QUALITATIVE PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL STUDY OF CROTON SPARSIFLORUS

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Abstract

Phytochemicals are the medicinally active compounds found in plant parts such as root, bark, leaves, flower and seeds. Present research study is based on phytochemical screening of *Croton sparsiflorus.* The plant extract was prepared in different solvents including ethanol, ethyl acetate and hexane. The ethanol plant revealed the presence of alkaloid, tannins, flavonoid and cardiac glycoside. Phytochemical test of ethyl acetate plant extract showed alkaloid, tannins, flavonoid and terpenoid were as hexane extract revealed the presence of alkaloid, reducing sugar, flavonoid. All three-leaf extract were tested against gram-positive *S. aureus* and gram-negative *E. coli*. Higher zone of inhibition was found for *E. coli* in ethanol extract.

Keywords: Phytochemical screening, Soxhlet, plant extract, antibacterial.

Introduction

Chandrapur forest region in (MS) India possesses vast array of plants. This forest found to contain vast number of medicinal plants and local people are utilizing these medicinal plants since year ago. Plants are natures “chemical factories” providing richest source of organic chemical on the earth. Most of the medicinal plants from this forest are used in traditional medicine to cure various sicknesses and diseases. The indigenous system of medicine namely Ayurveda, Unani and Siddha have been in existence in several centuries. These systems of medicine cater to the need of nearly seventy percent of our population residing in the villages. In Homeopathy system, 70% of the medicines are prepared from plants. Nature has bestowed on us a very rich botanical wealth and large number of diverse type of plants grow wild in different part of country. India is a country rich in indigenous herbal resources which grow on their varied topography and under changing climate condition permitting the growth of almost 20,000 plants are of medicinal value. In Indian scenario, it has been recognized that 25,00 plants have been found to be have medicinal value out of 17,000 plants. The world is looking toward India for new drugs to manage various challenging diseases because of its rich biodiversity of medicinal plants and abundance of traditional knowledge such as Sibbha, Ayurveda etc., to cure different diseases. The study is aimed at investigating phytochemical and antibacterial properties of *Croton sparsiflorus*leaves from Chandrapur district forest (MS) India. The plants species is used in traditional medicine since long. Leaf juice with honey and ginger oil is used to cure fever, headache, cough, tiredness, shivering, delirium and biliousness by the Irulars in Tamil Nadu. Leaf is used to cure diarrhea, wounds Urinary complaints. Mixture of leaf and root paste is given for headache.

Treatment of human diseases is becoming more complicated since the pathogenic microorganisms get multiple drug resistance. Hence, there is an increasing need to identify antimicrobial compounds from plants. Many phytochemicals have been isolated from weeds which possess antimicrobial activity. *Croton sparsiflorus* Morong (synonym= Crotonbonplandianum Bail) belongs to the family Euphorbiaceae is a weed found in southern parts of Indian subcontinent. It is known as Eliamanakkuin Tamil. This plant is one of the disturbances in agricultural fields which compete with crop plants for space and nutrition. However, the latex ofthis plant is being used in folk medicines by tribal people of Tamil Nadu totreat wasp sting [1]. According to

them, traditional medical practitioners use fresh plant parts than dried ones to treat most of the diseases generally [1] Whole plant extracts of *C. sparsiflorus* were evaluated for hypotensive activity [2], anti-pyretic and anti-inflammatory properties [3]. However, very meager number of studies on the antibacterial activity [4-6] and antifungal activity [7] *of C. sparsiflorus* are available in literatures. The validity of the medicinal properties of *C. sparsiflorus* by scientific analysis has been slowly coming up. Presence of alkaloids and other compounds have been reportedin this weed [8-11].

Previous studies on antibacterial activity of *C. sparsiflorus* were conducted using different solvent extracts of oven dried whole plant, dried leaf powder and fresh leaf extract. However, ethanolic fresh leaf extract of *C. sparsiflorus* has been tested for antibacterial studies. The present study was aimed at screening the antibacterial potential of extract isolated from fresh leaf of *C. sparsiflorus* using ethanol. Though, lignins and tannins are reported to have antimicrobial activity in manyplants but they are not yet identified in *C. sparsiflorus* with antimicrobial properties. Hence, we also carried out histo chemical localization of lignins and tannins in leaves.

