



Research Article

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EXTRACTION AND ANTIMICROBIAL POTENTIAL OF SECONDARY PLANT METABOLITES FROM *ARTABOTRYS HEXAPETALUS* (LINN. F.) BHANDARI

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ABSTRACT

Plants are the important sources for several drugs. In recent years many drug formulations are based on plant products. The present study was carried out with an objective to investigate the antibacterial and antifungal potentials of leaves of *Artabotrys hexapetalus* Linn. belonging to the family Annonaceae. It is widely distributed throughout the southern part of the Asia and china. In the present study, methanolic extracts of leaves of *Artabotrys hexapetalus* Linn was evaluated for potential antimicrobial activity against medically important bacterial and fungal strains. The antimicrobial activity was determined in the extracts using agar well diffusion method. The antibacterial and antifungal activities of extracts (25, 50, 75 and 100 µg/ml) of *Artabotrys hexapetalus* were tested against ten human pathogenic bacteria; and four fungal strains. The results revealed that the remarkable inhibition of the microbial growth was shown against the tested organisms. Phytochemical analysis of the plant was also carried out. The microbial activity of the *Artabotrys hexapetalus* was due to the presence of various secondary metabolites. Hence, this plant can be further studied to discover bioactive natural products that may serve as lead molecules in the development of new pharmaceutically important compounds.

Keywords: Antimicrobial activity, phytochemical analysis, Secondary metabolites, synthetic drugs, Traditional knowledge.

INTRODUCTION

Now a day's formulations of several new drugs are based on plant products. Medicinal plants are the richest bio-resources of drugs of traditional systems of medicine, modern medicines, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.¹ Nearly 80 % of the world's population relies on traditional Medicines for primary health care and most of which involve plant extracts.² In India 95 % of prescriptions were plant based in the traditional systems of Unani, Ayurveda, Homeopathy and Siddha.³ All plants produce chemical compounds as part of their normal metabolic activities. These phytochemicals are divided into (1) Primary metabolites such as sugars and fats, which are found in all plants; and (2) Secondary metabolites, which are found in smaller proportions in plants, serving a more specific function. Hence, these plants can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals research activities. *Artabotrys hexapetalus* is globally distributed in India, Sri Lanka, Java and South China. Within India, it is indigenous to South India and very commonly cultivated in gardens throughout the country for its fragrant flowers. *Artabotrys* is a climbing tree of 10m tall. Branchlets are glabrous. petiole is 4 - 8 mm; leaf blade is oblong, broadly lanceolate and papery, secondary veins are 8 - 16 cm on each side of midvein and adaxially prominent. Inflorescences is 1 or 2 flowered. Flowers are 2.5 - 3 cm in diameter and fragrant. Sepals are three or two, green, ovate, 5 - 8 mm, sparsely puberulent. Petals are six in number, greenish to yellowish, usually thick and fleshy, biseriolate, hypogynous. Stamens are many, adenate anthers connectively produced into an oblong head. Carpels are numerous or rarely few in number. Seeds are pale brown,

1.5 – 2 cm, smooth. The odour of these flowers is sweet and fresh. The flower oil is used in perfumes.

Artabotrys hexapetalus (Linn. F.) bhandari

Synonym - *Artabotrys odoratissimus* R. Br.

Family - Annonaceae.

Habitat - Southern India, largely grown in gardens.

Ayurvedic - Panasagandhi, (Harit) Champaka.

Siddha/Tamil - Manoranjidam.

Folk - Haraa champaa (north), Kathari champaa; Hirvaa champaa (Maharashtra).

Action - Cardiac stimulant, uterine stimulant, muscle relaxant

Taxonomic Position

Kingdom	-	Plantae
Super-division	-	Spermatophyte
Sub division	-	Magnoliophyta
Class	-	Magnoliopsida
Sub class	-	Magnolidae
Order	-	Magnoliales
Family	-	Annonaceae
Genus	-	<i>Artabotrys</i>
Species	-	<i>hexapetalus</i>

Useful Parts

All parts of the plant are useful.

