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ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF ACALYPHA INDICA ANDWRIGHTIA TINCTORIALEAVES EXTRACTS: A COMPARATIVE STUDY

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ABSTRACT
In the present study, the bioactive compounds in the leaves of *Wrightiatinctoria* and *Acalyphaindica* were extracted with chloroform and ethanol as solvents. The crude extractswere tested against, bacterial strains such as *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* and fungal strains such as *Candida albicans, Microsporumgypseum*and *Trichophyton* which are being the causative agents of various skin infections in humans using agar disc diffusion method. The antibacterial and antifungal activity of extracts of two leaf samples were compared. This study showed that the ethanol extract of *A.indica* exhibited a maximum zone of inhibition of 27mm at 1000µg/ml against the *Tricophyton* than the *W.tinctoria* leaf extracts. The chloroform extract of *W.tinctoria* displayed a maximum zone of inhibition of 14mm, 10mm, 18mm, 14mm and 15mm at 1000µg/ml against *Staphylococcusaureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans*and *Microsporumgypseumrespectively* than the *Acalyphaindica* leaf extracts.

KEYWORDS: *Acalyphaindica, Wrightiatinctoria,* Antibacterial, Antifungal, Skin infection.

INTRODUCTION
Many of the plants used today were known to the people of ancient cultures throughout the world and were highly considered for their preservative and medicinal powers. Scientific experiments on the antimicrobial properties of plants and their components have been documented in the late 19th century (Zaika, 1975). India has a rich flora that is widely distributed throughout the country. Herbal medicines have been the basis of treatment and cure for various diseases and physiological conditions in traditional methods practiced such as Ayurveda, Unani and Siddha. Medicinal components from plants play an important role in conventional as well as inwestern medicine. Plant derived drugs have been a part of the evolution of human, healthcare for thousands of years (Duraipandian and Ignacimuthu, 2007). Today, nearly 88% of the global populations turn to plant derived medicines as their first line of defense for maintaining health and combating diseases. One hundred and nineteen secondary plant metabolites derived from plants are used globally to prepare drugs.Among them 15% are angiospermsand they have been investigated chemically and in them 74% of pharmacologically active plant derived components were discovered (Perumalsamy*et al*., 2008). Currently, pupil of Asia and India are utilizing plants as part of their routine health management (Perumalsamy*et al*., 2008).

Kannan*et al*., (2006) have studied the antimicrobial activity of *Wrightiatinctoria* of the extract of leaf using hexane, methanol and ethanol against skin bacteria and dermatophytes by *in- vitro*. They reported that *Wrightiatinctoria* leaves possessed potent antimicrobial properties against dermatophytic microbes. In particular, methanol and ethanol extracts were active against bacteria and hexane extract was active against dermatophytic fungi, suggesting that the active principles may be useful in the topical treatment of superficial skin infection. Govindarajan*et al*., (2008) have tested the Hexane, chloroform, ethyl acetate and methanol extracts of the leaves of *Acalyphaindica*against Grampositive (*Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, Streptococcus faecalis*) and Gram-negative (*Klebsiellapneumoniae, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa*) bacteria. All the extracts exhibited antibacterial activity against Gram-positive organisms with minimum inhibitory concentrations (MIC) between 0.156 to 2.5 mg/ml. Among the Gram-negative bacteria, only the *Pseudomonas aeruginosa*was susceptible to the extracts. Somchit*et al*., (2010) have investigated the antimicrobial activity of water, ethanol and chloroform extracts of *Acalyphaindica*against four bacterial and fungal strains and reported that gram positive bacteria was more pronounced ($p < 0.05$) in water and ethanol extracts. Antifungal activity was more significant ($p < 0.05$) only in chloroform extract. Ravishankar*et al*., (2010) have

investigated the hexane, methanol, chloroform, ethyl acetate and water extracts of *Wrightiatinctoria* against plant pathogenic bacteria by *in vitro* method and showed that the leaves of *Wrightiatinctoria* possessed potent antimicrobial properties against plant pathogenic bacteria suggesting that the active principles may be useful in the control of diseases. Rajaselvam*et al*., (2012) have investigated the antibacterial activity of *Acalyphaindica*and reported that the acetone extract of *Acalyphaindica*showed the maximum zone of inhibition against *Staphylococcus aureus*and *Bacillus subtilis*, minimum inhibition of *Escherichia coli* and *Klebsiella sp*. Moorthy*et al*., (2012) have studied the antimicrobial activity of *Wrightiatinctoria* using methanolic and petroleum ether extracts from the leaves of *Wrightiatinctoria*. Vedhanarayanan*et al*., (2013) have investigated the antibacterial activity of different extracts (Chloroform, ethanol and methanol) of *Wrightiatinctoria*against the human pathogenic bacterial strains. Indira PriyaDarsini (2015) have studied the antibacterial properties and phytochemical evaluation of *Acalyphaindica*. Vijayarekha*et al*., (2015) have studied the antibacterial activity of petroleum ether, chloroform, acetone, methanol and ethanol extract of the medicinal plant *Acalyphaindica*.

