

**PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF BARK EXTRACT
OF WRIGHTIA TINCTORIA AND COMPARATIVE STUDIES WITH AMOXICILLIN**

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Article Received on 27/01/2025

Article Revised on 17/02/2025

Article Published on 10/03/2025

ABSTRACT

Plant extract have been used for a wide variety of purpose of many thousands of years. In particular the antimicrobial activity of plant extract has formed the basic of many application including raw and processed food preservation, pharmaceutical, alternative medicine and natural therapies. The bark of wrightia tinctoria is considered for anti-dirrheal, aphrodisiac, Anti-helmalit is febrifuge, stomachic, tooth ache, tonic. It has antibacterial, anti inflammatory, antioxidant, antifungal, anticancer, anti diabetes, antiviral activity. More over studies Pharmacognosy, antimicrobial activity and photochemistry part of wrightia tinctoria. Thebark of wrightia tinctoria contain bioactive compound such as alkaloids, saponins, quinine, tannins, glycosides, flavonoids, phenolic substance, terpenoids. It has antibacterial activity against both gram positive and gram negative studies have shown that extract from the bark effectively inhibit staphylococcus aureus and escherichia coli. The antimicrobial potential was assessed using the agar well diffusion method against bacteria. Among the different bacteria strains a more inhibitory action was observed against staphylococcus aureus and compare to standard drug amoxicillin.

KEYWORDS: Wrightia Tinctoria, Anti Microbial, Gram Positive, Anti inflammatory, Staphylococcus aureus, Escherichia Coli, Amoxicillin.

INTRODUCTION

In recent years, multiple drug resistance in human pathogenic microorganisms has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of such diseases. Over the last three centuries, intensive efforts have been made to discover clinically useful antimicrobial drugs. Plant extracts have been used for a wide variety of purposes for many thousands of years. In particular, the antimicrobial activity of plant extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine, and natural therapies.

Antimicrobials of plant origin are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. Wrightia tinctoria R.Br. and Wrightia arborea (Densst.) Mabb. belong to the family Apocynaceae. They are distributed in all districts of deciduous forest of India W. tinctoria is commonly called as "Indrajav" and locally as Pandhara kuda, while W. arborea is known as Tambda kuda. These

species have been important in the traditional healing. However, the former one is widely recognized medicinal plant.

The bark of W. tinctoria is considered for antidiarrhoeal, aphrodisiac, anthelmintic, febrifuge, stomachic, toothache, tonic and dog bite. It is employed in seminal weakness and flatulence, also used in piles and skin diseases. Whereas, a preparation of the bark made from Wrightia arborea is found useful in menstrual and renal complaints. Moreover, several studies viz. pharmacognosy, antimicrobial activity and phytochemistry on different parts of these two species studied earlier. This work however, is designed to evaluate the comparative account phytochemical components and antibacterial activity of bark of both species on selected bacterial strains.

The bark of Writghtia tinctoria contains bioactive compounds such as flavonoids, alkaloids and tannis which exhibit strong antibacterial activity against both gram positive and gram negative bacteria. Studies have shown that extract from the bark effectively inhibit

staphylococcus aureus, the primary bacteria responsible for boils as well as Escherichia coli.

Research works on phytochemical constituents and pharmacological activities of Wrightia Tinctoria were reviewed.

ANTIBACTERIAL ACTIVITY

C.S. Sailaja et al., (2024) This study evaluates the inhibitory properties of successive extracts from the leaves and bark of Wrightia tinctoria for phytochemical constituents and antimicrobial efficacy against four bacteria. The leaves and bark were shade-dried and extracted using methanol, petroleum ether, and water. Qualitative phytochemical analysis revealed the presence of primary and secondary metabolites, including proteins, phenols, tannins, flavonoids, terpenoids, glycosides, saponins, and coumarins. The antimicrobial potential was assessed using the agar well-diffusion method against four pathogenic bacteria: E. coli, Pseudomonas fluorescence, Bacillus subtilis, and Streptococcus mutans. Methanol and petroleum ether extracts exhibited significant inhibitory activities, with the highest inhibition observed in E. coli from leaf petroleum ether extract (30 mm inhibition zone). These findings suggest the potential of W. tinctoria extracts in developing novel phytomedicines.

M.S. Khyade et al., (2011) Comparative phytochemical and antibacterial activities of bark of Wrightia tinctoria and Wrightia arborea were investigated. The bark extracts showed the presence of alkaloids, phenolics, saponins and tannins in both the species. The antibacterial activities of bark of W. tinctoria and W. arborea in successive different solvent were tested against gram +ve and gram -ve organisms. The chloroform extracts of W. arborea showed the broader spectrum of antibacterial activity when compared with W. tinctoria. However, Wrightia tinctoria is a widely used medicinal plant.^[1]

ANTIINFLAMMATORY ACTIVITY

S.Rajkumar et al., (2023) In this present study, the phytochemical constituents and anti-inflammatory activity of Wrightia tinctoria leaf and bark samples are estimated. The solvents such as acetone, aqueous, ethanol, and methanol are added with leaf and bark extracts of W. Tinctoria plant and evaluated. The preliminary screening test is executed for the presence of secondary metabolites and was reported in order to understand the phytochemicals of leaf and bark. Both qualitative and quantitative analysis of alkaloids, coumarins, flavonoids, phenols, proteins, saponins, steroids, tannins, terpenoids, and quinones were done. Each extracted solvents are found to be recorded with alkaloid content. Aqueous and methanol extracts of W. tinctoria leaf and bark have been tested for their ability to replicate anti-inflammatory action. The medicinal plant W. tinctoria leaf and bark extract with aqueous solvent gave excellent anti-inflammatory properties when

compared with methanolic solvents of bark and leaf at 500 µg/ml concentration respectively. Obviously, W. tinctoria aqueous leaf extract showed extraordinary anti-inflammatory activities. Hence the medicinal plant W. tinctoria is more suitable as a drug for biomedical properties in the human era.

