



PHYTOCHEMICAL AND ANTIMICROBIAL EVALUATIONS OF *DICHROSTACHYS CINEREA*

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ABSTRACT

In underdeveloped countries, traditional and herbal medicines including folk medicinal practice scatters to nearly 70% of the population because of accessibility, affordability and the time tested dependability. They still depend on herbal medicine because of the side effect of most of the modern drugs. The medicinal activities are due to the presence of active principle in them. We have chosen the plant *Dichrostachys cinerea* (L) belonging to the family Mimosaceae for preliminary phyto chemical screening and to study the antimicrobial activity. *Dichrostachys cinerea* are traditionally used for antimicrobial, anticonvulsant. astringent (root); antihelminthic, purgative, laxative and diuretic (bark). In medicine, bark is used to alleviate headache, toothache, dysentery, elephantiasis and root infusions are consumed to treat leprosy, syphilis coughs, as an anthelmintic, purgative and strong diuretic. The leaves are particularly useful and can be taken to treat epilepsy and can also be taken as a diuretic and laxative, and its powder can be used in the massage of fractures. Based on the ethano pharmacological information's, the present study is focused to evaluate Preliminary phytochemical and antimicrobial activity.

KEYWORDS: *Dichrostachys cinerea*, Antimicrobial, Methanol, Ethyl Acetate.

INTRODUCTION

Traditional system of medicine remains the major source of healthcare for more than two thirds of world's population. Impressive progress has been made in certain developing countries like China and India through integration of traditional with western system of medicines and application of modern science and technology to the promotion and development of traditional medicine.

Plant has been one of the important sources of medicines since the beginning of human civilization. In spite of tremendous developments in the field of allopathy during the 20th century, plants till continue as one of the major source of drugs in modern as well as traditional medicine throughout the world. Approximately one third of all pharmaceuticals are of plant origin. If fungi and bacteria are also included, over 60% of all pharmaceuticals are plant based.

In spite of the overwhelming influence and our dependence on modern medicine and tremendous advances in synthetic drugs, a large segment of the world population (80% of about four billion) can not afford the products of western pharmaceuticals industry and have to rely upon the use of traditional medicines, which are mainly derived from plant material. That fact is well recognized by the WHO, which has recently compiled an inventory of medicinal plants listing over 20,000 species.

In present trend is more towards everything "NATURAL". Medicinal plants are value added for the content and chemical composition of their active components. In a wider context, there is a growing demand for plant based medicines, health products, pharmaceuticals, food supplements, cosmetics etc. international market if medicinal plant is over \$60 billion per year, which is growing at the 7% of rate.

Indian subcontinent is endowed with varied agro climates and topography. Therefore, it is one of the richest plant biodiversity of the world and rightly acclaimed as 'Botanical garden of the world'^{1,2}.

A Brief Introduction and Guide to the Tri Doshas

Ayurveda:

Ayurveda is a Sanskrit word, derived from two roots: ayur, which means life, and Veda, knowledge. Knowledge arranged systematically with logic becomes science. During

the due course of time, Ayurveda became the science of life. It has its root in ancient Vedic literature and encompasses our entire life, the body, mind and spirit.

Ayurveda is considered by many scholars to be the oldest healing science. Ayurveda is a Sanskrit word which means "The Science of Life." Ayurvedic knowledge originated in India more than 5,000 years ago and is often called the "Mother of All Healing". It stems from the ancient Vedic culture and was taught for many thousands of years in an oral tradition from accomplished masters to their disciples. Some of this knowledge was set to print a few thousand years ago, but much of it is inaccessible. The principles of many, if not all, natural healing systems now familiar in the West, such as Homeopathy and Polarity Therapy, have their roots in Ayurveda.

Ayurveda places great emphasis on prevention and encourages maintaining health by paying close attention to balance in one's life through right thinking, diet, lifestyle and herbs. Knowledge of Ayurveda enables one to understand how to create balance of body, mind and consciousness according to one's own individual constitution and how to make lifestyle changes to bring about and maintain this balance.