Classification of natural product

The most important natural sources of drugs are higher plants, microbes & animals and marine organisms. Some useful products are obtained from minerals that are both organic and inorganic in nature. To follow the study of the individual drugs, one must adopt some particular sequence of arrangement and this is referred to a system of classification of drugs. 1.Alphabetical classification 2.Morphological classification 3.Taxonomic classification 4. Pharmacological classification 5.Chemical classification 6.Chemo-taxonomical classification

1. Alphabetical Classification: Alphabetical classification is the simplest way of classification of any disconnected items. Crude drugs are arranged in alphabetical order of their Latin and English names (Common names) or sometimes local some of the pharmacopoeias, dictionaries and reference books which classify crude drugs according to this system are as follows:

1.1 Indian Pharmacopoeia 1.2 British Herbal 1.3 British pharmaceutical Codex 1.4European Pharmacopoeia

2. Morphological classification: In this system the drugs are arranged according to the morphological or external characters of the plant parts nor animal parts i.e. Which part of the plant is used as a drug e.g. (leaves, roots, stems etc.)The drugs which obtained from the dried parts of the plants & containing cellular tissues are called as Organized Drugs e.g. Rhizomes, barks, leaves, fruits, entire plants, hairs, & fibers. The drugs which are prepared from plants by some intermediate physical processes such as incision, drying or extraction with a solvent and not containing any cellular plant tissues are called as unorganized drugs such as (Aloe juice, opium latex, agar, gambir, gelatin, tragacanth, benzoin, honey, beeswax, lemon grass oil etc.) Organized Drugs.

Woods: Quassia, Sandalwood, Red Sandalwood.

Leaves: Digitalis, Mint, Senna, Spearmint, Squill, Tea.

3. Taxonomical Classification: Taxonomical classification is purely a botanical classification; it's based on principles of natural relationship & evolutionary developments. They are grouped in Kingdom, Phylum, Order, Family, Genus & Species. As all the entire plants are not used as drugs, parts of plant are used as a drug. For example, Cinnamon bark. This it is of no significance from identification point of view to put plants in a taxonomic order.

4. Pharmacological Classification: In this system grouping of drugs according to their pharmacological action or of most important constituent or their therapeutic use is termed as pharmacological or therapeutic classification of drug. This classification is more relevant and is mostly followed method. Drugs like digitalis, squill and strophanthus having cardiotonic action are grouped together irrespective of their parts used or phylogenetic relationship or the nature of phytoconstituents they contain.

5. Chemical classification:The crude drugs are divided into different groups according to the chemical nature of their most important constituent. Since the pharmacological activity and therapeutic significance of crude drugs are based on the nature of their chemical constituents. The chemical classification of drugs is dependent upon the grouping of drugs with identical constituents. An out of this classification is as follow:

1.carbohydrate,
2.Glycosides,
3.Tannin,
4.Lipids,
5.Fats,
6.wax

6. Chemotaxonomic Classification:

This system of classification relies on the chemical similarity of taxon i.e. it is based on the existence of relationship between constituents in various plants. There are certain types of chemical constituents that characterize certain classes of plants. This gives birth to entirely new concept of chemotaxonomy that utilizes chemical facts / characters for understanding thetaxonomical status, relationships and the evolution of the plants. For example, tropane alkaloidsgenerally occur among the members of Solanaceae thereby, serving as a chemotaxonomic marker.

Materials and Method

Plants Collection:

The plant named *Croton Sparsiflorus*was collected from Chandrapur forest region. The leaves of *Croton Sparsiflorus* were thoroughly washed with water and dried under shade for about ten days. The dried plant sample were milled into a fine powder in a mixture grinder. The powder was stored in air sealed polyethylene bag at room temperature before extraction.

Preparation of Ethanol extract:

100 g of the dried powder of leaf *Croton Sparsiflorus* were extracted at room temperature with 500 ml absolute ethanol for 72 h. extraction was done using the Soxhlet apparatus briefly 100gm of powder steam was stored inair sealed polyethylene bag & placed in Soxhlet& extracted with absolute ethanol The extraction was done until the solvent in the Soxhlet turned colorless. The extract was concentrated by recovering the solvent using the Soxhlet apparatus until the extract became just pourable. It was poured into a beaker & this was then used for the analysis.