A. Jyotriam et al (2015) Since free radicals play such an important role in the disease scenario of an individual, a thorough understanding of the various physiologically significant free radicals is of paramount importance before the search of the radical scavengers or the antioxidant principles to treat the physiological disorders caused by them such as inflammation.

G.R. Rajalaskshmi et al., (2012) Ethyl alcohol and aqueous extract of Wrightia tinctoria were investigated for anti-inflammatory activity by HRBC method. The prevention of hypo tonicity induced HRBC membrane lysis was taken as a measure of antiinflammatory activity and these extracts shows biphasic effects. Their activities are compared with standard drug diclofinac sodium.

P.R. Tharkar et al (2010) In the present study, the bark of Wrightia tinctoria was investigated for anti-inflammatory activity by carrageenan- induced rat paw oedema and cotton pellet induced granuloma method. The various extracts showed inhibition of rat paw oedema and percent granuloma changes at dose of 200mg/kg when compared to control group. The activity was compared with that of standard drug diclofenac sodium (13.5 mg/kg /b w, p.o).^[2]

ANTIDERMATOPHYTIC ACTIVITY

M. Deventhiran et al., (2016) Medicinal plants are the most important source of life saving drugs for the majority of the world population. Dermatophytes is common contagious fungal diseases, discovery of new drugs are essential against dermatophytes. The aim of the present study is to screen the phytochemical compounds in the Chloroform leaf extracts of Wrightia tinctoria by GC-MS analysis and carry out in-vitro antifungal activity against seven dermatophytes fungi. The chemical composition of W.tinctoria Chloroform leaf extract using PerkinElmer Gas Chromatography - Mass Spectroscopy analysis revealed the presence of eight major compounds. The antifungal activity was carried out by agar well diffusion method against seven fungi and the tested sample showed remarkable activity in comparison to standard antibiotic drug Clotrimazole. The obtained result interprets that W. tinctoria possess potential anti-dermatophytic activity.^[3]

ANTIOXIDANT ACTIVITY

S. Rajkumar et al., (2023) In the Indian traditional system of medicine, Wrightia tinctoria R.Br. (Apocyanaceae) is recognized as a biologically effective therapeutic plant for the treatment of jaundice. It is a natural medicinal tree possessing antidiabetic, anti-inflammatory, hepatoprotective, antinociceptive,

antifungal, antibacterial, antipsoriatic, antiviral, anthelmintic, anticancerous, analgesic, antipyretic, and aphrodisiac activities. Methanolic extract of *W. tinctoria* plant was investigated with Gas Chromatography– Mass Spectrometry (GC-MS), Fourier transform infrared spectroscopy (FTIR) and the antioxidant activity of *W. tinctoria* was analysed by most probable hydrogen peroxide scavenging (H₂O₂) activity, DPPH activity and Thiobarbutaric acid. The phytochemical in *W. tinctoria* leaf and bark have been assessed by GC-MS analysis. GC-MS analysis of *W. tinctoria* methanolic extract exposed the existence of the GC-MS chromatograms of 10 peaks in the leaf and 20 peaks in the bark. The FTIR spectroscopy analyses were identified by various functional compounds in the extracts with distinctive peak values. The FTIR analysis of methanolic leaf extracts of *W. tinctoria* confirmed the presence of alcohol, amine, alkane, carboxylic acid, sulfur compounds, halogen compounds and alkyne which showed major peaks. Also the methanolic bark extracts of *W. tinctoria* confirmed the presence of amines, phenol, alcohols, alkane, aldehydes, carboxylic acid, nitrogen compounds, sulfur compounds, and halogen compounds which also exhibited major peaks. Significant antioxidant activity is displayed by the plant part of leaf and bark sample. The results obtained in the determination of antioxidant activity of MEWT displayed considerable free radical scavenging capacity against DPPH which generated free radicals. This study includes the identification of phytochemicals and antioxidant potential of methanolic and aqueous extract of *W. tinctoria* which assist in therapeutic claims about this species in the traditional medicinal plant system.

Nausheen Khan et al., (2021) *Wrightia tinctoria* R.Br. (Apocyanaceae) is known as a biologically effective plant for the treatment of jaundice in the Indian traditional system of medicine. It is a wild medicinal tree possessing anti-inflammatory, antidiabetic, antinociceptive, hepatoprotective, antibacterial, antifungal, antiviral, antipsoriatic, anticancerous, anthelmintic, aphrodisiac, analgesic, and antipyretic activities. Its constituents are of utmost interest to pharmaceutical industries owing to their many actions and biological activities.^[4]

ANTIFUNGAL ACTIVITY

K.V. Devika et al., (2012) Nature is a valuable source of active ingredients that needs to be explored, especially its utilization in the medical field. Owing to the limited availability of antifungal drugs and also considering their side effects, there is always a constant need for a safe and competent alternative. *Wrightia tinctoria*, a medicinal tree, has been reported to possess potent antifungal activity against commercially available candida strains [American type culture collection (ATCC) and microbial type culture collection (MTCC)]. This could be beneficial clinically only if its antifungal activity could be proved against candida species isolated from clinical samples as the commercially available candida strains

might have lost its pathophysiological characteristics on repeated subcultures. Hence, with this background, we performed this study to determine the antifungal efficacy of the extracts obtained from the leaves of *W. tinctoria* against the candida species isolated from the oral cavity. The aim of this study was to determine the antifungal efficacy of *W. tinctoria* on candida species isolated from the oral cavity.^[5]