Just as everyone has an individual face or thumb print, according to Ayurveda, each person has a particular pattern of energy--an individual combination of physical, mental and emotional characteristics--which is his or her constitution. This constitution is determined at conception by a number of factors and is the same throughout one's life. Many factors, both internal and external, act upon us to disturb this balance and are reflected as a change in one's constitution from the balanced state. Examples of some of these emotional and physical stresses are: one's emotional state, diet and food choices, seasons and weather, physical trauma, work and family relationships. Once these factors that can cause imbalance are understood, one can take appropriate actions to nullify or minimize their effects or eliminate the causes, and re-establish one's original constitution. Balance is the natural order; imbalance is disorder. Health is order; disease is disorder. Within the body there is a constant interaction

between order and disorder. Once one understands the nature and structure of disorder, one can re-establish order.

Ayurveda identifies three basic types of energy or functional principles that are present in everybody and everything. There are no single words in English to describe these principles, so we use the original Sanskrit words vata, pitta and kapha. Energy is required to create movement so that fluids and nutrients get to the cells, enabling the body to function. Energy is also required to metabolize the nutrients in the cells, and is called for to lubricate and maintain cellular structure. Vata is the energy of movement, pitta the energy of digestion or metabolism and kapha the energy of lubrication and structure. All people have vata, pitta and kapha, but one is usually primary, one secondary and the third least prominent. The cause of disease in Ayurveda is viewed as the lack of proper cellular function because of an excess or deficiency of vata, pitta or kapha and/or the presence of toxins. In Ayurveda, body, mind and consciousness work together in maintaining balance. They are simply viewed as different facets of one's being.

To learn how to balance the body, mind and consciousness then requires an understanding how vata, pitta and kapha work together. According to Ayurvedic philosophy the entire cosmos is interplay of the energies of the five great elements -Space, Air, Fire, Water and Earth. Vata, pitta and kapha are combinations and permutations of these five elements that manifest as patterns present in all creation.

Devanāgarī: the 'science of life') is a system of traditional medicine native to India, and practiced in other parts of the world as a form of alternative medicine. In Sanskrit, the word Ayurveda comprises the words āyus, meaning 'life' and Veda, meaning 'science' evolving throughout its history, Ayurveda remains an influential system of medicine in South Asia. The earliest literature of Ayurveda appeared during the Vedic period in India. The Sushruta Samhita and the Charaka Samhita were influential works on traditional medicine during this era. Ayurvedic practitioners also claim to have identified a number of medicinal preparations and surgical procedures for curing various ailments and diseases.

Ayurveda is considered to be a form of complementary and alternative medicine (CAM) within the western world, where several of its methods-such as herbs, massage, and Yoga as exercise or alternative medicines are applied on their own as a form of CAM treatment^{3,4,5}.

AIM OF THE WORK

In underdeveloped countries, traditional and herbal medicines including folk medicinal practice scatters to nearly 70% of the population because of accessibility, affordability and the time tested dependability. They still depend on herbal medicine because of the side effect of most of the modern drugs. The medicinal activities are due to the presence of active principle in them. We have chosen the plant *Dichrostachys cinerea* (L) belonging to the family Mimosaceae for preliminary phyto chemical screening and to study the antimicrobial activity.

Dichrostachys cinerea are traditionally used for antimicrobial, anticonvulsant, astringent (root); antihelminthic, purgative, laxative and diuretic (bark). In medicine, bark is used to alleviate headache, toothache, dysentery, elephantiasis and root infusions are consumed to treat leprosy, syphilis coughs, as an anthelmintic, purgative and strong diuretic. The leaves are particularly useful and can be taken to treat epilepsy and can also be taken as a diuretic and laxative, and its powder can be used in the massage of fractures.

