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PHARMACOGNOSTICAL AND ANTI MICROBIAL STUDIES ON THE LEAF OF WRIGHTIA TINCTORIA Br.,

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Abstract

Pharmacognostical and antimicrobial studies on the leaf of Wrightia tinctoria, Br.,

Wrightia tinctoria, Br., belongs to the Family of Apocyanacea. Commonly known as veypale in Tamil Language, in Telugu: ankuduchettu, chiti-anikudu, kondajemudu and Indrajau in Hindi Language. It has been used for many diseases and disorders. Particularly the wrightia tinctoria, Br., Bark is used for anti dysenteric, Flatulence, bilious affection dropsy and the leaves has been using for releave the toothache, and proteolytic activity. But there is little evidence of leaf on Antimicrobial studies. In present study we have attempt was made to study its pharmacognostical features including Macroscopic, Microscopic Features, Physio-Chemical parameters and Phyto chemical studies ,little extensive of Anti-Microbial studies. The present study on pharmacognostical characters and Anti microbial studies of wrightia tinctoria, Br., leaf has given useful information inregard to its correct identity and help to differentiate from the closely related other species of wrightia. The present study will direct the several scientific to do the further, in great way of (the exact mechanism and molecular aspects)

Key Words: Microscopy, W.tinctoria., Br., Stomatal no, Microbial studies.

Introduction

The isolation of secondary plant metabolites begins with the selection of a plant, is the most critical aspect of the project. In order to locate a plant, previously utilized in folklore practices, one should turn to the discipline of ethno botany. It can include present day and involves inter – disciplinary study surrounding a core of botany with chemistry, pharmacology and anthropology among others. The plant family of w.t, (Apocyanaceae) which has been used

traditionally for its various there properties like antidysentric, bilious affection, stomachic Rubbed over the body for dropsy and the seed is used for the treatment of flatulence, aphrodisiac and for anthelmentic. Several pharmacognostical and Biological activity has studied by several authors on the wrightia tinctoria Bark and seeds, But in leaves only numberable scientist have studies. That's why we have planned the study on leaves. Thus the present investigation was aimed at evaluating the pharmacognostical features and phytochemical analysis for authentication and identification of the plant and also to evaluate the extract responsible for the biological activity[12]

Materials and Methods

Plant Material

The plant material was collected From Pulianzolai in the month of June. The Collected leaf of plant was dried in the shade For one month. Then the shade-dried leaf was powdered to get a coarse powder. The coarse powdered drug was subjected to hot continuous percolation by using soxhlet apparatus. Different Solvents were used according to their polarity. Herb authentication tests were done at the Botany Department; St.Joseph's College, Trichirappalli and was identified with the help of botanist. The plant was subjected to various morphos – anatomical, Physio-Chemical and Antimicrobial characterization for which the materials and methods are presented below. Air dried powdered leaf of W.tinctoria, Br., was subjected to the Following Analysis. Histological Features of the leaf of Wrightia tinctoria, Br., Physio-chemical standards, preliminary phytochemical investigation of the leaf of Wrightia tinctoria, Br., and Quantitative microscopy.

Pharmacognostical Studies

Systemic position, Macroscopy of the leaf of Wrightia tinctoria, Br.,

The plant w.t.Br., belongs to the Family of Apocyanaceae known by several names in the vernacular language. Hyamaraka in Sanskrit, mitha, indrajav in Hindi, and vey pale, in Tamil. The Habit is: As small, deciduous tree Generally up to 1-8m tall and often 40cm in grith, some times upto 7.5m high ,Bark is light grey ,scaly and smooth ,the leaves are Elliptic obovate ablong , 7.5-12.5 cm long.(Figure-2).

Histological Features of leaf and stem

The Histological Features of the leaf of wrightia tinctoria, Br., its T.S of leaf shows a thin walled cuticle that covers upper epidermis. The cell arrangement shows that dorsiventral type of leaf, below that upper epidermis palisade cells are present. In the lamina region is composed of palisade cells, vascular strands and spongy parenchyma. The midrib portion contains collenchymas presents both upper and lower epidermis and also vascular bundles, are composed of lignified xylem, non-lignified phloencers. Described in (Figure-2, 3).

