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Original article

Study on the qualitative phytochemical analysis of ethanolic extract of Terminalia paniculata bark

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Abstract

Aim- The present paper deals with phytochemical studies on the ethanolic extract of Terminalia paniculata, a potential medicinal plant from Sengottai, Tamil Nadu, India.

Materials & methods - The stem bark was screened for phytochemical composition. The extraction method followed was the Soxhlet method. A small quantity of ethanol extract was dissolved in 5 ml of distilled water and then filtered. The filtrate was tested to detect the presence of different phytochemical constituents in the sample.

Results- Phytochemical analysis of test extract revealed presence of valuable phytocompounds like alkaloids, saponins, terpenoids, flavonoids, tannins which can be useful in managing many ailments.

Conclusion- The results of the present study show that, the presence of many valuable phytocomponents of Terminalia paniculata which could be well considered for further investigation for its medicinal and therapeutic efficacy.

Keywords: Terminalia paniculata, phytochemicals, flavonoids, antioxidant.

Introduction

Medicinal plants usage has great importance since ancient times as they are widely used for the treatment of diseases all over the world[1]. According to a WHO report above 80% of the world's population is taking interest in indigenous medicinal plant remedies[2]. Some chemical components in plants have therapeutic significance because they have a specific physiological effect on people[3]. During the past few decades, the traditional system of medicine has drawn tremendous attention for in vivo studies. For this reason, more research is carried out in order to determine the toxicity of medicinal plants and their products.

Therefore, it is essential to investigate traditional medicine to identify and exploit safe and effective remedies for ailments of both microbial and non-microbial origin[4]. The use of phytochemicals with established actions could be of great significance in therapeutic approaches. Alkaloids, flavonoids, saponins, terpenoids, phenolics, tannins, etc., and other natural bioactive substances found in plants called phytoconstituents function in concert with nutrients and fibers to form an integrated aspect of the body's defence mechanism and considered as major constituents in crude drugs. Antioxidants are common low-concentration chemicals that can stop free radical-mediated processes from oxidatively damaging biomolecules (proteins, nucleic acids, polyunsaturated lipids, and carbohydrates)[5]. The massive, 20–30 m tall, deciduous *Terminalia paniculata* Roth., Syn. Pentaptera paniculata (Family: Combretaceae) tree has rough, peeling, brown to dark brown bark, a clear bole of about 10 m, and simple, upper alternate, lower opposite, oblong or elliptic, acute or acuminate, pale brown with two glands near the base of the midrib below, main nerves 10-15 pairs; flowers reddish brown, sessile, fruits reddish brown-winged, one wing broad and the other two narrow, widely distributed along the Western Ghats of India[6,7]. Many parts of plant have seen to contain phytochemicals which might be having antioxidant properties and can be beneficial in various acute and chronic ailments. In the past, cholera, diabetes, inflammatory parotid

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glands, menstruation problems, cough, germs, wounds, ulcers, worms, skin diseases, leprosy, and anaemia have all been treated with *Terminalia paniculata* flower juice and bark. In addition to its spermicidal action, *Terminalia paniculata* is used to treat diabetes, hepatitis, bronchitis, cough, and heart debility.[6,7,8].

The present was conducted to investigate the preliminary phytoconstituents of ethanol bark extract of *T. paniculata*

Materials and methods

Collection and extraction of plant material

The bark of Terminalia paniculata (1 Kg) was dried in the shade, and pulverized to coarse powder with the help of a suitable grinder. The powder obtained was stored in an airtight and clean glass container and used in the process of extraction. It was extracted with 99% ethanol for 48 hrs using a Soxhlet extractor. The extract obtained was evaporated under a vacuum to remove the solvent completely and concentrated to obtain a dark reddish semisolid residue. The plant was authenticated by Dr.Sharadha, Professor, Department of Pharmacognosy, Challa Malla Reddy College of Pharmacy, Hyderabad.

Soxhlet extraction procedure

We refer to this procedure as continuous heat extraction. It is a Soxhlet extractor, a glass-based apparatus. It is made up of a top condenser, extraction chamber, syphon tube, and round bottom ask. The porous bag (thimble), composed of strong filter paper or clean fabric, is filled with dried, ground, and fine coarsed powder plantmaterial and sealed tightly. The bottom flask is filled with the extraction solvent, and then the thimble is placed inside the extraction chamber. The medication is extracted by the solvent in the extraction chamber after it is heated from the bottom flask, evaporates, and condenses there before slowing down and passing through the condenser. Consequently, when the level of solvent in the extraction chamber reaches the top of the siphon, the solvent and the extracted plant material flow back to the flask[9-12,13,14,15]. When there is no more trace of the drug left behind after the solvent pours out of the extraction chamber, the process is repeated until the drug is completely removed.