Based on the ethano pharmacological information's, the present study is focused to evaluate Preliminary phytochemical and antimicrobial activity.

DESCRIPTION OF THE PLANT

Dichrostachys cinerea Wight & Arn

Cailliea cinerea Macb., Cailliea glomerata (Forsskal) J.F. Macbr., *Dichrostachys glomerata* (Forssk.) Chiov., *Dichrostachys nutans* (Pers.) Benth., *Mimosa cinerea* L. Mimosaceae

It is native mostly to Africa and parts of Southeast Asia

English: bell mimosa, Chinese lantern tree, Kalahari Christmas tree, marabou thorn, sickle bush

Hindi: khairi, kunlai, nutan

Tamil: vadataram

Shrub or small tree to 8 m tall. Branchlets densely to sparsely puberulous; lateral shoots to 8 cm long

Leaves axes puberulous to minutely pilose, sometimes with red hairlets, especially near base of pinnae; glands peg-like at base

Upper flowers of a hanging spike are sterile, and are of a lilac or pale purple. Pods are usually a mustard brown and are generally twisted or spiralled and may be up to 100 x 15 mm.

Seeds biconvex, elliptic to subcircular, 4 mm long, 2-4 mm wide, pale tan, glossy; pleurogram Elliptic

USES:

Food: Fruit and seeds from *D. cinerea* are edible. Fodder: Cattle, camels and game (giraffe, buffalo, kudu, Lichtenstein's hartebeest, nyala, impala, klipspringer, red duiker and Damara dik-dik) relish the juicy pods that drop to the ground and even eat the young twigs and leaves. Leaves are highly palatable, rich in protein (11-15% crude protein) and mineral content. Young shoots and pods are also browsed by smaller domestic animals. Pods and seeds do not contain hydrocyanic acid, minimizing the chance of poisoning animals. Apiculture: The flowers are a valuable honey source. Fuel: The wood is dense, burns slowly with few sparks and emits a non-toxic smoke, making it excellent firewood. It often grows many small trunks, ideal in size for carrying in a head load. Fibre: The bark yields a strong fibre used for various applications such as twine. The debarked roots are used for strong plaiting work such as for racks and baskets. Timber: *D. cinerea* yields a medium to heavy, durable hardwood with a density of 600-1190 kg/cubic m at 15% mc. Heartwood red or dark purple with darker streaks, sharply differentiated from the yellowish-brown sapwood; grain straight or slightly interlocked; texture rather fine and even. Due to its generally small dimensions, its utilization is limited making such items as walking sticks, handles, spears and tool handles. Fencing posts are durable and termite resistant, easily lasting up to 50 years. Medicine: The bark is used to treat dysentery, headaches, toothaches, elephantiasis and acts as a vermifuge. Root infusions are taken for leprosy, syphilis coughs, as an anthelmintic, purgative and strong diuretic. Pounded roots and leaves are used to treat epilepsy. The roots are chewed and placed on the sites of snakebites and scorpion stings, and the leaves, which are believed to produce a local anaesthesia, are used for the same purpose and also as a remedy for sore eyes and toothache. Leaves are taken as a diuretic and laxative, and used for gonorrhoea and boils; powder from leaves is used in the massage of fractures. The plant is used as a veterinary medicine in India^{6,7,8}.

MATERIALS AND METHODS

The plant *Dichrostachys cinerea* are widely found throughout India along road sides. For our work the plant was collected from Bhavani area in the month of November 2008.

The plant was identified by a botanist, botanical survey of India, Coimbatore. Who authenticated plant with available literature, the insects and dirt free leaves were collected from the shrub.

TREATMENT

The leaves were dried in shade the dried, leaves were powdered by mixer grinder and powder was passed through sieve number 40 and the powder was used for extraction work.

MATERIALS

- Shade dried powder of leaves of *Dichrostachys cinerea*.
- Petroleum ether
- Ethyl acetate
- Methanol.