Based on the above review, the plants *Wrightiatinctoria* and *Acalyphaindica*exhibited the antimicrobial activity against the chosen pathogen. The present study is to compare the antibacterial and antifungal activity of chloroform and ethanol extract of *Acalyphaindica* and *Wrightiatinctoria* leaves.

Acalyphaindica

Acalyphaindica [Fig 1.1] known as Kuppaimani in tamil is an annual weed. It belongs to the family *Euphorbiacea*. *Acalyphaindica* is an herbaceous annual that has catkin-like inflorescences with cup-shaped involucres surrounding the minute flowers (Schmelzer and Gurib-fakim, 2008). It is a common weed in many part are reported to be useful in treating pneumoniae, asthma, rheumatism and several other ailments. The dried leaves of *Acalyphaindica* was made into a poultice to treat bedsores and wounds and the juice of *Acalyphaindica* is added to oil or lime to treat a variety of skin disorders. Leaves possess laxative properties (a substitute for senega) and used in the form of powder or decoction. *Acalypha* cures diseases of the teeth and gums, burns, toxins of plant and mixed origin, stomach pain, eases due to pitta, bleeding piles, irritations, stabbing pain, wheezing, sinusitis and neutralizes predominance of the Kabha factor. The ethanolic extracts of *Heliotropiumindicum*, *Plumbagozeylanicum* and *Acalyphaindica* were evaluated for their wound healing activity in rats (Suresh Reddy *et al*., 2002). The major phytochemical constituents are alkaloids Acalypus and aclyphine (Kirtikar and Basu, 1975). This plant is used as diuretic, antihelmintic and for respiratory problems such as bronchitis, asthma and pneumonia (Varier, 1996). The roots of *Acalyphaindica* is used as laxative and leaves for scabies and other cutaneous diseases (Perry, 1980). The plant has many traditional medicinal uses. In Madagascar, the crushed plant is used for skin parasites. In Mauritius, the sap of crushed leaves mixed with salt, or a decoction of plant, is used for scabies and other skin problems. In Seychelles, a root infusion or decoction is taken for asthma, and also to clean the liver and kidneys. The root decoction is also taken for intestinal worms and stomach ache. The leaf sap is taken as an emetic. An infusion together with the roots of *Tylophoraindica* is taken as an emetic in the case of poisoning. A leaf infusion is also taken as a purgative and vermifuge in Madagascar. In East Africa sap of the leaves is used for eye infections. Leaf powder is used for maggot-infested wounds. *Acalyphaindica* is listed in the Pharmacopoeia of India as an expectorant to treat asthma and pneumonia.

This plant is held in high esteem in traditional Tamil Siddha medicine as it is believed to rejuvenate the body. The plant has also been eaten as a vegetable in Africa and India, but care needs when eating it since it contains several alkaloids as well as hydrocyanic acid.