ANTICANCER ACTIVITY

R.Mini et al., (2019) *Wrightia Tinctoria* belongs to the family Apocynaceae is a small deciduous tree with pale grey, smooth bark, distributed in tropical Africa and Asia. The plant is commonly known as Paalai and generally called “Sweet Indrajao”. The juice of the tender leaves is used as an effective drug for treatment of jaundice. The crushed fresh leaves when filled in the cavity of decayed tooth relieve toothache. The leaves are a fodder for the cattle, goat and sheep. In south India the plant is used for green manuring rice fields. The leaves of this tree yield a blue dye called pala indigo. *W. Tinctoria* leaves were soaked in coconut oil for few hours and applied for eczema psoriasis and other skin diseases. The *W. tinctoria* flower has been reported to have a good anti-inflammatory activity. Bark and seeds are used to cure bilious infections, psoriasis, leprosy, asthma and various skin diseases. Many authors reported pharmacological activity of *W. Tinctoria* plant like antiulcer activity, anti-inflammatory activity, wound healing activity, anthelmintic activity and antimicrobial activity.

E.S Jesy et al., (2017) The present study is based on the evaluation of preliminary phytochemical screening, antitumor, cytotoxic and antioxidant activities of leaf oil of *Wrightia tinctoria* (Oil B). The oil B from *Wrightia tinctoria* leaves was subjected to phytochemical screening tests by using standard procedures. The free radical scavenging activity of both the extracts was measured by using the 1, 1- diphenyl-2-picrylhydrazyl (DPPH) assay. The dose dependent increase was observed in DPPH activity of leaf oil B (0.125, 0.25, 0.50, 1 µg/ml). Oil B was investigated for its short term cytotoxicity on DLA cells by trypan blue dye exclusion method. In vivo antitumor activity was studied on DLA tumor bearing mice. Activity was assessed by monitoring the mean survival time and solid tumor volume. *Wrightia tinctoria* leaf oil was proved to be an effective anticancer and antioxidant agent. These results suggest that the oil B has potential of antioxidant and antitumor activity that support the ethno pharmacological uses of this plant. The remarkable activity showed by the plant could be attributed to the synergic effect of the active compounds present in it.^[6]

ANTIMICRIBIAL ACTIVITY

A.Thambidurai et al., (2023) Antimicrobial textiles are functionally active textiles, which may kill the microorganisms or inhibit their growth. The present article explores the applications of natural antimicrobial compounds used to prepare antimicrobial textiles.

Different types of antimicrobial textiles including: antibacterial, antifungal, and antiviral have also been discussed. Different strategies and methods used for the detection of a textile's antimicrobial properties against bacterial and fungal pathogens as well as viral particles have also been highlighted. These antimicrobial textiles are used in a variety of applications ranging from households to commercial including air filters, food packaging, health care, hygiene, medical, sportswear, storage, ventilation, and water purification systems. Public awareness on antimicrobial textiles and growth in commercial opportunities has been observed during past few years. Not only antimicrobial properties, but its durability along with the color, prints and designing are also important for fashionable clothing; thus, many commercial brands are now focusing on such type of materials. Overall, this paper summarizes the scientific aspect dealing with different fabrics including natural antimicrobial agents along with their current functional perspective and future opportunities.

Sravanthi Maddila et al., (2017) The crude hexane, ethyl acetate and aqueous leaf extracts of medicinal plant *Wrightia tinctoria* R.Br. were evaluated for in vitro antimicrobial activity against selected bacterial and fungal strains. Preliminary phytochemical screening of the three crude leaf extracts revealed the presence of phenols, alkaloids, tannins, flavonoids, terpenoids and sterols. The extracts were screened against three Gram positive strains (*Bacillus subtilis*, *Bacillus licheniformis* and *Streptococcus pneumoniae*), two Gram negative strains (*Escherichia coli* and *Sphingomonas sanguinis*) and against three fungal strains (*Aspergillus flavus*, *Aspergillus niger* and *Candida albicans*). Antimicrobial activity of these crude extracts was determined using Agar well diffusion method. Agar broth and Potato dextrose broth dilution method was used to determine the minimum inhibitory concentration. Ampicillin and Flucanazole were used as standards for bacteria and fungi respectively. The results of in vitro antimicrobial activity showed that hexane, ethyl acetate and aqueous crude leaf extracts were active on all microbial strains tested at different concentrations, with hexane leaf extract exhibiting more activity. Results obtained from the present study showed that the leaf extracts of *Wrightia tinctoria* R.Br. have considerable antimicrobial activity and hence, they may be used for further extraction and isolation of antimicrobial compounds which may find a place in medicine industry as constituents of antibiotics.

K.Moorthy et al., (2014) An in-vitro antimicrobial study was done using methanolic and petroleum ether extracts from the leaves of *Wrightia tinctoria* (Roxb.) R.Br. BauerKirby (disc diffusion) and broth dilution methods were employed for the assessment of antimicrobial activity against 14 microorganisms. Methanolic extract of *Wrightia tinctoria* leaves showed significant antimicrobial activity against *Cryptococcus neoformans* (36.0mm), *Staphylococcus aureus* (27.2mm), *Candida*

albicans (25.0mm), *S.epidermidis* (23.2mm) and *Bacillus subtilis* (20.2mm), whereas petroleum ether leaves extract showed significant antimicrobial activity against *S.aureus* (25.0mm), *C.neoformans* (21.8mm), *S.epidermidis* (18.5mm) and *C.albicans* (16.0mm) were observed. According to broth dilution method, the methanolic extract of plant material showed the MIC values against *C.neoformans* (256µg/ml), *S.aureus* and *C.albicans* (512µg/ml) respectively. Whereas, the petroleum ether extract of *Wrightia tinctoria* showed the MIC values against *S.aureus* and *C.neoformans* (512µg/ml) and *S.epidermidis* and *C.albicans* (1,024µg/ml) with a significant inhibitory activity. The present result revealed that methanolic and petroleum ether extracts of *Wrightia tinctoria* possesses both antibacterial and antifungal activity.