- Leaves are mainly used against cholera and as antifungal.
- In Chinese traditional medicine the roots are used for treating malaria.
- Flowers yield essential oil which has antifungal activity.
- The use of flowers is mentioned in the perfume industry also used for preparing tea like beverage.

- Fruits are eaten by tribal and rural folk and have cardiac depressant activity.⁴⁻⁶

Ethno-medicinal Use

- Drug in the form of leaf decoction is used for cholera by the tribal's of Malay Archipelago.
- In the Chinese traditional system fruits are used in scrofula.
- Pulp of ripened fruits has been used traditionally in some part of Assam for the fungal infections in the domestic animals.⁴⁻⁶
- Decoction of leaves has been found to exhibit antifertility effects in rats.⁷
- The essential oil of this plant has show excellent antihelminthic property against tapeworms, earth worms and round worms.⁸
- Fruits of this plant are recoded as containing fixed and volatile oil, glycosides and resins. Extracts are reported to exhibit hypotensive and sparmogenic as well as cardiac stimulating effects on some animals and cardiac depressant on others.⁹
- Ethanol and benzene extracts of leaves has shown irregular oestrus cycle in albino rats.
- The essential oil from the flower is used in the aroma therapy; even the essential oil obtained from the flower is used as substitute for the famous Ylang-Ylang oil.¹⁰

MATERIALS AND METHODS

Collection of Sample

The fresh and healthy leaves of the plant *Artabotrys hexapetalus* Linn were collected between March and June months from various areas of Guntur and Krishna districts, Andra Pradesh, India. The plant was identified accordingly to various literatures, including other pertinent taxonomic literature. Plant parts were collected on the basis of the information provided in the ethnobotanical survey of India.

Collection of Microorganisms

The micro organisms were collected from Department of Biotechnology, Acharya Nagarjuna University, Guntur, India and they were reconfirmed by gram staining and sub culturing in appropriate elective media.

Preparation of Extracts

The leaves were washed thoroughly 2 - 3 times with running tap water, and then leaf material was shade dried. After that the plant material was grinded with suitable pulveriser and powder was kept in small plastic bags with paper labelling.

Cold Extract

Methanol, Ethyl acetate, Chloroform, Acetone, Hexane, hydroalcohol and Water extract were prepared by soaking 1 g of the dry powered plant material in 10 ml of respective solvents at room temperature for 48 hours and filtered through a Whatman filter Paper No: 1. The filtrate was stored at 4°C for further use.

Hot Extracts

The grinded leaves material of 25 g weighed using an electronic balance and it was extracted with 900 ml of methanol by using a Soxhlet extractor for 72 hours at a temperature not exceeding the boiling point of the solvent. The extracts were filtered using Whatman filter paper (No.1) while hot, concentrated in vacuum under reduced pressure using rotary flask evaporator, and dried in a desiccator. The extracts were then kept in sterile bottles, under refrigerated conditions, until further use. The dry weight of the plant extracts was obtained by the solvent evaporation and used to determine concentration in µg/ml. The extract was preserved at 2 - 4°C. This crude extracts of methanol was used for further investigation for potential of antimicrobial properties

Qualitative Phytochemical Analysis

Phytochemical Screening

Preliminary qualitative phytochemical screening was carried out with the following methods by using cold extract.

Detection of Alkaloids: Extract was dissolved individually in dilute Hydrochloric acid and solution was clarified by filtration.

- **Mayer's Test:** Filtrate was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.
- **Wagner's Test:** Filtrate was treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.
- **Dragendorff's Test:** Filtrate was treated with Dragendorff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.
- **Hager's Test:** Filtrate was treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

Detection of Phenols

- **Ferric Chloride Test:** The filtered solution of extract was treated with three drops of freshly prepared 1 % ferric chloride and potassium ferrocyanide. Formation of bluish-green colour was taken as positive.
- The methanol extract was dissolved in water. Few crystals of ferric sulphate were added to the mixture. Formation of dark-violet colour indicated the presence of phenolic compounds.