Wrightiatinctoria

Wrightiatinctoria is a potential medicinal plant distributed in tropical region belongs to the family *Apocynaceae*. It is originated in India and is also a native of Burma (Vedhanarayanan*et al*., 2013). *W. tinctoria* has another botanical name called *Holarrhenaantidysenterica*. *W.tinctoria* is given its name after William Wright. He was a Scottish physician and botanist. *Wrightiatinctoria* is a flowering plant and is deciduous, i.e., its leaves fall off after reaching maturity. It is a small tree which grows about 3 meters high. The stem of the plant has a pale bark and color of ivory. It has branches and has the flowers at the tip of branches. The leaves of *W.tinctoria* [Fig 1.2] are oval and oppositely arranged. The leaves grow up to 20 cm long. Flowers are whitish with five petals which are 2-3 cm long. They havegood fragrance and by aging theyturn into creamy yellow. The tree bears fruits with long follicles which are up to 50 cm long. The fruits have seeds which are dark and 1-2 cmlong**.** The flowers of the tree resemble snowflake. The Ayurvedic properties of the plant are Rasa**:** Tikta **(**bitter**),** Kashaya **(**astringent**),** Guna:Rooksha **(**dry**),** Virya**:**Seeta**,** Vipaka: Katu**.** Chemical constituents of *Wrightiatinctoria* are wrightial–triterpenoid, cycloartenone, cycloeucalenol, beta-amyrin, betasitosterol**.** The parts of the plant which are used for various purposes are Leaves, Seeds, and Bark. The plant is used in making hair oils as it has anti-dandruff and anti-inflammatory properties. The plant is the best remedy for balancing tridoshas. Properties of *W.tinctoria*include anthelmintic, anodyne, antipyretic, aphrodisiac, astringent, carminative, and depurative. *W.tinctoria* is the best medicine for Diarrhoea and Blood Pressure**.** It is also used in the treatment of piles and skin diseases like ringworm, leprosy, etc. It is used as an

effective remedy for urinary problems. It is used in the treatment of Rheumatoid Arthritis and Osteoarthritis**.** It is an effective treatment for Psoriasis and any other non-specific skin problems. It is also a good remedy for fever, toothache, constipation, and stomach ache. Leaves indicated the presence of flavonoids, glycoflavones, iso-orientin and phenolic acid, the leaves of this tree yield a blue die called pala indigo and its leaves were soaked in coconut oil for few hours and applied for eczema psoriasis and other skin disease.

MATERIAL AND METHODS

Collection and processing of plant material

Leaves of *Acalyphaindica* was collected from Egattur village, Thiruvallur district and leaves of *Wrightiatinctoria* was collected from Poondi, Thiruvallurtaluk. The collected leaf of *Acalyphaindica* and *Wrightiatinctoria*was washed with distilled water and shade dried for two week. The shade dried leaves of both the plants [Fig 1.1 and Fig 1.2] was grounded using mortar and pestle to powder. The powdered leaf was stored for further use.

Fig 1.1: Shade Dried Leaves of *Acalypha indica.*

Fig 1.2: Shade Dried Leaves of *Wrightia tinctoria.*

Bacterial culture

Stock culture of bacterial strains such as *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*and fungal strains such as *Candida albicans, Microsporumgypseum* and *Trichophyton* was collected from Life teck research centre, Chennai.

Sterilization

Media and glasswares were sterilized in autoclave at 15psi pressure at 121ºC for 20 min.

Preparation of inoculum

Stock cultures were maintained at 4°C on Nutrient agar (NA) Slant. Active cultures for experiments were prepared by transferring a loop full of culture from the stock cultures into the test tubes containing nutrient broth (NB), that were incubated at 24hrs at 37ºC.

SOLVENT EXTRACTION (CHLOROFORM AND ETHANOL) EXTRACT OF *Acalyphaindica* LEAVESPREPARED BY COLD SOLVENT EXTRACTION

Chloroform extraction of *Acalypha indica* leaf

A conical flask containing 10g of powdered leaf of *Acalyphaindica* was immersed in 50ml of chloroform solvent. The mixture was shaken for 2 hours using orbital shaker at 8000 rpm for 7 days. After 7 days the filtrate [Fig 1.3] was collected by filtering the mixture using Whatmann filter paper 1. The collected filtrate was condensed for further use.

Ethanol extraction of *Acalyphaindica* leaf

A conical flask containing 10g of powdered leaf of *Acalyphaindica* was immersed in 50ml of ethanol solvent. The mixture was shaken for 2 hours using orbital shaker at 8000 rpm for 7 days. After 7 days the filtrate [Fig 1.3] was collected by filtering the mixture using Whatmann filter paper 1. The collected filtrate was condensed for further use.

Fig 1.3: Ethanol and Chloroform filtrate of *A.indica.*

Fig 1.4: Extraction of *Wrightia tinctoria*leaves using soxhlet extractor.

CHLOROFORM AND ETHANOL EXTRACT OF *Wrightiatinctoria* LEAVES PREPARED USING SOXHLET EXTRACTOR

Extraction of *Wrightiatinctoria*leaf using Chloroform as solvent

The finely ground powder (25gm) was placed in a porous bag or thimble made out of strong filter paper, which was placed in the chamber of the soxhlet apparatus. The extracting solvent Chloroform (250ml) in flask was heated at its boiling point 61.2ºC and its vapors condense in condenser. The condensed extractant drips into the thimble containing the plant powder and extracts it by contact. When the level of the liquid in chamber have reached the top of siphon tube, the liquid content of chamber sip-hon into the flask. After 72 hours the dried compound was concentrated in the flask. After extraction the solvent was removed, typically by evaporation, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and

was usually discarded. The obtained extract was condensed for further use.