Stem shows epidermis layer covered with thin walled cuticle, below this hypodermis (collenchymas) is present with intracellular spaces are filled with starch and pectin. Hypodermis (collenchymas) cortex, sclerenchymas are present and composed of phloem, cambium and xylem (proto and Meta xylems) air cavities are also present in this region below that wood parenchyma and pith are present Figure-4.

Physico – Chemical standards

Air dried coarsely powdered leaf of Wrightia tinctoria, Br., was subjected to the Following Analysis. Analysis of determination of total ash, determination of water soluble ash, determination of acid insoluble ash, determination of sulphated ash, determination of loss on drying, determination of alcohol soluble extractive, determination of water soluble extractive and determination of crude fiber content by Dutch process and fluorescence analysis,. Separate procedure was Followed to study the physico -Chemical standard described in (Table -, Fluoresce analysis was also carried out for the powder and for extract as per standard procedures.

Preliminaryphytochemical screening[11], 2

Extract was prepared by weighing 1kg of the dried powdered leaves and were subjected to hot successive continuous extraction with different solvents as per the polarity, petroleum ether ,ethyl acetate, chloroform methanol and finally with aqueous. The extracts were filtered in each step, concentrated and the solvent removed by rotary evaporator. The extracts were dried over dessicator and the residues were weighed. The presence or absence of the primary and secondary phytoconstituents was detected by usual prescribed methods. The shade dried powder and various extracts of the leaf of Wrightia tinctoria, were subjected to chemical tests for identification of its Active constituents. (Alkaloids,

Carbohydrates and glycosides, phytosterol, fixed oils and Fats, saponins, Tannins and phenolic compounds ,proteins and Free amino acids, Gums and Mucilage's, Flavonoids, and lignin.(table-6)

Quantitative microscopy

The quantitative microscopy of the leaves vein islet number, vein termination number, stomatal number and stomatal index where on Fresh leaves using standard procedure the values are described in (Table -1,2) (Figure -1,2)

Anti-Microbial Activity

Materials

E–Eoli, Klebsiella Pneumoniae,Staphylococcus aureus, Candida albicans,Nutrient broth,Nutrient Agar media (H, Media).

Pert dishes, sterilized borer, Autoclave, incubator, Zone readerOfloxacin (50, 75 µg/ml), Alcohol and A aqueous extracts of the leaf of wrightia tinctoria, Br.,Img/ml stock solution of extracts were taken (50 mg/me)

Preparation of inoculums

For pure culture from secondary plate 4 to 5 identical colonies[8] 4 to 5ml of nutrient broth was included for 2 to 4 hours at 37^0C. This subscriber broth was used as the inoculums for seeding the CUP Plate method.

Preparation of sub culture broth[8]

Ingredients for the SUB culture Broth is yeast extract – 5 Gms, meat (Beef) extract (10 gms), Peptone -5gms, sodium chloride (5 gms), and Distilled water – q.s. All the ingredients were dissolved in distilled water; PH was adjusted to 7.6 transferred to a suitable container and auto claved at 121^0C for 25 minutes. After sterilization was over the bacteria was inoculated into the nutrient broth by means of inoculating 100P and incubated at 37^0C for 2 to 4 hrs. Preparation of the culture media: (HI media)[1]Ingredients of the culture media is Nacl – 3gms, meat (Beef) extract – 5 gms, peptone – 5gms, Ager – 25sms Distilled H_2O (q.s)

Methodology

All the ingredients were accurately weighed and dissolved in Distilled water, the Ph was adjusted to 7.6 and this was transferred to a suitable container and autoclaved at 121^0C for 15mts. It meets at 90^0C and solidified only when cool to about 40^o C. The media was poured in a large sized ;petri dished to a uniform depth of 4mm and then allowed to

solidity at room temperature just prior to use, cu tone plates in an incubator was dried at 37° C with a lid partly. Opened until the surface was free from visible moisture (15 to 20 mts).The plates were incubated within 15 minutes after preparing the inoculation with a wax pencil the plate was divided into section according to the number of standard and sample solutions to be used. Sterilized cotton swab was dipped into the nutrient broth, excess fluid was removed by notating the tube above third level Test and control drugs were added into the cup plate by using micro pipette. Then the plates were incubated at 37°C in incubator.