Beena Jose et al., (2014) *Wrightia tinctoria* has been extensively used in Folk medicine. It has been reported to have good analgesic, anti-inflammatory, anthelmintic, antiulcer, antidysentric, antidiabetic, anticancer, antipyretic activities and also effective in the treatment of psoriasis. The present investigation was undertaken to statistically evaluate the antimicrobial activity of the leaf and bark extracts of *Wrightia tinctoria*.

P.Vedhanarayanan et al., (2013) In the present investigation, antibacterial activity of different extracts (Chloroform, ethanol and methanol) of *Wrightia tinctoria* has been studied against the human pathogenic bacterial strains, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* by disc diffusion method on agar. The findings showed potential antibacterial properties of the extracts against the organisms tested. Among the three solvents tested, ethanol extract of leaf showed higher inhibition zone. Ethanol extract of *Wrightia tinctoria* exhibits maximum zone of inhibition against *Escherichia coli* (29 mm), *Bacillus subtilis* (24 mm) *Staphylococcus aureus* (30 mm) and *Pseudomonas aeruginosa* (24 mm). Preliminary phytochemical analysis of *Wrightia tinctoria* showed the presence of alkaloids, flavonoids, phenols, saponins, steroids and tannins.^[7]

ANTIDIABETIC ACTIVITY

A. Shruthi et al., (2012) In the present study, investigation has been carried out to evaluate the effect of the different extracts of the leaves of *Wrightia tinctoria* on alloxan induced diabetic rats of wistar strain. The experiment was carried out using six groups of albino rats. Chloroform extract showed a significant anti-diabetic activity when compared to the standard drug glibenclamide.

M.Sandhya Rani et al., (2012) The present work was undertaken to investigate various extracts of fruit of *Wrightia tinctoria* (F-Apocyanacea) for anti-diabetic activity in alloxan induced diabetic rats. A comparison was made between the action of extracts and known anti-diabetic drug glibenclamide (10mg/kg body weight).

Oral administration of methanolic extract at a dose of 300 mg/kg/b. wt and ethyl acetate extract at a dose of 200 mg/kg/b. wt exhibited a significant ($p < 0.001, p < 0.001$) hypoglycemic activity in normal rats and significant ($p < 0.001, p < 0.001$) anti-hyperglycemic activity in alloxan induced diabetic rats respectively. The maximum reduction in blood glucose level was observed after 4 hours in case of methanolic and ethyl acetate extracts with a percentage protection of 37% and 42% respectively. In long term treatment of alloxan induced diabetic rats the degree of protection was determined by measuring blood glucose on 0, 1, 2, 4, 7, 14th day. Both the extracts showed a significant anti-diabetic activity comparable with that of glibenclamide. These results indicate that the *W. tinctoria* fruit extracts possess significant anti – diabetic activity.^[8]

ANTI PSORIATIC ACTIVITY

S.P. Dhanabal et al., (2012) Antipsoriatic activity was performed at a dose 200 mg/kg body weight in mice (25-30 g). Isoretinoic acid (0.5 mg/kg) was used as the standard. Degree of orthokeratosis, drug activity and the relative epidermal thicknesses were calculated and statistically analyzed. The extract was also evaluated for its antioxidant potential by DPPH, nitric oxide and hydrogen peroxide radical scavenging assays.^[10]

ANTI PLASMODIAL ACTIVITY

Battu Ganga Rao et al.,(2019) An indole alkaloid of tryptanthrin is naturally found in *W.tinctoria* leaves had been found to be active against *Leishmania* species and *Plasmodium falsiparum*. Various tryptanthrin derivatives are being used for the drug development against these protozoans.^[11]

ANTIVIRAL ACTIVITY

Battu Ganga Rao et al.,(2019) The methanol extract of the *W.tinctoria* leaves was found to be have anti-viral activity against hepatitis C virus using Huh 5.2 cell line (a cell line with a persistent viral replication).The aqueous leaf extract having flavonoids, particularly isatin and its derivatives found to have anti-HIV activity by inhibiting HIV-1 integrase enzyme during its 3' processing and strand transfer with IC₅₀ of 1.9 ± 0.5 $\mu\text{g/ml}$ and 1.4 ± 0.3 $\mu\text{g/ml}$ respectively.Hence *W.tinctoria* can also be used for the current menace, AIDS.^[12]

ANTIDIARRHOEAL ACTIVITY

Battu Ganga Rao et al.,(2019) Ethanol extract of *Wrightia tinctoria* bark and steroidal alkaloid fraction derived from it were found to have anti-diarrhoeal activity by showing its effect on prostaglandin inhibition and decreasing intestinal propulsive / spasmodic movement.^[13]

ANTI ANALGESIA ACTIVITY

Battu Ganga Rao et al.,(2019) *Wrightia tinctoria* bark was evaluated using acetic acid-induced writhing test, in which methanol extract showed to have Anti-nociceptive

activity comparable to acetyl salicylic acid. More work is needed to know about the active compounds responsible for the activity. The ethanol extract of bark showed Anti-nociceptive effect and moderate analgesic effect against thermal and chemical stimuli but not mechanical stimulus, which may be due to the presence of steroids. The study also concluded that *W.tinctoria* didn't have any sedative effects. Ethyl acetate fraction of *W.tinctoria* leaves showed analgesic activity and was found to be effective in inhibiting both centrally and peripherally acting pain mechanisms.^[14]