Qualitative Phytoconstituents evaluation of Ethanolic extract of *T. paniculata*(ETP) bark

The standard procedure was followed to screen Ethanolic *T. paniculata*(ETP) bark for the presence of various phytoconstituents like carbohydrates, flavonoids, polyphenolic compounds, tannins, triterpenoids, etc.

Test for Carbohydrates

Fehling's test

Fehling's A (0.5ml) was added to of Fehling's B (0.5ml) solution and to this mixture, ETP(2ml) extract was added. The mixture was heated in boiling water for 5-10 minutes and observations were noted. A yellow followed by a brick-red precipitate shows the presence of carbohydrates.

Benedict's test

ETP(0.5ml) was taken in a test tube and to this, 0.5ml of Benedict's reagent was added. The mixture was boiled for 5 minutes and a brick-red precipitate shows the presence of carbohydrates.

Molisch's test

ETP (2-3 ml) was taken in a test tube and a few drops of α-naphthol solution were added. The test tube was shaken and conc. H_2SO_4 was added to the walls. Carbohydrates can be detected by a violet ring at the intersection of two liquids.

Test for Saponins(Froth test)

ETP(3ml) was taken in a test tube and was shaken vigorously and is kept aside for 3 minutes. Formation of honeycomb-like froth indicates the presence of saponins.

Test for Flavonoids

Shinoda test

0.5ml ETP is taken in a test tube and to it, 0.5ml dil. hydrochloric acid was added followed by the addition of few pieces of magnesium turnings. Observation were noted. A pink or reddish pink color shows the presence of flavonoids.

Lead acetate test

Lead acetate solution was added to 1 millilitre of ETP, and the presence of flavonoids is indicated by a yellow precipitate.

Sodium hydroxide test

To 200mg of ETP, 2ml of dil. NaOH was added and formation of yellow color which gets decolorized on the addition of dil. HCl shows the presence of flavonoids.

Tests for steroids

Salkowski reaction

Chloroform and conc. H_2SO_4 were added to 2 ml of ETP. firmly shaken. Acid layer exhibits fluorescence that is greenish yellow whereas the chloroform layer appears red.

Liebermann-Burchard reaction

2ml of the ETP was mixed with chloroform. 1-2ml of acetic anhydride was added and 2 drops conc.

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H_2SO_4 was added from the sides of the test tube. First red, then blue and finally green color appears.

Test for Tannins and Phenolic compounds

To 2ml of ETP, following reagents were added:

- 5% $FeCl_3$ solution ⟶ Deep blue-black color
- Lead acetate solution ⟶ White precipitate
- Bromine water ⟶ Decoloration of bromine water
- Dil. Iodine solution ⟶ Transient red color

Legal's Test for glycosides

To ETP, 1ml pyridine and 1ml sodium nitroprusside were added. Pink to red color appears.

Test for Triterpenoids

About 0.5 g of ETP in a separate test tube was taken with 2 ml of chloroform; 5 ml of concentrated sulphuric acid was carefully added to form a layer and observed for the presence of reddish-brown color interface to show positive results for the presence of terpenoids.

Test for Proteins

Biuret's test

1ml of hot ETP was taken in a test tube and to it, add 0.5ml of w/v of sodium hydroxide and 0.1ml of 3% copper sulfate solution. A red or violet color shows the presence of proteins.

Millon's test

A small quantity of plant leaf extract was treated with a few drops of Million's reagent and observed for the formation of a white precipitate which indicates the presence of protein.

Ninhydrin test for Amino acids

3ml of ETP was heated and to it 2-3 drops of 5% Ninhydrin solution was added. For ten minutes, the mixture was heated in a water bath. The purple or bluish color indicates the presence of amino acids.

Test for Fats and Oils

The extract is taken on a filter paper and a permanent stain on the filter paper indicated the presence of fats and oils.

Test for Gums

ETP was hydrolysed using dil. HCl. This solution was subjected to Fehling's test and red color indicates the presence of gums[16-19].

Results

The present study was designed to evaluate the phytochemical constituents of the ethanolic extract of *Terminalia paniculata* bark. Phytochemical studies revealed that the ethanol extract exhibited a positive reaction for the maximum number of metabolites. Alkaloids, triterpenoids, anthracene glycosides, coumarins phenols, and saponins were present in ethanol solvent.