Detection of Flavonoids

- **Alkaline Reagent Test:** The Extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute HCl acid, indicates the presence of flavonoids.
- **Lead Acetate Test:** The Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Detection of Anthroquinones

- **Free Anthroquinones Test: (Borntrager's test)** The extract of the plant material (equivalent to 100 mg) was shaken vigorously with 10 ml of benzene, filtered, and 5 ml of 10 % ammonia solution added to the filtrate. Shake the mixture and the presence of a pink, red, or violet colour in the ammonia (lower) phase indicated the presence of free anthroquinones.
- **Modified Borntrager's Test:** The Extract was treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

Detection of Phytosterols

- **Salkowski's Test:** The extract was dissolved in 2 ml chloroform in a test tube. Conc. Sulphuric acid was carefully added on the wall of the test tube to form a lower layer. A reddish brown colour at the interface indicated the presence of a steroid ring (i.e., the aglycone portion of the glycoside).
- **Liebermann Burchard's Test:** The Extract was treated with chloroform and filtered. The filtrate was treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

Detection of Terpenoids: The extract was added to 2 ml of acetic anhydride and conc. H₂SO₄. Formation of blue, green rings indicate the presence of Terpenoids.

Detection of Fatty acids: The extract was mixed with 5 ml of ether. These extract was allowed for evaporation on filter paper and dried the filter paper. The appearance of transparency on filter paper indicates the presence of fatty acids

Detection of Tannins

- **Ferric Chloride Test:** The extract was dissolved in water. The solution was clarified by filtration; 10 % ferric chloride solution was added to the clear filtrate. This was to observe a change in colour to bluish black.
- **Lead Acetate Test:** The extract was dissolved in water and to that 10 % Lead acetate solution was added. The appearance of yellow precipitate confirms the tannins.
- **Potassium Dichromate Test:** The extract was dissolved in water to that strong potassium dichromate solution was added, a yellow colour precipitate indicates the presence of tannins and Phenolic compounds.

Detection of Saponins

Froth Test: Extract was diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15

minutes. Formation of 1 cm layer of "honey comb" froth indicates the presence of saponins.

Anthocyanins: The extract was added to 2 ml of 2 N HCl and ammonia. The appearance of pink-red colour which turns into blue-violet colour indicates the presence of anthocyanins.

Leucoanthocyanins: The extract was added to 5 ml of isoamyl alcohol. Upper layer appears red in colour which indicates the presence of leucoanthocyanins.

Coumarins: 3 ml of 10 % NaOH was added to the extract, formation of yellow colour indicates the presence of coumarins

Emodins: 2 ml of NH₄OH and 3 ml of Benzene was added to the extract. Appearance of red colour indicates the presence of emodins

Detection of Reducing Sugars: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

- **Fehling's Test:** Filtrates were hydrolyzed with dil. HCl, neutralized with alkali and heated with Fehling's A and B solutions. Formation of red precipitate indicates the presence of reducing sugars.
- **Keller - Kiliani Test (for de-oxy sugars in cardiac glycosides):** Methanol extract was obtained and the extract was reduced to dryness. 50 mg of this was dissolved in 2 ml chloroform. H₂SO₄ was added to form a layer and the colour at inter phase was recorded. Brown ring at inter phase is characteristic of deoxysugars in cardenolides.

Preparation of Inoculum

The inoculums for the experiment were prepared in fresh nutrient broth and fresh Sabouraud's broth from pure bacterial and fungal cultures respectively before 24 hours.

Anti Microbial Activity

Agar well Diffusion Method

Agar well-diffusion method was used to determine the antimicrobial activity. Both Nutrient agar (NAM) and Sabouraud Dextrose Agar (SDA) media were prepared then the media was poured into the plates and allowed to solidify. Inoculum of 24 h culture was spreaded on the plate with the help of L shaped rod. Wells were punctured using 0.05 mm cork borer on each plate. The plant extracts were added to the wells. The plates were inoculated in an upright position at 37°C for 24 h for in the incubator. Microbial growth was determined by measuring the diameter of zone of inhibition using microbial scale.

RESULTS AND DISCUSSION

Qualitative Phytochemical Analysis

The results of preliminary phytochemical screening were given in Table 1.