ANTI ULCER ACTIVITY

Battu Ganga Rao et al.,(2019) Hydrochloric acid extract of *W.tinctoria* bark at 1000 mg/ml was found to possess significant antiulcer activity based on evaluating the factors relating to gastric juice, acidity level, protein and carbohydrate content in gastric mucous substances. The abdominal distress caused by ulcer due to bacterial action and food habits is an important area of concern. However, more work in *W.tinctoria* is required to gain insight into its efficacy against ulcer.^[15]

PLANT PROFILE

VITEXNEGUNDOLINN

Common Name	: Sweet Indrajao, pala indigoplant, Dyers oleander, Jaundice curative tree in south india.
Synonyms	: <i>Holarrhena tinctoria</i> R.Br, <i>wrightia antidysenterica</i> , <i>migg wrightia tomentosa</i> (Roxb) Roem and schult, <i>Nerium tinctorium</i> Roxb
Family Name	: Apocynaceae.
Parts used	: Stem Bark.

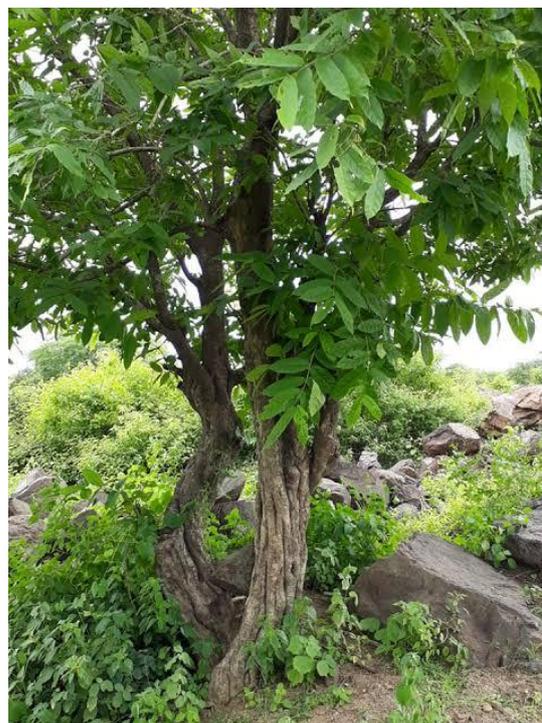


Fig. 1: Vitexnegundolinn.

VERNACULARNAMES

Kannada	:	Veppale, Beppalli, Kodamurki.
Malayalam	:	Ayyapala, Dentappala
English	:	Pala indigo plant.
Telugu	:	Akupala, Amkudu, Dodda pala.
Hindi	:	Mitaindrajou, Dohi, Inrajau.
Tamil	:	Vetpalai, Veppalai, Irumpaalai, Nelapalai, Palai.
Oriya	:	Pita karuan, Duda kerunain, kryo, set kure.
Sanskrit	:	Shweta kudaja.
Marathi	:	Kala-kuda.
Urdu	:	Indearjao shireen.

TAXONOMICAL CLASSIFICATION

Kingdom	:	Plantae.
Clade	:	Eudicots
Clade	:	Angiosperms.
Clade	:	Asterids.
Subfamily	:	Apocynoideae.
Order	:	Gentianales.
Family	:	Apocynaceae.
Genus	:	<i>Wrightia</i> .
Species	:	<i>Wrightia tinctoria</i> .
Tribe	:	<i>Wrightieae</i>

Botanical description: *Wrightia tinctoria* is an ever green tree, which is a medium sized tree and it produces milky white latex.

Leaves: Leaves are simple with opposite leaf arrangement, petiolate, glands axillary and Glabrous ovate, obtusely acuminate and are 10-20 cm long and 5cm width. Sometimes pubescent beneath. Leaf stalks are very short.

Bark: Bark is smooth and yellowish-brown coloured and about 10 mm thick Producing Milky-white latex.

Inflorescence: Inflorescence is terminal and flowers are white, bisexual, actinomorphic and hypogynous. Appear in India from March to May, peaking flowers appears flowers appear in corymb-like cymes, 5-15 cm across, at the end of branches. Flowers have oblong petals which are rounded at the tip, and are similar to the Flowers of frangipani.

Fruits: Fruiting is in august and fruit is cylindrical, blackish-green speckled with white long horn like and united at the tip.

Seeds: The seeds are brown and flat with bunch of white hairs at chalazal end. Seed dispersal is by Wind.

Pollination: Pollination is entomophilous without any direct contact of the biotic factor With pollen or stigma

Fruits: Fruits are mericarpous in origin, dark green in colour and have paired Pendulous follicles joined at the tips which may be long upto 50 cm.

Propogation: Propogation can be done by seeds and stem cuttings. The wood of *W.tinctoria* has uniformly distributed grains and is light in weight. It grows in wide range of soil types and are found to grow as undergrowth in forest and under various plantations like oil plantation etc. The karyotype of *W.tinctoria* (intraspecific polyploidy, disomy) is $2n=20, 22$. They are found in India, Myanmar, Australia, Tropical Africa, Nepal and China.

Traditional uses

- Bark and seeds are used to treat flatulence.
- Root bark extract is used orally as an antidote for snakebite.
- Powder of the bark is used for treatment of kidney stones.
- The plant is used in making hair oils as it has anti-dandruff and anti-inflammatory properties. *Wrightia tinctoria* is the best medicine for Diarrhoea and blood pressure.
- It is used to cure piles by mixing sunthi with decoction of bark of the plant.
- The bark of the plant mixed with cow's urine and applied on skin, turns to be very effective.
- Fresh juice of the bark is mixed with cow milk and used to cure urine problems.
- It is used in the treatment of Rheumatoid Arthritis and osteoarthritis.
- It is also a good remedy for fever, toothache, constipation and stomach ache.
- The milk of this plant is used to treat tooth gum problems.