Observation

50, 75 µG/m concentration of alcohol and equals extracts of the root of wrightia tinctoria, Br). Were tested for anti bacteria activity against E-coli, Klebsiella-pneumonia, staphylococcus aureus and Candida albicans, Ofloxacin (50, 75 µg/ml) served as standard drug.

Results

Histology Features of the leaf of Wrightia tinctoria, Br.,

The histological features of the leaf and stem have studied and the result has shown in figure-2, 3, and 4.

Physiochemical and Phyto-chemical Analysis.[6, 8]

The extract obtained with each solvent is lighted and its percentage is calculated .The color and consistencies of extracts were noted. The present study on the leaf of Wrightia tinctoria, Br., gave the pharmacognostical identity of the plant and preliminary Phytochemical screening shown that the presence of alkaloids, glycosides, tannins, alcoholic and aqueous (50, 75µg/ml). separate procedure was followed to study the physio-chemical standards described in (Table-1,2,3).for the determination of Total ash, Water soluble ash, Acid insoluble ash, sulphated ash, loss on drying, Alcohol soluble extractive, water soluble extractive, crude fiber content by Dutch process.[5]

Fluorescence Analysis[4]

Fluorescence analysis of leaf was observed in daylight and UV light (365nm) and distributed in (Table-1, 2.)

Quantitative Analysis[1]

The fresh leaf samples were subjected to quantitative analysis for various leaf constants like Stomatal number, Stomatal index, Vein islet number, Vein termination number. The results are shown in table-4, figure-5, and 6.

Anti microbial Activity[1, 8, 11]

50,75µg/ml concentration of alcohol and aqueous extracts of the leaf of W t,Br., were tested for anti bacterial against E.Coli,Klebsiella,Pneumoniae,Staphylococcus aureus and Candida albicans , ofloxacin(50,75 µg/ml) served as a standard drug and it has figured in (7,8,9,10)

Discussion

The present study which was studied on Wrightia tinctoria, Br., belongs to the family of Apocyanaceae,Many authors have discussed the pharmacognostical studies on the bark, root, seed and there is very little information and document on leaf,Our studies gave a detailed report on its pharmacognostical characters such as (macroscopy, histology and physio-chemical standards) linear measurements.Above report gave a detailed data of this plant .About the physical constant values gave the further support for easy identification and authentication of this plant. The present study on the leaf of Wrightia tinctoria, Br., gave the Pharmacognostical identity of the plant and preliminary phyto chemical screening shown that the presence of alkaloids, glycosides, tannins, alcoholic and aqueous (50, 75 µg/ml) leaf extract shown significant antimicrobial activity against E.Colli, Klebsiella pneumonia, Staphylococcus aureus and Candida albicans.

Table -1: Fluorescence Analysis of the leaf extracts of wrightia tinctoria, Br.,

Sl.no	Sample	Color in day light	Color in short UV 365nm
1	Pet ether	Green	Pale green
2	Benzene	Green Light	Reddish brown
3	Chloro Form	Green	Dark green
4	Acetone	Green	Bluish green
5	Alcohol	Pink	Pinkish violet
6	Aqueous	Green	Yellow wish green

Table- 2: Fluorescence Analysis of leaf powder.