Table 1: Qualitative Phytochemical analysis of various solvents

Phytochemical compound	Methanol	Ethyl acetate	Chloroform	Hexane	Acetone	Water	Hydroalcohol
Alkaloids	+	+	-	-	+	+	+
Phenolics	+	-	-	-	+	+	+
Flavonoids	-	-	-	-	-	+	+
Anthraquinones	-	-	-	-	-	-	+
Steroids	+	-	-	-	-	+	+
Fatty acids	-	-	-	-	-	+	+
Tanins	+	+	-	-	+	+	+
Saponins	-	-	-	-	-	-	+
Anthocyanins	-	-	-	-	-	-	+
Leucoanthocyanins	-	-	-	-	-	-	+
Coumarins	-	-	-	-	-	-	+
Emodins	-	-	-	-	-	-	+
Reducing sugars	+	-	-	-	-	+	+

+ indicates Positive and - indicates Negative

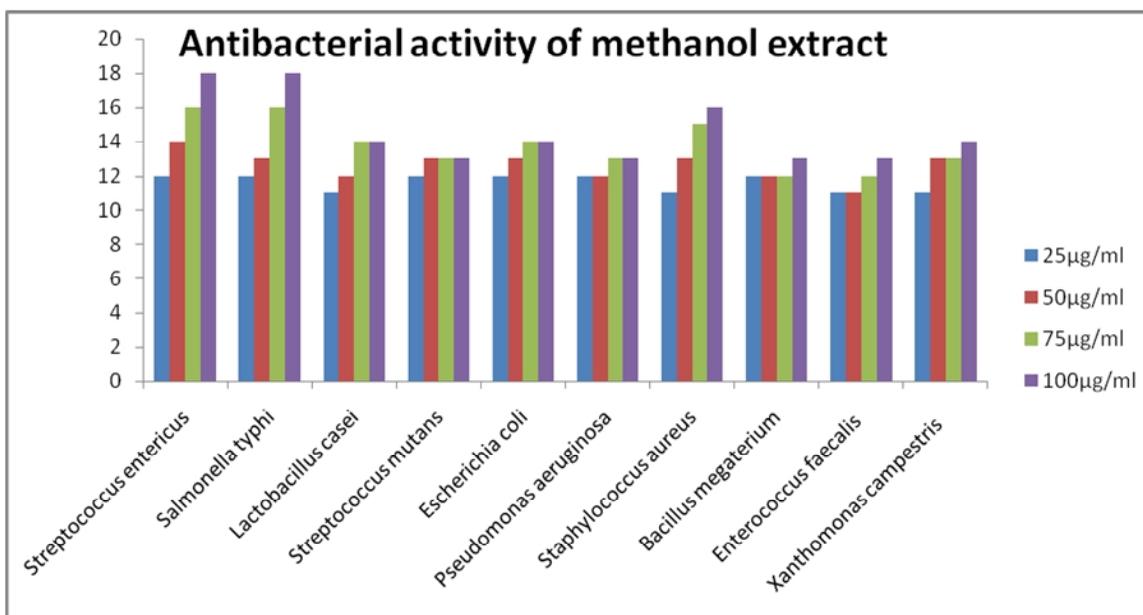


Figure 1: Graphical representation of antibacterial activity

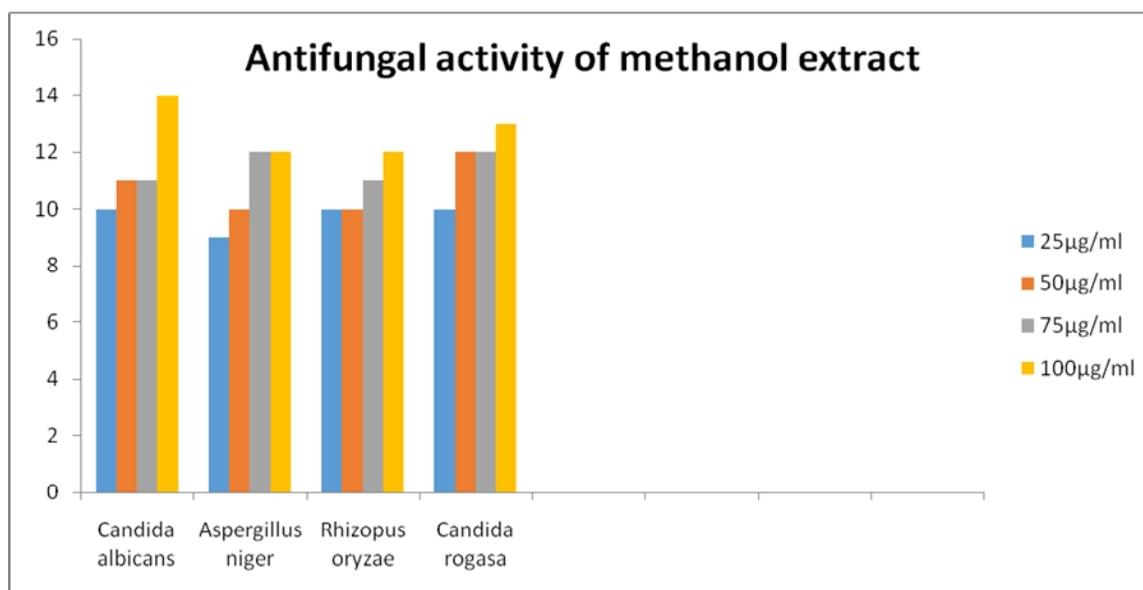


Figure 2: Graphical representation of antifungal activity

It shows the presence of flavonoids, alkaloids, phenolic compounds, tannins, anthroquinones, reducing sugars, fatty acids and steroids of *Artabotrys hexapetalus*. Reducing sugars, alkaloids, phenolics and tannins were present in methanol extract. Alkaloids, steroids, tannins were present in ethyl acetate extract. Chloroform and acetone extracts shows the presence of only tannins and alkaloids respectively. Water extract shows the presence of alkaloids, steroids, Flavonoids, tannins, and saponins and hydroalcoholic extract shows the presence of all compounds.

Antimicrobial Activity

Determination of Zone of Inhibition

The main objective of this study was to investigate the antibacterial and antifungal potentials of leaves of *Artabotrys hexapetalus* Linn. *In vitro* antibacterial and antifungal activities were examined by using methanol extract. These antibacterial and antifungal activities of plant leaf extracts were done against ten pathogenic bacteria and four pathogenic fungi by using the agar well diffusion method.