Table no. 1.

Plant part	Uses
Leaf	Skin disease, Eczema, Jaundice, Wounds
Bark	Boils, Galactagogue, Antipyretics, Antiote, Leprosy, Antidiarrhoeal
Stem	Toothbresh, Cure stomach disorder
Latex	Skin disease, Wound healing
Root	Epilepsy, Laxative
Whole plant	Antioxidant, Antinociceptive
Seed	Piles, Worm infestation, Indigestion
Unspecified	Wounds



Fig. 2: Leaf.



Fig. 3: Bark.



Fig. 4: root.



Fig. 5: Fruit.



Fig. 6: Seed.



Fig. 7: Flower.

Species of *Wrightia*

- *Wrightia angustifolia*
- *Wrightia annamensis*
- *Wrightia antidysenterica*
- *Wrightia arborea*
- *Wrightia candollei*
- *Wrightia coccinea*
- *Wrightia collettii*
- *Wrightia cunninghamii*
- *Wrightia demartiniana*
- *Wrightia dolichocarpa*
- *Wrightia dubia*
- *Wrightia flavirosea*
- *Wrightia hanleyi*
- *Wrightia indica*
- *Wrightia karaketii*
- *Wrightia laevis*
- *Wrightia lanceolata*
- *Wrightia lecomtei*
- *Wrightia natalensis*
- *Wrightia novobritambica*
- *Wrightia palawanensis*
- *Wrightia poomae*
- *Wrightia puberula*
- *Wrightia pubescens*
- *Wrightia religiosa*
- *Wrightia saligna*
- *Wrightia siamensis*
- *Wrightia sikkimensis*

- *Wrightia siritiae*
- *Wrightia tinctoria*
- *Wrightia tokiae*

Table no. 2.^[16]

S.no	Plant part used	Compounds isolated
1.	Leaves	Lupeol, α - and β - amyryn, Indigotin, Indirubin, tryptanthrin, Isatin, Rutin, β sitosterol, Triacntanol, Myristic acid, Palmitoleic acid, Palmetic acid, Stearic acid, Behenic acid, Arachidic acid.
2.	Flowers	Lupeol, α - and β - amyryn, Indigotin, Indirubin, tryptanthrin, Isatin, Rutin, β sitosterol, Triacntanol, Myristic acid, Palmitoleic acid, Palmetic acid, Stearic acid, Behenic acid, Arachidic acid.
3.	Stem	Lupeol, α - and β - amyryn, Indigotin, Indirubin, tryptanthrin, Isatin, Rutin, β sitosterol, Triacntanol, Myristic acid, Palmitoleic acid, Palmetic acid, Stearic acid, Behenic acid, Arachidic acid.
4.	Bark	Lupeol, α - and β -amyryn
5.	Seed	Lupeol, Chlorogenic acid, Dihydrocanaric acid, Glycerol, Erythritol, Thritol, Dgalactose, D-mannose, 14 α -methyl zymosterol, Desmosterol, Clerosterol, 24- methylene-25-methyl cholesterol, 24-dehydropollinastanol, 24-methylcholesterol, 24-methylene cholesterol, 24-ethyl cholesterol, 24 ethyl 22 Edehydrocholesterol, Isofucosterol, cholesterol, Palmetic acid, stearic acid, Behenic acid, Arachidic acid
6.	Mature seedpods	α - and β -amyryn, Lupeol, Ursolic acid, Oleanolic acid, Isoricinolic acid, β sitosterol.
7.	Imature seedpods	α - and β -amyryn, Cycloartenone, Cycloeucaenol, Wrightial, β -sitosterol.

PREPARATION OF EXTRACT

1. SOXHLET METHOD EXTRACTION

Ethanolic extract was made by soxhlet extraction method. About 25 g of powdered material mixed with 250ml of ethanol at constant temperature 40 to 60°C. The extract obtained was filtered through Whatmann filter paper and residue water content was evaporated (40°C) by using heating mandle. This extract was stored in refrigerator and used for screening of phytochemicals and antibacterial activity.



Fig. 8: Soxhlet extraction of wrightia tinctoria bark.

PRELIMINARY PHYTOCHEMICAL SCREENING

Preliminary phytochemical analysis was performed for all two solvent extracts of *Wrightia tinctoria*. All two extracts were qualitatively analysed for identification of various phytoconstituents such as alkaloids, saponins, quinines, tannins, glycosides, flavonoids, phenolic

compounds, proteins, terpenoids and steroids by using standard protocols.

Mayer's test (Alkaloids Test)

A 2 mL of 1% HCl was added to 5 mL of extract. The mixture was then mixed with Mayer's reagent. Alkaloids are indicated by turbidity.

The foam test (saponin test)

After mixing 5 mL of extract with 5 mL of distilled water, the mixture was heated. The presence of saponins is indicated by the production of stable foam.

Quinine testing

A few drops of strong sulfuric acid were poured along the test tube's edges to 1 mL of extract. Quinines are indicated by their red appearance.

Tannin test (ferric chloride test)

A few drops of FeCl₃ were added after 2 mL of the extract and 2 mL of distilled water were mixed together. Tannins are indicated by green precipitate.

Glycosides test (Fehling's test)

A 1 mL of Fehling's solution was boiled and added to 1 mL of extract. Glycosides are indicated by orange precipitate.