Sl.no	Reagents	UV light(365nm)
1	Leaf powder	Pale green
2	Powder + In naoh (aq)	Greenish Yellow
3	Powder + INACl	Pale green
4	Powder + 50% HNO_3	Green
5	Powder + 50% H_2SO_4	Light green
6	Powder + methanol	Green
7	I_2 Solution	Dark Green
8	$Fecl_3$	Light Green

Table-3: Physiochemical evaluation of the Crude drug wrightia tinctoria, Br.,

Sl.no	Standardization Parameters	% percentage W/W
1	Total Ash	40
2	H_2O Soluble	12.5
3	Acid insoluble ash	9
4	Sulphated Ash	14
5	Loss on drying	12.83
6	H_2O soluble extractive	2.54
7	Alcohol	4.60
8	Crude Fiber content	12.38

Table-4: Quantitative analysis of leaf contents of wrightia tinctoria, Br.,

Sl.no	Particulars	value
1	Stomatal upper epidermis Number Lower epidermis	5
2	Stomatal upper epidermis Number Lower epidermis	5
3	Stomatal Upper epidermis Index lower epidermis	33
4	Vein islet number	12
5	Vein Termination number	12

Table -5: Extractive values of leaf extracts of w.t with Different solvents.

Sl.no	Extracts	% W/W
1	Alcohol	2.37
2	Aqueous	2.12

Table-6: Behavior of the w.t leaf powder with Different chemical reagents.

Sl.No	Reagent	Color / Precipitate	Constituent
1	Picric acid		presence
2	Mayer's reagent	Cream precipitate	presence
3	Dragendroff's reagent	Orange brown precipitate	presence
4	Hager's reagent	Yellow precipitate	presence
5	Wagner's reagent	Reddish brown precipitate	presence

6	Con H_2SO_4	-	-
7	1% alcoholic alpha naphthol	-	-
8	Lml fehling's solution	Orange precipition	presence
9	$CHcd_3$	$CHCl_3$ layer sepereated	presence