Preparation of Ethyl acetate extract:

100 g of the dried powder of leaf *Croton Sparsiflorus* were extracted at room temperature with 500 ml absolute ethyl acetate for 72 h. extraction was done using the Soxhlet apparatus briefly 100gm of powder steam was stored in air sealed polyethylene bag & placed in Soxhlet& extracted with absolute ethanol The extraction was done until the solvent in the Soxhlet turned colorless. The extract was concentrated by recovering the solvent using the Soxhlet apparatus until the extract became just pourable. It was poured into a beaker & this was then used for the analysis.

Preparation of hexane extract:

100 g of the dried powder of leaf *Croton Sparsiflorus* were extracted at room temperature with 500 ml hexane solution for 72 h. extraction was done using the Soxhlet apparatus briefly 100gm of powder steam was stored inair sealed polyethylene bag & placed in Soxhlet& extracted with absolute ethanol The extraction was done until the solvent in the Soxhlet turned colorless. The extract was concentrated by recovering the solvent using the Soxhlet apparatus until the extract became just pourable. It was poured into a beaker & this was then used for the analysis. The entire phytochemical analysis test is common for the above solvent Ethanol, Ethyl acetate and Hexane.

Phytochemical Analysis:

The extracts were analyzed for the presence of Alkaloids, Terpenoids, Tannin, Saponin, Flavonoid, Anthraquinone, Reducing Sugar, Glycoside and Cardiac glycoside [8 to 11].

Alkaloid: About 0.2 g of the extracts was warmed with 2% $H2SO_4$ for two minutes. It was filtered and few drops of dragendoff reagent were added. Orange red precipitated indicates the presence of alkaloids.

Tannin: Small quantity of extracts was mixed with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green solution indicates the presence of tannins.

Anthraquinones: About 0.5 g of the extracts was boiled with 10% HCl for few minutes in a water bath. It was filtered and allow to cool. Equal volume of $CHCl_3$ was added to the filtrated Few drops of 10% NH_3 were added to the mixture and heat. Formation of rose-pink color indicates the presence of anthraquinones.

Glycoside: The extracts were hydrolyzed with HCl solution and neutralized with NaOH solution. A few drops of Fehling's solution A and B were added. Red precipitate indicates the presence of glycoside.

Reducing Sugars: The extracts were shaken with distilled water and filtered. The filtrate was boiled with drop of Fehling's solution for minutes. An orange red precipitate indicates presence of reducing sugar.

Saponin: About 0.2 g of the extract was shaken with 5 ml of distilled water and then heated to boil. Frothing (appearance of creamy miss of small bubbles) shows the presence of saponins.

Flavonoids: Extracts of about 0.2 g was dissolved in diluted NaOH and HCl was added. A yellow solution that turns colorless, indicates the presence of flavonoids.

Phlobatannins: The extracts (0.5 g) were dissolved in distilled water and filtered. The filtrate was boiled with 2%HCl solution. Red precipitated show the presence of Phlobatannins.

Terpenoids (Salkowski test): 0.2 g of extracts was mixed with 2 ml Chloroform ($CHCl_3$) and 3 ml concentrated $H2SO_4$ was carefully added to form a layer. A reddish-brown coloration of the interface was formed to indicate positive results for the presence of terpenoids.

Cardiac glycosides: Five ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated H2SO4. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brow ring, while in acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Antimicrobial screening of extracts:

Susceptibility test was carried out. The modified agar well diffusion method [6,7] is usedto test the antimicrobial activity of the extracts. The medium employed was diagnostic sensitivity agar. The culture was prepared in triplicate and incubated at 37 °C for 24 to 72 h. 0.2 ml of the both culture of the test organism was put in a sterile Petri-dish and 18 ml of sterile molten diagnostic sensitivity agar, was added. Well were bored into the medium using 0.1 ml of the extracts. Streptomycin and Chloramphenicol were used as the standard antimicrobial agents at a concentration of 10 mcg/disk, 30 mcg/disk respectively. The plates were kept in sterilized inoculation chamber for 2 h to facilitate diffusion of the antimicrobial agents into the medium. The plates were then incubated at 37 °C for 24 h and the diameter of zone of inhibition of microbial growth were measured in the plates in millimeters.