Flavonoid test (lead acetate test)

One millilitre of 10% lead acetate solution was added to one millilitre of extract. The presence of flavonoids is shown by the production of a yellow precipitate.

Phenolic substances(ferric chloride)

A tiny amount of the extract was diluted with water. A solution of ferric chloride was used the dilute. Phenolic substances are indicated by a violet colour.

Protein test (Biuret test)

Two drops of 0.1% copper sulphate and 10% sodium hydroxide were used to treat the extract. Proteins are indicated by a violet or pink tint.

Terpenoids (salkowski test)

A 5 mL of extract was mixed with 2 mL of chloroform and 3 mL of strong sulfuric acid in that order. Terpenoids are indicated by a reddish-brown interface in the solution.

Steroid (liberman test)

Two millilitres of acetic anhydride and one millilitre of extract were combined with 2 mL of sulfuric acid. Steroids are present if the colour changes from violet to blue or green.



Fig. 9: phytochemical test of extract of wrightia tinctoria bark.

RESULT

Preliminary phytochemical analysis

Preliminary phytochemical analysis was performed for ethanolic extract. This were qualitatively analysed for identification of various phytoconstituents. The preliminary phytochemical studies showed the presence of alkaloids, quinine, tannis, flavonoids, phenolic substance, terpenoids, Steroids.this the result were showed in table(4).

Table no:3.

S.no	Secondary Metabolites	Phytochemical Test	Ethanolic Extract of Bark
1.	Alkaloids	Mayer test	+
2.	Saponins	Foam test	-
3.	Quinies	Few drops of strong H ₂ SO ₄ + 1 ml of extract	+
4.	Tannins	Ferric chloride test	+
5.	Glycosides	Fehling's test	-
6.	Flavonoids	Lead acetate test	+
7.	Phenolic substances	Ferric chloride test	+
8.	Terpenoids	Salkowski test	+
9.	Steroids	Liebermann-Burchard test	+

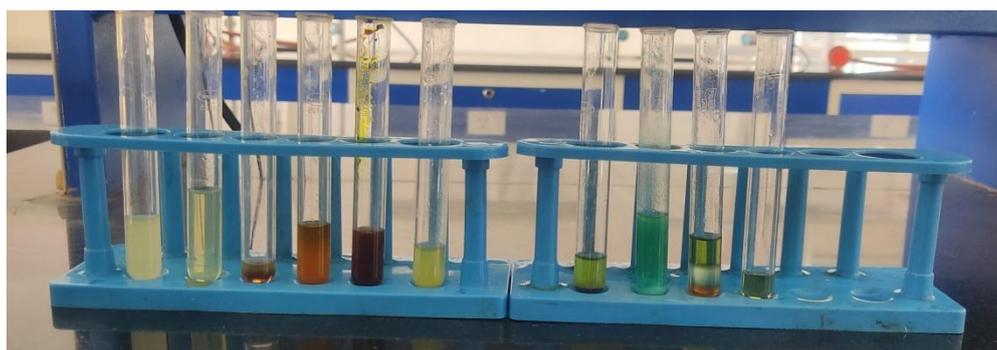


Fig. 10: Phytochemical test.^[17]

Table no: 4: Invitro Antibacterial Activity Extract of Writghtia Tinctoria.

S.NO	Test Organisms	Zone of Inhibition In (Mm) Against Different Concentration				
		Pc	25µl	50µl	75µl	100µl
1.	Staphylococcus aureus	31	13	23	26	27
2.	E.coli	17	-	-	11	14

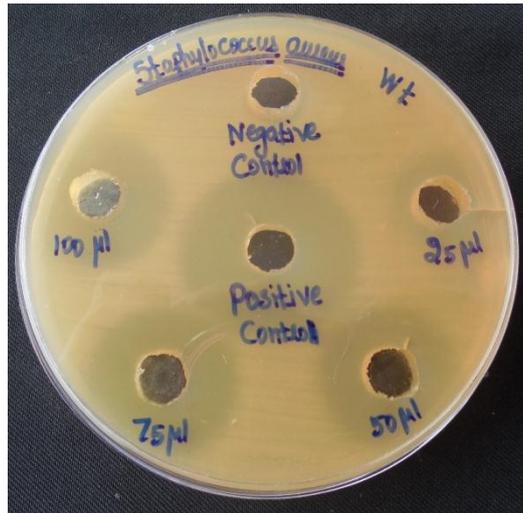


Fig. 11: Wrightia tinctoria extract of stephylococcus aureus.