EXTRACTION

PREPARATION OF SAMPLE:

The air dried leaves of *Dichrostachys cinerea* plant was taken separately which has been separated from insect and dirt particles and it was made into powder separately this was utilized for extraction process.

EXTRACTION METHOD:

Method: Soxhlet's extraction (continues hot percolation)

Solvents Used: Pet ether, ethyl acetate, methanol.

PREPARATION OF PETROLEUM ETHER EXTRACT:

100gms of crude dry powder of leaves of *Dichrostachys cinerea* was extracted by soxhlet extraction (continuous hot percolation) by petroleum ether solvent, for 18 hours. After completion of extraction the extract were distilled for removing the solvents. Concentrated the extract of drug by keeping in room for removing remaining solvents. Then yielded dark green extract of leaves it was used for further experiments^{9,10}.

PREPARATION OF ETHYL ACETATE EXTRACT:

100gms of crude dry powder of leaves of *Dichrostachys cinerea* was extracted by soxhlet extraction (continous hot percolation) by ETHYL ACETATE solvent extracted for 18 hours. After completion of extraction the extract was taken and was distilled by distillation process for removing the solvents. Concentrate extract of drug by keeping in room for removing remaining solvents. Then yielded dark green extract of leaves it was used for further experiments^{11,12}.

PREPARATION OF METHANOL EXTRACT:

100gms of crude dry powder of leaves of *Dichrostachys cinerea* was extracted by soxhlet extraction (continous hot percolation) by solvents methanol separately for 18 hours. After completion of extraction the extract was distilled by distillation process for removing the solvents. Concentrate each extract of drug by keeping in room for removing remaining solvents. Then we yield dark green extract of leaves it was used for further experiments^{13,14}.

PRELIMINARY PHYTOCHEMICAL STUDIES

All the extracts were taken for preliminary phytochemical test and tested for the following constituents using the common chemical identification tests.

DETECTION OF CARBOHYDRATES

A small quantity of extract was dissolved in 5 ml of distilled water and filtered. The filtrate was subjected to Molisch's and Fehling's solution test.

a) Molisch's test

To the filtrate, few drops of alcoholic alpha-naphthol and 2 ml of concentrated sulphuric acid was added slowly through the sides of the test tube.

b) Fehling's test

A small portion of filtrate was dissolved in water and treated with Fehling's solution I and II and heated.

DETECTION OF PROTEINS AND AMINO ACIDS

a) Biuret Reaction

Addition of a very dilute solution of copper sulphate to an alkaline solution of an extract produces red (or) violet colour. This reaction is due to the presence – CONH-CHR-CO-NH-group. At least two peptide linkages (-CONH) must be present.

b) Xantho protic Reaction

Extract usually produce a yellow colour when warmed with concentrated Nitric acid and the colour becomes orange when the solution is made alkaline. This reaction is due to nitration of the benzene ring in phenyl hydrazine, tyrosine and tryptophan.

c) Millon's Reaction

Millon's reagent (mercuric nitrate in nitric acid containing trace of nitrous acid). Usually, produces pink-red precipitate on addition to a protein is characteristic of phenol and so given by protein containing tyrosine. (This is the only phenolic amino acid that occurs in protein)

d) Ninhydrin Test

Proteins (and peptide) give this test but the colour is different from that of the amino acid.

DETECTION OF SAPONIN

a) Frothing Test

To a small quantity of the extract, few ml of water was added and shaken well.

b) Haemolysis Test

The substance was spreaded over the glass slide to form a thin film layer on which a drop of human blood is placed. Observe under microscope for a change in structure and shape of RBC. A control was maintained to see the change in RBC structure for haemolysis.

DETECTION OF PHYTOSTEROL

a) Salkowski Reaction

When concentrated sulphuric acid is added to a solution of extract in chloroform a red colour is produced in the chloroform layer.

b) Liebermann-Burchard Reaction

A greenish colour is developed when a solution of extract in chloroform is treated with concentrated sulphuric acid and acetic anhydride.