Extraction of *Wrightiatinctoria*leaf using Ethanol as solvent

The finely ground powder (25gm) was placed in a porous bag or thimble made out of strong filter paper, which was placed in the chamber of the soxhlet apparatus. The extracting solvent Ethanol (250ml) in flask was heated at its boiling point 78·37ºC, and its vapors condense in condenser. The condensed extractant drips into the thimble containing the plant powder and extracts it by contact. When the level of liquid in chamber have reached the top of siphon tube, the liquid content of chamber sip-hon into flask. After 72 hours the dried compound is concentrated in the flask. After extraction the solvent was removed, typically by evaporation, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and was usually discarded. The obtained extract was condensed for further use.

PHYTOCHEMICAL ANALYSIS OF *Acalyphaindica* AND *Wrightiatinctoria* extracts

TEST FOR ALKALOIDS

Hager′s test: A few millilitres of the filtrate were taken, a drop of Hager′s reagent was added along the sides of test tube. A yellow precipitate [Fig 3.5(a)] indicated the presence of alkaloids. Chloroform and Ethanol extract of *Acalyphaindica*and Chloroform extract of *Wrightiatinctoria* indicated the presence of alkaloids.

TEST FOR FLAVANOIDS

Alkaline reagent test: A 1ml of extract was taken and a few drop of dilute sodium hydroxide was added. Appearance of yellow color [Fig 3.5(b)] indicated the presence of flavonoids. Ethanol extract of *Wrightiatinctoria* indicated the presence of flavonoids.

TEST FOR PHENOL

Phenolic test: A 5ml of extract, 1% ferric chloride (1ml) was taken and 1% potassium ferrocyanide(1ml) was added. Appearance of fresh radish blue color [Fig 3.5(c)] indicated the presence phenol. Ethanol extracts of *Acalyphaindica* and *Wrightiatinctoria* indicated the presence of phenol.

TEST FOR TERPINOID

Salkowski test: A 5ml of extract, 2ml of chloroform was taken and few drops of concentrated sulphuric acid were added to form a layer. A reddish brown color [Fig 3.5(d)] indicated the presence of terpenoid. Chloroform and Ethanol extract of *Acalyphaindica* and Ethanol extract of *Wrightiatinctoria* indicated the presence of terpenoid.

TEST FOR TANNIN

Ferric chloride test: A 2ml of extract was taken and a few ml of 10% ferric chloride solution was added drop by drop. A blue black precipitate [Fig 3.5(e)] indicated the presence of tannin. The Ethanol and Chloroform extract of *Wrightiatinctoria* indicated the presence of tannin.

Fig 1.5(a): Screening of alkaloids.

Fig 1.5(b): Screening of flavonoids.

Fig 1.5(c): Screening of phenols.

Fig 1.5(d): Screening of terpenoids.

Fig 1.5(e): Screening of tannins

Fig 1.5: Screening of phytochemicals of *Acalyphaindica* and *Wrightiatinctoria* leaves extract.

AGAR DISC DIFFUSION METHOD FOR ANALYZING ANTIBACTERIAL ACTIVITY

Antibacterial activity of the extracts was determined by disc diffusion method on Mueller Hinton Agar (MHA). MHA medium was poured into the petriplates. After the medium was solidified, the inoculums of bacterial strains was spread on the solid plates with sterile swab moistened with the bacterial suspension. Amphicillin was taken as positive control. Ethanol and Chloroform extract of *Acalyphaindica* and *Wrightiatinctoria* and positive control 20µl each was added in sterile disc and placed in MHA plates. The plates were incubated at 28ºC for 24 hours. Then Antibacterial activity was determined by measuring the diameter of zone of inhibition.

AGAR DISC DIFFUSION METHOD FOR ANALYZING ANTIFUNGAL ACTIVITY

Antifungal activity of the extracts was determined by disc diffusion method on Sabouraud Dextrose Agar (SDA). SDA medium was poured into the petriplates. After the medium was solidified, the inoculum of fungal strains was spread on the solid plates with sterile swab moistened with the bacterial suspension. Amphotericin-B was taken as positive control. Ethanol and Chloroform extract of *Acalyphaindica* and *Wrightiatinctoria* and positive control 20µl each was added in sterile disc and placed in SDA plates. The plates were incubated at 28ºC for 24 hours. Then Antifungal activity was determined by measuring the diameter of zone of inhibition.