Result and Discussion

The qualitative analysis of extracts from ethanol and leaf of *Croton Sparsiflorus* showed the presence of photochemical constituents such as alkaloid, tannins, flavonoid, cardiac glycoside. The results are summarized in **Table 1**. The above results indicate that, the leaf of plant investigated are rich in alkaloid, tannins, flavonoid, cardiac glycoside. The Ethanol extracts have showed absence of anthraquinone, Glycoside, saponin, and terpenoid, reducing sugar. Extracts of leaf were tested against gram-positive *S. aureus* and gram-negative *E. coli* and foundmoderately sensitive against Gram-positive *S. aureus* and gram-negative *E. coli* with zone of inhibition 13 mm & 17 mm respectively (as shown in **Table 2**). Ethanol extract was showed more antimicrobial activity than standard antibiotics streptomycin and chloramphenicol. The inhibitory activity of this extract confirmed the potential use of the plant in the treatments of microbial induced ailments.

Phytochemical screening of ethanol, ethyl acetate, hexane extracts of *Croton Sparsiflorus* leaf is shown in **Table 1**.

Table1: Phytochemical tests of ethanol, Ethyl acetate, hexane extract of plant *Croton Sparsiflorus* leaves

Chemical Composition	Ethanol Extract	Ethylacetate Extract	Hexane Extract
Alkaloid	Present	Present	Present
Tannin	Present	Present	absent
Anthraquinone	Absent	Absent	Absent
Glycoside	Absent	Absent	Absent
Reducing sugar	Absent	Absent	Present
Saponin	Absent	Absent	Absent
Flavonoid	Present	Present	Present
Phlobatannins	Absent	Absent	Absent
Terpenoid	Absent	Present	Absent
Cardiac glycoside	Present	Absent	Absent

Table 2. Antimicrobial activity of leaf extract in ethanol

Sr. No.	Bacterial strain	Zone of inhibition (mm in diameter)
1.	*S. aureus*	13
2.	*E. coli*	17

The qualitative analysis of extracts from ethyl acetate and leaf of *Croton Sparsiflorus* showed the presence of photochemical constituents such as alkaloid, tannins, flavonoid, terpenoid. The results are summarized in **Table 1**. The above results indicate that the leaf of plant investigatedrich in alkaloid, tannins, flavonoid, terpenoid. The Ethyl acetate extracts have showed absence of anthraquinone, glycoside, saponin, cardiac glycoside and reducing sugar. Extracts of leaf were tested against gram-positive *S. aureus* and gram-negative *E. coli*. Ethyl acetate was found activity against *S.aureus* and *E. coli*with zone of inhibition 09 mmand 15mm respectively(as shown in **Table 3**). Ethanol extract was showed more antimicrobial activity than standard antibiotics streptomycin and chloramphenicol. The inhibitory activity of this extract confirmed the potential use of the plant in the treatments of microbial induced ailments

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Table 3. Antimicrobial activity of leaf extract in ethyl acetate

Sr. No.	Bacterial strain	Zone of inhibition (mm in diameter)
1.	*S. aureus*	09
2.	*E. coli*	15

The qualitative analysis of Hexane extracts and leaf of *Croton Sparsiflorus* showed the presence of photochemical constituents such as alkaloid, flavonoid, reducing sugar. The results are summarized in **Table 1**. The above results indicate that the leaf of plant investigated are rich in alkaloid, flavonoid, reducing sugar. The Hexane extracts have showed absence of tannin, anthraquinone, glycoside, saponin, terpenoid, cardiac glycoside. Extracts of leaf were tested against gram-positive *S. aureus* and gram-negative *E. coli*. Hexane were found to be moderately sensitive against S. aureus and *E. coli* with zone of inhibition 11 mm & 13mm respectively (as shown in **Table 4**). Hexane extract was showed more antimicrobial activity than standard antibiotics streptomycin and chloramphenicol. The inhibitory activity of this extract confirmed the potential use of the plant in the treatments of microbial induced ailments.

Table 4. Antimicrobial activity of leaf extract in hexane

Sr. No.	Bacterial strain	Zone of inhibition (mm in diameter)
1.	*S. aureus*	11
2.	*E. coli*	13

Conclusion

Screening of plant revealed the presence of several phytochemical compounds. Our present investigation is focused on the topic that showed the presence of chemicals in plant extract which have antibacterial value and can play important role in the treatment of human ailment.The ethanol plant revealed the presence of alkaloid, tannins, flavonoid and cardiac glycoside. Phytochemical test of ethyl acetate plant extract showed alkaloid, tannins, flavonoid and terpenoid were as hexane extract revealed the presence of alkaloid, reducing sugar, flavonoid. All three-leaf extract were tested against gram-positive *S. aureus* and gram-negative *E. coli*. Higher zone of inhibition was found for *E. coli* in ethanol extract.

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