DETECTION OF ALKALOIDS

A small portion of solvent free extract was stirred, treated with a few drops of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloidal reagents.

- Mayer's reagent
- Dragendroft's reagent
- Hager's reagent
- Wagner's reagent
- With tannin

TEST FOR FLAVONOLS

The alcoholic extract when treated with aqueous sodium hydroxide produces yellow colour which changes to orange. This indicates the presence of Flavonols.

The alcoholic extract when treated with concentrated sulphuric acid produces yellow colour which changes to orange. This indicates the presence of Flavonols.

The alcoholic extract when treated with Magnesium metal and Hydrochloric acid produces red colour which changes to magenta. This indicates the presence of Flavonols.

TEST FOR PHENOLIC COMPOUNDS

The ethylacetate extract when treated with 0.2ml of neutral ferric chloride produces black precipitate. This indicates the presence of phenolic compounds.

The extract when treated with 0.2ml of neutral ferric chloride produces black precipitate. This indicates the presence of phenolic compounds^{15,16}.

OBSERVATION

TABLE 1. PRELIMINARY PHYTOCHEMICAL SCREENING

Test for	Result		
	A	B	C
Carbohydrates:			
Molisch's test	+	+	+
Fehling's solution test	+	+	-
Protein and Amino acids:			
Millon's test	-	-	-
Biuret test	-	-	-
Ninhydrin test	-	-	-
Saponins:			
Frothing test	+	+	-
Haemolysis test	+	+	-
Phytosterol:			
Libermann burchard test	+	-	+
Salkowski test	+	+	-
Alkaloids:			
Mayers reagent	-	-	-
Dragendroft's reagent	-	+	+
Hager's reagent	-	-	-
Wagner's reagent	-	-	-
Flavonones:			
With NaOH	+	+	-
With H ₂ SO ₄	+	+	+
Phenolic Compounds:	+	+	-
Glycosides:	+	-	-
Tannins	+	+	-

A-Ethyl acetate extract

B-Methanol extract

C-Petroleum ether extract

STUDY OF ANTIMICROBIAL ACTIVITY

Antimicrobial drugs are the greatest contribution of the present century to therapeutics. Drugs in this class differ from all others in that they are designed to inhibit/kill the infecting organism and to have no/minimal effect on the recipient.

The inhibition of microbial growth under standard conditions may be utilized for demonstration the therapeutic efficacy of antibiotics. Any subtle change in the antibiotic molecules which may not be detected by chemical methods will be revealed by a reduction in the antimicrobial activity and

ANTIMICROBIAL SUSCEPTIBILITY TEST

TABLE-2. ETHYLACETATE EXTRACT

Bacteria	1 (1000µg)	2 (500µg)	3 (250µg)	4 (125µg)	5 (62.5µg)	Std (1000µg)
<i>Shigelle sonellii</i> (Gram Negative)	16mm	10mm	8mm	6mm	2mm	20mm
<i>Staphylococcus aureus</i> (Gram Positive)	18mm	16mm	10mm	7mm	5mm	30mm

hence microbial assays are very useful for resolving doubts regarding possible loss of potency of antibiotics and their preparation.

PRINCIPLE

The microbial assays are based upon a comparison of inhibition of growth of bacteria (test organisms) by measured concentration of the antibiotics to be examined with that production by known activity. Two general methods are usually employed, the cylinder plate (cup plate) method and the turbidimetric method (tube assay).

The assay method used is proven, valid by statistical analysis of actual data construction the assay, i.e, the dose response line produced by the standard and the preparation to be examined must be cylinder- plate method. This method depends on the diffusion of an antibiotic from a vertical cylinder or a cavity through a solidified agar layer of a Petri-dish or plate to an extend such that growth of added (inoculated) microorganism is prevented entirely in a circular area of "zone" around the cylinder or cavity containing a solution of antibiotics^{17,18}.