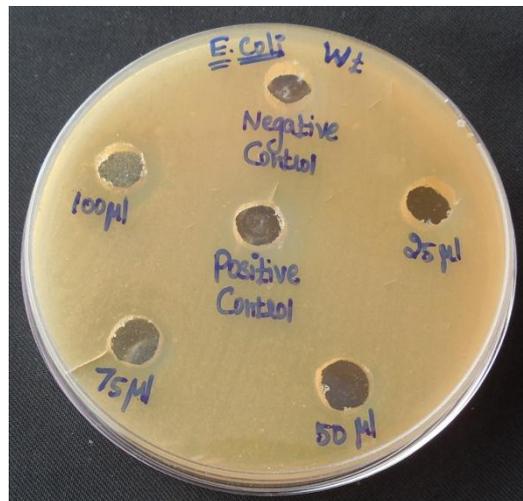
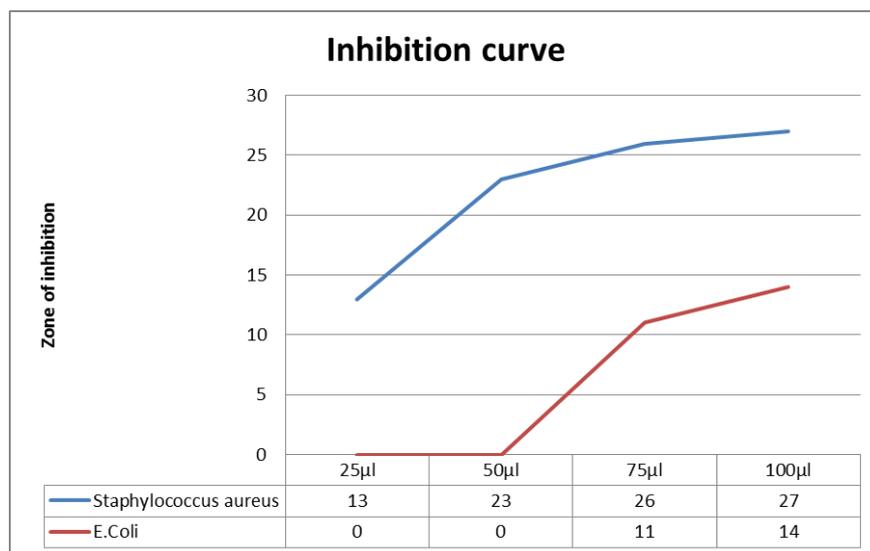


Fig. 12: Wrightia tinctoria extract on E.coli.

GRAPH

The graph shows the antibacterial activity of ethanolic extract of wrightia tinctoria plant.



Graph 1.

COMPARISON STUDIES

Table no: 5.

S.no	Drug	Zone of inhibition in (mm) Against Different Concentration				
		PC	2.5 μ l	5.0 μ l	7.5 μ l	10 μ l
1	Amoxicillin	31	24	26	28	29

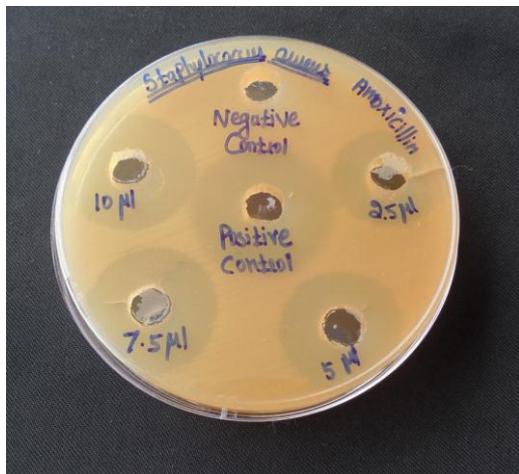


Fig. no. 13: Amoxicillin against Staphylococcus aureus.

The 2.5 μ l of standard drug gives 24mm zone of inhibition on staphylococcus aureus. The 5.0 μ l of

standard drug gives 26mm zone of inhibition on staphylococcus aureus. The 7.5 μ l of standard drug gives 28mm zone of inhibition on staphylococcus aureus. The 10.0 μ l of standard drug gives 29 mm zone of inhibition on staphylococcus aureus.

2.5 μ l < 5.0 μ l < 7.5 μ l < 10.0 μ l

The 50 μ l of ethanolic extract of wrightia tinctoria gives zone of inhibition of 23 is more often equal to the 5.0 μ l of standard drug of zone of inhibition of 29.

The 75 μ l of ethanolic extract of wrightia tinctoria gives a zone of inhibition of 26 is more often equal to the zone of inhibition of 7.5 μ l standard drug gives 28.

The 100 μ l of ethanolic extract of wrightia tinctoria gives a zone of inhibition of 27 is more often equal to the 10. μ l of standard drug gives zone of inhibition gives 29

Table No: 6: The Comparison Studies Of Ethanolic Extract Of Wrightia Tinctoria And Standard Drug (Amoxicillin).

S.no	Sample	Zone of Inhibition In Mm Against Different Concentration			
1	Staphylococcus aureus	PC	50 μ l	75 μ l	100 μ l
		31	23	26	27
2	Amoxicillin	PC	5.0 μ l	7.5 μ l	10 μ l
		31	26	28	29

CONCLUSION

- Antibacterial properties of medicinal plants are being increasingly reported from different parts of world.
- The World Health Organization (WHO) estimates the plant extract or active constituents are used as folk medicine in traditional therapies of 80% of the world population.
- In the present work the ethanolic extract obtained from Wrightia tinctoria bark showed strong activity against the selected bacteria strains.
- The result of phytochemical in the present investigation showed that the plant contains effective constituents like alkaloid, quinine, tannin, flavonoids, phenolic compounds, terpenoids, steroids.
- In this study different concentration of ethanolic bark extract 25 μ l, 50 μ l, 75 μ l, 100 μ l. Were used against the different bacterial strains a marked increase in zone of inhibition was observed on increasing the ethanolic bark extract.
- The 2.5 μ l of standard drug gives 24mm zone of inhibition on staphylococcus aureus. The 5.0 μ l of standard drug gives 26mm zone of inhibition on

staphylococcus aureus. The 7.5 μ l of standard drug gives 28mm zone of inhibition on staphylococcus aureus. The 10.0 μ l of standard drug gives 29 mm zone of inhibition on staphylococcus aureus.

- Among the different bacterial strains a more inhibitory action was observed against Staphylococcus aureus and a minimal inhibitory action against E.coli.
- In the different bacterial strains which zone of inhibition of inhibitory action was observed of extract against Staphylococcus aureus is more often equal to the zone of inhibition of extract against Staphylococcus aureus
- However further studies are needed to better evaluate the potential effectiveness of the crude extract as anti bacterial agents.
- The present result will form the basis for selection investigation in the potential discovery of new natural bio-active compound.

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