RESULTS AND DISCUSSION

Table 1.1: Phytochemical analysis of *A.indica* and *W.tinctoria* leaf extracts.

LEAF EXTRACTS	PHYTOCHEMICAL COMPOUNDS				
	ALKALOIDS	FLAVONOIDS	TERPENOIDS	PHENOL	TANNIN
Ethanol extract of *A.indica*	+	-	+	+	-
Chloroform extract of *A.indica*	+	-	+	-	-
Ethanol extract of *W.tinctoria*	-	+	+	+	+
Chloroform extract of *W.tinctoria*	+	-	-	-	+

(+ indicates Presence, - indicates absence)

Phytochemical analysis of *A.indica* and *W.tinctoria*leaf extracts

Ethanol and chloroform extracts of *Wrightiatinctoria*and *Acalyphaindica*showed the presence of alkaloids, flavonoids, terpenoids, phenol and tannin [Table 1.1]. The presence of phytochemical compounds indicated the antimicrobial activity of *Acalyphaindica* and *Wrightiatinctoria*.

ANALYSIS OF SAMPLE USING THIN LAYER CHROMATOGRAPHY

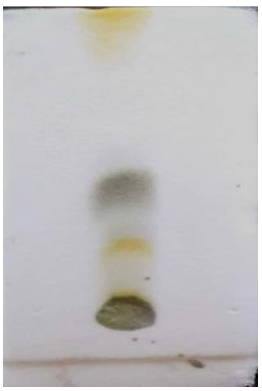
TLC was performed for the chloroform and ethanol extracts of *Acalyphaindica* and *Wrightiaindica*, the following results were observed:

1. Ethanol extract of *Acalyphaindica*exhibited 3 spots [Fig 1.1] with R.F of 0.63 [Table 1.2],
2. Chloroform extract of *Acalyphaindica*displayed 4 spots [Fig 1.2] with R.F of 0.53 [Table 1.2],
3. Ethanol extract of *Wrightiatinctoria* exhibited 2 spots [Fig 1.3] with R.F of 0.53 [Table 1.2],
4. Chloroform extract of *Wrightiatinctoria* displayed 5 spots [Fig 1.4] with R.F of 0.63 [Table 1.2].

Table 1.2: Retardation Factor of Chloroform and Ethanol Extracts of *A.indica* and *W.tinctoria.*

SR.NO	LEAF EXTRACTS	RETARDATION FACTOR
1.	**Ethanol extract of *A.indica***	0.63
2.	**Chloroform extract of *A.indica***	0.53
3.	**Ethanol extract of *W.tinctoria***	0.53
4.	**Chloroform extract of *W.tinctoria***	0.63

1.1 TLC for Ethanol Extract of *Acalypha indica.*



1.3 TLC for Ethanol Extract of *Wrightia tinctoria.*

1.2 TLC for Chloroform Extract of *Acalypha indica.*

1.4 TLC for Chloroform Extract of *Wrightia tinctoria.*

DISCUSSION

The results of the present study showed that *Acalyphaindica* and *Wrightiatinctoria* has antibacterial and antifungal activity. These results corroborate withearlier investigations by Vedhanarayanan*et al*., (2013) and Somchit*et al*., (2010).

The chloroform extracts of *Acalyphaindica* and *Wrightiatinctoria* have a greaterantimicrobial effect than that of ethanol. It indicates that the active plant compounds in these plants can be readilydissolved or extracted in chloroform when compared to ethanol.

The leaves extract of *Acalyphaindica* possess major phytochemical constituents include alkaloids, tannin and flavonoids (Kiritikar and Basu, 1975) and the *Wrightiatinctoria* leaves indicated the presence of flavonoids, glycoflavones, iso-orientin and phenolic acid (Veerapur*et.al*., 2004) responsible for its antimicrobial activity. The phytochemical screening of study showed that the presence of flavonoids, alkaloids, and tannins in the leaves extract supports for the antibacterial and antifungal reports of (Rajaselvam*et al*., (2010). In this study, the antibacterial and antifungal activity was compared and based on the analysis it is identified that the chloroform extract of *Wrightiatinctoria* has more antibacterial and antifungal activity than the extracts of *Acalyphaindica*.

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