Table 1: Results of Phytochemical screening of ETP

S. No	Name of the Phytochemical	ETP
1.	Carbohydrates	+
2.	Amino acids	-
3.	Proteins	+
4.	Alkaloids	+
5.	Cardiac glycosides	+
6.	Triterpenoids	+
7.	Saponins	+
8.	Flavonoids	+
9.	Phenolic compounds	+
10.	Tannins	+
11.	Steroids	+
12.	Gums	-

ETP: ethanolic extract of terminalia paniculata Where (+) means positive & (-) means negative.

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Discussion:

From immemorial times plants and their products are used by man as medicines. Chronic illness patients tend to favour therapeutic drugs from the traditional/conventional medical system. India is the world's top producer of medicinal plants among other nations. Approximately 20,000 medicinal plants have been identified as of today in India, and 25,000 potent plant-based remedies are employed in traditional medicine. The Combretaceae family of plants is extremely rich in phytochemicals, which have a variety of essential physiological and pharmacological effects, according to published research reports. Natural chemicals play a vital part in therapeutic applications due to their minimal side effects. In recent years because of their biological and pharmacological significance, people have become more aware of the role that carbohydrates play in the biological system and in medicine. Anti-inflammatory, anticoagulant, and antithrombotic properties are possessed by carbohydrates.

Flavonoids have been linked to improve health in both humans and animals, and there is currently interest in using them for chemoprevention and illness treatment. Approximately 6000 flavonoids are known to be responsible for the vibrant colors of fruits, vegetables, herbs, and medicinal plants. Inhibiting acetylcholinesterase (AChE), a crucial enzyme in the central nervous system, raises neuronal acetylcholine levels, which is one treatment option for mild to severe AD symptoms[21]. The protective effects of flavonoids on the central nervous system are well documented, particularly with regard to neurological conditions resulting from the combination of oxidative stress, inflammation, and transition metal buildup. Citrus flavanones, including naringenin and hesperidin, may be able to cross the blood-brain barrier and be useful in treating neurodegenerative illnesses, according to another study[22]. The role of flavonoids in antidiabetic activity and anti-aging has also been reported[23-26]. Glycosides engage in a variety of pharmacological processes, including immune regulation, CNS and cardiac stimulation, and broad-spectrum antibacterial action against bacteria, fungi, viruses, and parasites[27].

Another well-known phytoconstituent is tannins which possess antioxidant properties being widely used in both food and medicine. Numerous investigations have been carried out in the last few years to determine tannins having pertinent antioxidant activity. Due to their ability to act as antioxidants, which can help prevent cancer, heart disease, and osteoporosis, tannins have gained a lot of attention [26,28]. According to a study by Phung et al., Japanese chestnut extracts had the most remarkable capacity to scavenge DPPH. Based on the findings, tannins appeared to have promising antioxidant properties as a possible natural preservative[29].As a polyphenolic component, tannic acid is one of the most significant kinds of tannins. Because of its ability to prevent monoamine oxidase and reduce neurodegeneration, this substance is referred to as a potent antidepressant. A prevalent psychological condition that affects a lot of people is memory loss. It can be challenging to identify the underlying reasons of the condition, which makes effective treatment much more challenging. A person with this chronic condition may have disruptions in their emotions, thinking, and physical demeanour, which might culminate in a severe amnesia cycle. Throughout ancient times, numerous herbs and mixtures have been utilized to cure depression [30].

According to published findings, several triterpenoids, saponins have antibacterial and anti-inflammatory properties. Their varied functions have been discovered, encompassing hepatoprotective, anti-inflammatory, anti-bacterial, anti-allergic, immunomodulatory, anti-tumor, molluscicidal, and anti-Alzheimer's disease properties, in addition to hemolytic toxicity[31-34]. Some of the Terminalia genus species have been employed as effective therapeutic drugs due to their presence phytochemicals. The alcoholic extract of *T. paniculata* bark was utilized to investigate the plant's potential for protection in a broad range of applications in the field of complementary and alternative medicine due to its rich and diverse phytochemical features, which were indicated by previous researchers as well as by the detailed investigation carried out in this work.

Conclusion

The results of the current study showed that *Terminalia paniculata* bark has a wide range of phytochemical components, including alkaloids, gallic acid, tannins. The plant bark were also rich in phenols and flavonoids, a major group of antioxidants. In medication, the safety and mode of the use of medicine are very important. When

compared to pharmaceutical drugs, natural plant compounds usually show negligible side effects. Based on the phytoconstituent's results and existing literature, it can be concluded that *T. paniculata* bark is an excellent medicinal plant and further studies can be continued to reveal its therapeutic efficiency.

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