
Proteins Amino acids	Biuret test Ninhydrin test	Biuret reagent Ninhydrin reagent	Violet or pink colour. Deep blue colour(Ruhemann's purple)

Result and Discussion

Table No. 1

Preliminary phytochemical screening of *Tridax procumbens* leaves.

	Extracts			
Plant constituent	Chloroform	Ethanol	Aqueous	Test
Alkaloids	+	-	-	Mayers test
Flavonoids	+	-	-	Ammonia test
Tannins	-	-	-	Ferric chloride test
Phenolic compound	+	+	+	Gelatine test
Terpenoids	-	-	-	Salkowaski test
Carbohydrates Reducing sugars	+ +	+ +	+ +	Molish test Benedicts test
Saponins	+	+	+	Lead acetate test Foam test
Glycosides	+	+	+	Keller killani test
Proteins	+	+	+	Biuret test

Qualitative tests mentioned in table no. 1 shows the presence of phytochemicals in the ***Tridax procumbens*** having medicinal property which can be used for inflammation and wound healing hence, hypothesis 1 is accepted.

Fig. 1 Extraction and test results of *T. procumbens*

Table No. 2

Preliminary phytochemical screening of *Ficus racemosa* leaves.

	Extracts			
Plant constituent	Chloroform	Ethanol	Aqueous	Test
Alkaloids	-	+	+	Mayers test
Flavonoids	+	+	+	Ammonia test
Tannins	-	-	-	Ferric chloride test
Phenolic compound	+	+	-	Gelatine test
Terpenoids	-	-	-	Salkowaski test

Carbohydrates Reducing sugars	+	+	-	Molish test Benedicts test
Saponins	+	+	+	Lead acetate test Foam test
Glycosides	+	+	+	Keller killani test
Proteins	+	+	+	Biuret test

Qualitative tests mentioned in table no. 2 shows the presence of phytochemicals in the ***Ficus racemosa*** having medicinal property which can be used for wound healing hence hypothesis 2 is accepted.

Fig. 2 Extraction and test results of *F. racemosa*

Table No. 3

Preliminary phytochemical screening of *Nyctanthes arbor-tristis*.

	Extracts			
Plant constituent	Chloroform	Ethanol	Aqueous	Test
Alkaloids	+	-	-	Mayers test
Flavonoids	+	-	+	Ammonia test
Tannins	-	-	-	Ferric chloride

				test
Phenolic compound	+	+	+	Gelatine test
Terpenoids	+	-	-	Salkowaski test
Carbohydrates Reducing sugars	+	-	+	Molish test Benedicts test
Saponins	+	+	+	Lead acetate test Foam test
Glycosides	+	+	+	Keller killani test
Proteins	+	+	+	Biuret test

Qualitative tests mentioned in table no.3 shows the presence of phytochemicals in the ***Nyctanthes arbor-tristis Linn.*** Having medicinal property which can be used for joint and bone pain hence hypothesis 3 is accepted.

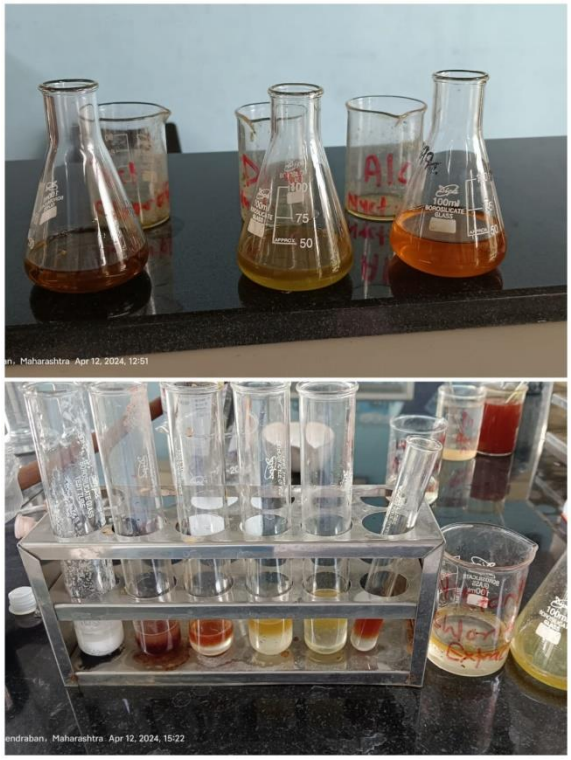

Fig.3 Extraction and test results of *N. arbor-tristis*

Discussion-

The findings from given study shows that in selected plant presence of diverse phytochemicals with medicinal property in them. After 5 days dose of fresh leaf juice of ***Nyctanthes arbor-tristis Linn*** a selected person for sample testing with severe joint pain can feel reduced bone pain and easy movements of joints as well as on inflammation and swelling ***Tridax procumbens*** shows effective results . After

systematically analysing the effect of phytochemicals with richest medicinal properties on human health we can assume that it will be helpful in medicinal field with less side effects and precise application. This will also marks the beneficial application for further research and clinical applications. After finding of various qualitative test, selected plant species rich in glycosides, flavonoids, alkaloids , terpenoids , carbohydrates and saponines. The diverse variety of phytochemicals and their mechanism of action undergo the importance of plants and their bioactive compounds in the medicinal field. Future studies should focus on the use of phytochemical for production of various therapeutic drugs instead of chemical use as natural phytochemicals will show same effect on various diseases with less or no side effect as compare to chemical containing medicines.

Conclusion:

The result obtained from the whole study of extraction and analysis of phytochemicals with medicinal uses from selected sample of plants shows that secondary metabolites are having some medicinal property such as anti-microbial, anti-fungal, anti-inflammatory, anti-oxidative etc. The plant shows medicinal property is due to presence of some bioactive compounds known as secondary metabolites like alkaloids, flavonoids, tannins, lignin's etc. the selected plant extract contain following secondary metabolites with having medicinal property after performing various qualitative tests.

Plant Samples	Obtained secondary metabolites
Tridax procumbens	Alkaloid, flavonoid, glycosides, carbohydrates, saponine
Ficus racemosa	Alkaloids, flavonoids, glycosides, saponines
Nycthanthes arbor-tristis	Alkaloids ,flavonoids, terpenoids, glycosides, saponines

Hence from the above study it is assume that due to presence of anti-bacterial, anti-inflammatory, anti-bacterial property in some plants can be as traditional medicines for various physiological conditions as well as diseases.

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