¹¹⁻¹³ Each purified extracts were dissolved in dimethyl sulfoxide, sterilized by filtration using sintered glass filter, and stored at 4°C. For the determination of zone of inhibition, all the extracts were screened for their antibacterial activity against the *Salmonella typhi*, *Streptococcus entericus*, *Lactobacillus casei*, *Streptococcus mutans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus megaterium*, *Enterococcus faecalis* and *Xanthomonas campestris* and antifungal activity against *Candida albicans*, *Aspergillus niger*, *Rhizopus oryzae* and *Candida rogasa* (Figure 1 and 2). The sets of four dilutions (25, 50, 75 and 100 µg/ml) of *Artabotrys hexapetalus* extract were used and control was prepared by taking only DMSO without plant extract. The zones of growth inhibition around the wells were measured after 18 to 24 hours of incubation at 37°C for bacteria and 48 to 96 hours for fungi at 28°C. The sensitivities of the microorganism species to the plant extracts were determined by measuring the sizes of inhibitory zones (including the diameter of well) on the agar surface around the wells, and values < 8 mm were considered as not active against microorganisms. The results showed remarkable inhibition of the microbial growth against the tested organisms and lack of inhibition in control. The phytochemical analysis of the plant was also carried out. The microbial activity of the *Artabotrys hexapetalus* was due to the presence of various secondary metabolites. Hence, these plants can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals research activities.

CONCLUSION

A rapid search of new antimicrobial substances, natural products may act as alternative for several drugs, antibiotics and chemotherapeutic agents in certain circumstances. The results showed that the methanolic extract of *Artabotrys hexapetalus* Linn leaf was able to inhibit all of the 10 bacterial and 3 fungal strains used in this study with different degrees of inhibition. Our comparative study revealed that *Artabotrys hexapetalus* Linn methanolic extract is more effective against the bacterial strains than the fungal strains.

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