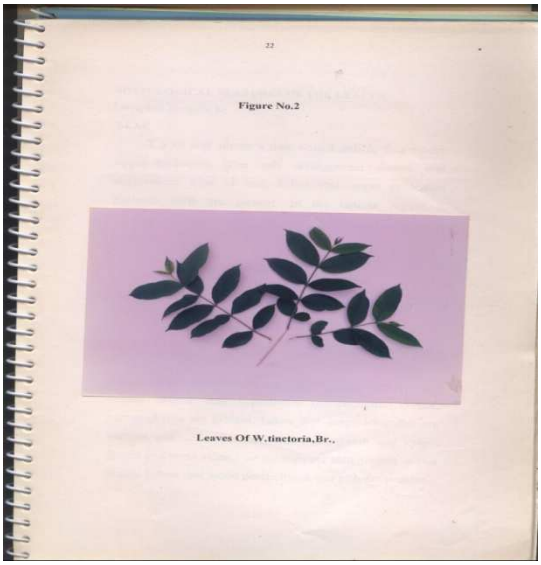

Figure-1 Leaves of W.tinctoria, Br.,

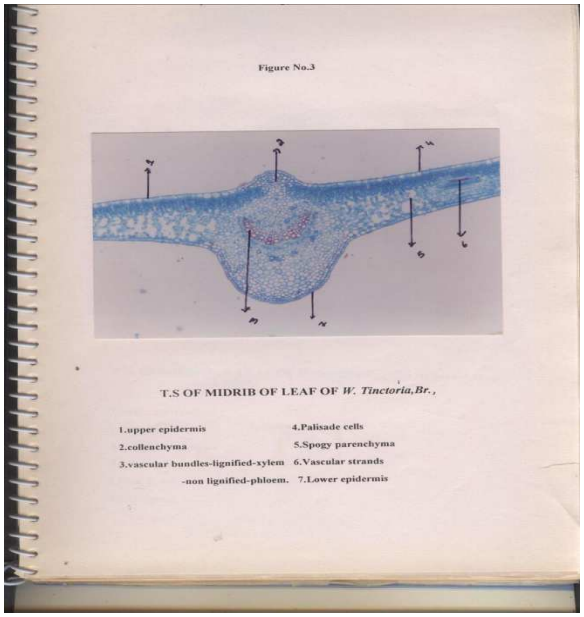

Figure-2 T.S MIDRIB LEAF OF W.tinctoria., Br.,

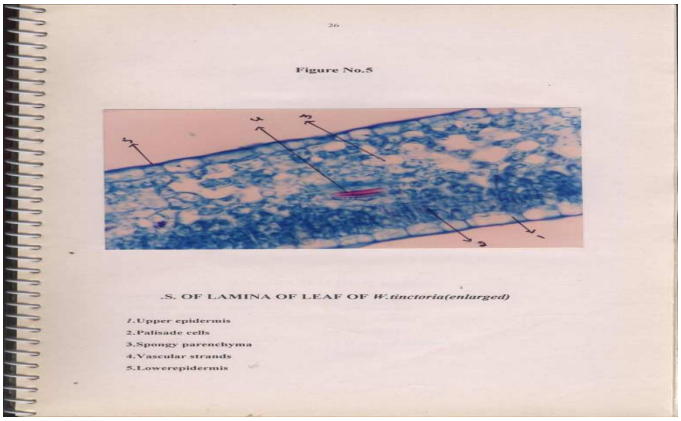

Figure-3 S.of Leaves of W.tinctoria., Br.,(enlarged)