ANTI MICROBIAL STUDY

CUP PLATE METHOD

- Nutrient broth was prepared and inoculated with different species of bacteria and incubated at 37°C overnight.
- From this overnight culture 1 % stock culture was prepared (99 ml of sterile nutrient broth + 1ml of overnight culture).
- Nutrient agar was prepared and 25 ml was poured in sterile Petri plates and allowed to cool.
- Each agar plates were inoculated with 0.2ml of 1% bacterial culture and spreaded by spreader.
- Using a sterile cork borer, 6mm diameter of holes were made in the solidified agar plates containing respective bacterial culture (1%).
- A total volume of 0.2ml of ethyl acetate and methanol extract was poured into the wells with the concentrations as 1000µg / ml, 500µg /ml, 250µg /ml.
- One well was poured with standard antibiotic and incubated at 37°C for 24 Hrs. After 24 Hrs of incubation Zone of Inhibition was measured in millimeter^{19,20}.

BACTERIAL STRAINS USED:

Gram Positive Bacteria : *1.Staphylococcus aureus*

Gram Negative Bacteria: *1.Shigella sonellii*

Standard Drug : Antibiotic : Cipro Floxacin. (1000 µg / ml)

Solvent : DMSO (Dimethyl Sulfoxone)



Fig. 1: Ethylacetate extract activity on *Staphylococcus aureus*

ANTIMICROBIAL SUSCEPTIBILITY TEST

TABLE-3. METHANOL EXTRACT

Bacteria	1 (1000µg)	2 (500µg)	3 (250µg)	4 (125µg)	5 (62.5µg)	std (1000µg)
<i>Shigelle soneii</i> (Gram Negative)	14mm	8mm	7mm	5mm	3mm	22mm
<i>Staphylococcus aureus</i> (Gram Positive)	20mm	15mm	12mm	8mm	2mm	35mm

Note: When compared to Methanoll extract, Ethyl acetate extract having significant antimicrobial (antibacterial) activity.

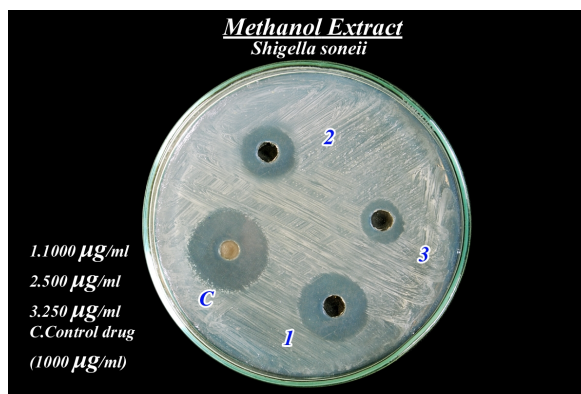


Fig. 2: Methanol extract activity on *Shigella soneii*

RESULTS AND DISCUSSION

Based on literature review the leaves of the *Dichrostachys cinerea* (L) –mimosaceae were selected and project work was carried out. The plant *Dichrostachys cinerea* (L) belonging to the family mimosaceae was collected and authenticated.

Leaves were collected, dried, extracted with petroleum ether, ethyl acetate and methanol and tested for its phytochemical constituents with different chemical tests. The ethyl acetate and methanol extracts were showed the presence of carbohydrates, phenols, tannins, saponins, flavonols etc., is shown in the table.

The ethylacetate, methanol extracts were selected and taken for antimicrobial activity studies against *Shigelle soneii* (Gram Negative) & *Staphylococcus aureus* (Gram Positive).

CONCLUSION

When compared to Methanol extract and Ethyl acetate extracts having significant antimicrobial (antibacterial) activity. Both the extracts were shown better activity against gram positive organism than the gram negative.

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