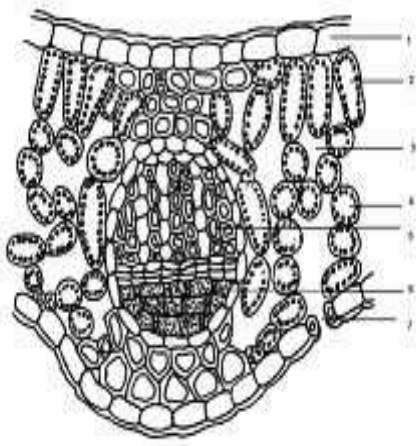

Figure-4 Parenchyma.of Leaves of W.tinctoria.,Br.,

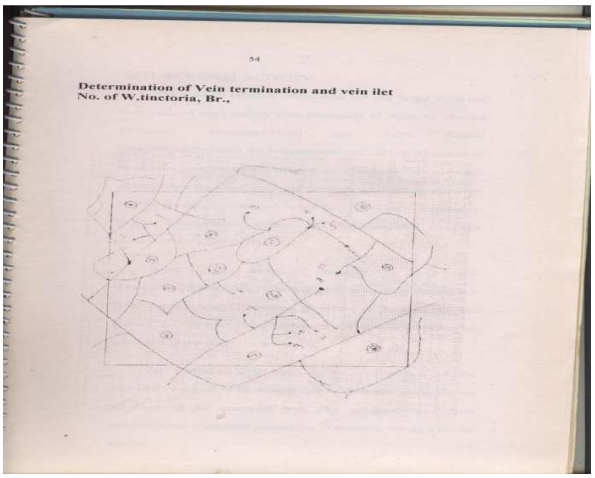

Figure-5: Det of V termination and v islet no W.tinctoria.,Br.,

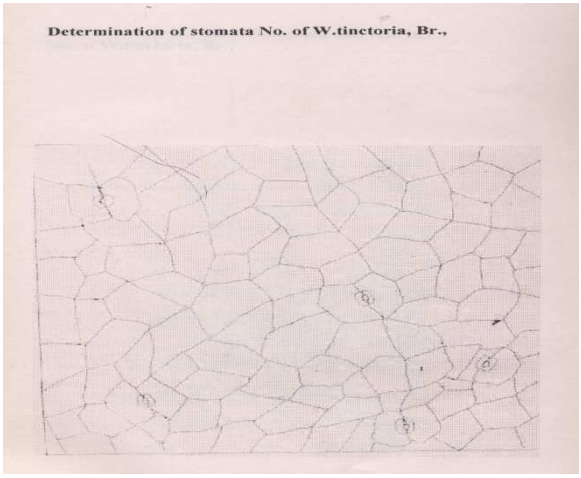

Figure-6: Det of Stomatal no W.tinctoria.Br.,

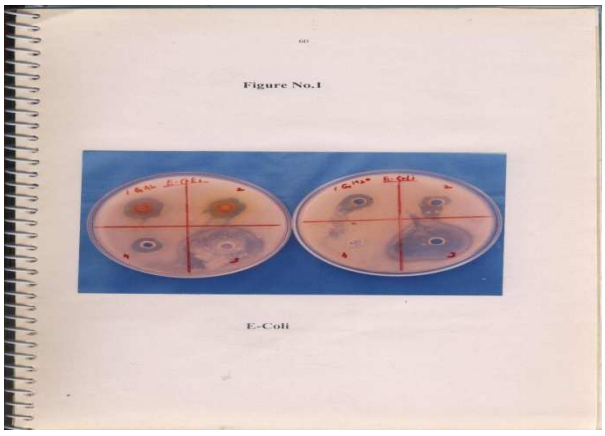

Figure-7(E-COLI)

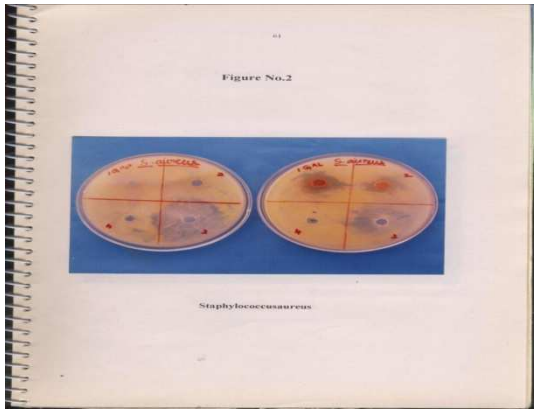

Figure-8(Staphylococcus aereus)

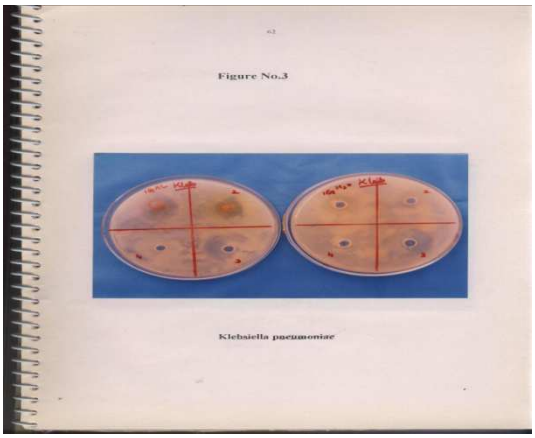

Figure-9 Klebsiella Pneumoniae

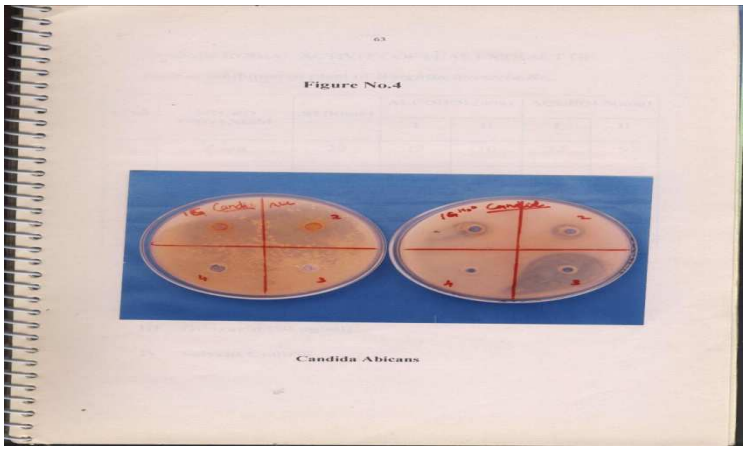

Figure-10 Candida Albicans

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