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EXTRACTION, ISOLATION AND *IN VITRO* ANTIBACTERIAL ACTIVITY OF CASALPINIA BONDUC SEEDS

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Abstract: Now a day's skin disease is a worldwide problem especially in developing and under-developed countries. Generally Eczema, Scabies, Ringworm, Clavus or Corn, Prurigo, Yaws, Warts, Pruritis and Psoriasis are common skin diseases found in tropical and subtropical countries. Most of the skin diseases are caused due to certain microorganisms (bacteria and fungi) ¹⁻². From the literature survey it is evident that medicinal plants have enormous biological activities. They have been proved to be instrumental in the control of diseases caused by microorganisms³⁻⁴. In the present study, seeds of *Caesalpinia bonduc* plant were isolated for various ingredients i.e. *terpenoids and phenolic, fats and waxes, alkaloids, quaternary alkaloids and N-oxides*. These ingredients were characterized and screened for their antibacterial activity against certain pathogens viz., *S. pyogenes, N. calcareo, B. subtilis, P. aeruginosa* by cup plate agar well diffusion method. The bacteria selected are generally the causative agents for skin diseases. All the ingredients showed moderate to good antibacterial activity. The result shows that the test plant might be useful in curing the skin ailments.

Keywords: *Caesalpinia bonduc*, antibacterial, seeds, skin diseases.



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INTRODUCTION

Ceasalpinina bonduc (Linn.) Roxb., an armed liana, up to 15 m in height, found wild throughout the plains of India. Branchlets glossy, black, armed with recurved prickles at the base of pinnae and elsewhere; pinnae 6-11 pairs, leaflets stalked, coriaceous, base rounded to acute, apex mucronate, margins curved, upper surface shining, lower dull; flowers yellow, fragrant, in axillary and terminal racemes 30-60 cm long; pods somewhat swollen, dark brown to black, oblong, 1 or 2 seeded, beaked; seeds orbicular or ovoid to reniform, black, polished, hard⁵.

The fruits and seeds of *Caesalpinia bonduc* are found to float in sea-water up to a period of one year and to drift to coastal areas. The seeds exhibit dormancy which can be overcome by acid scarification, light and temperature treatment, or treatment with conc. Sulphuric acid for 30-90 min. Bonduc nuts have been used as an antiperiodic for a long time. Pharmacological trials have revealed diuretic and anti-pyretic activity of the nuts, and have also proved efficacious in diarrhoea.

The seeds have a bitter taste, and are valued in indigenous medicine as a tonic. They are an ingredient of 'Ayush-64' an ayurvedic compound preparation used as an anti-malarial drug. They are also made into an ointment for treating hydrocele. As an infusion they are used for curing cerebral haemorrhage and infantile convulsions. The seeds are reported to be abortifacient. The seeds contain proteins, amino acids. The seeds yield thick, yellow fatty oil having a disagreeable odour. The oil is emollient and used as a cosmetic preparation, and also for stopping discharge from ears. It is anti-rheumatic. The seeds and leaves are reported to be used in skin diseases and rheumatism⁶⁻⁸.

EXPERIMENTAL SECTION

The seeds of *Ceasalpinina bonduc* plant were collected from the Melghat forest region of Amravati, District of Maharashtra, India seasonally and the plant material was authenticated by the taxonomists Dr. S. P. Rothe from the Department of Botany, Shri Shivaji College Akola. A voucher specimen (ML - 103) was deposited in the herbarium of Department of Botany, Shri Shivaji College, Akola.

EXTRACTION AND ISOLATION:

The seeds of *Ceasalpinina bonduc* plant were shade dried at room temperature and ground in a manual mill to get coarse powder. The powder was kept in the air tight polythene bags and stored at a dry place. The coarse powdered material of seeds were treated with methanol and filtered. The residue thus left was again treated with ethyl alcohol and filtered to get fats and waxes. The filtrate was acidified, extracted with chloroform and separated by using separating funnel. The chloroform layer contains terpenoids and phenolics whereas acidic aqueous layer on basic treatment with ammonia followed by chloroform treatment gave alkaloids, whereas methanolic treatment gave Quaternary alkaloids and N- Oxides. All the isolated constituents were analyzed phytochemically and screened for their antibacterial activity⁹.

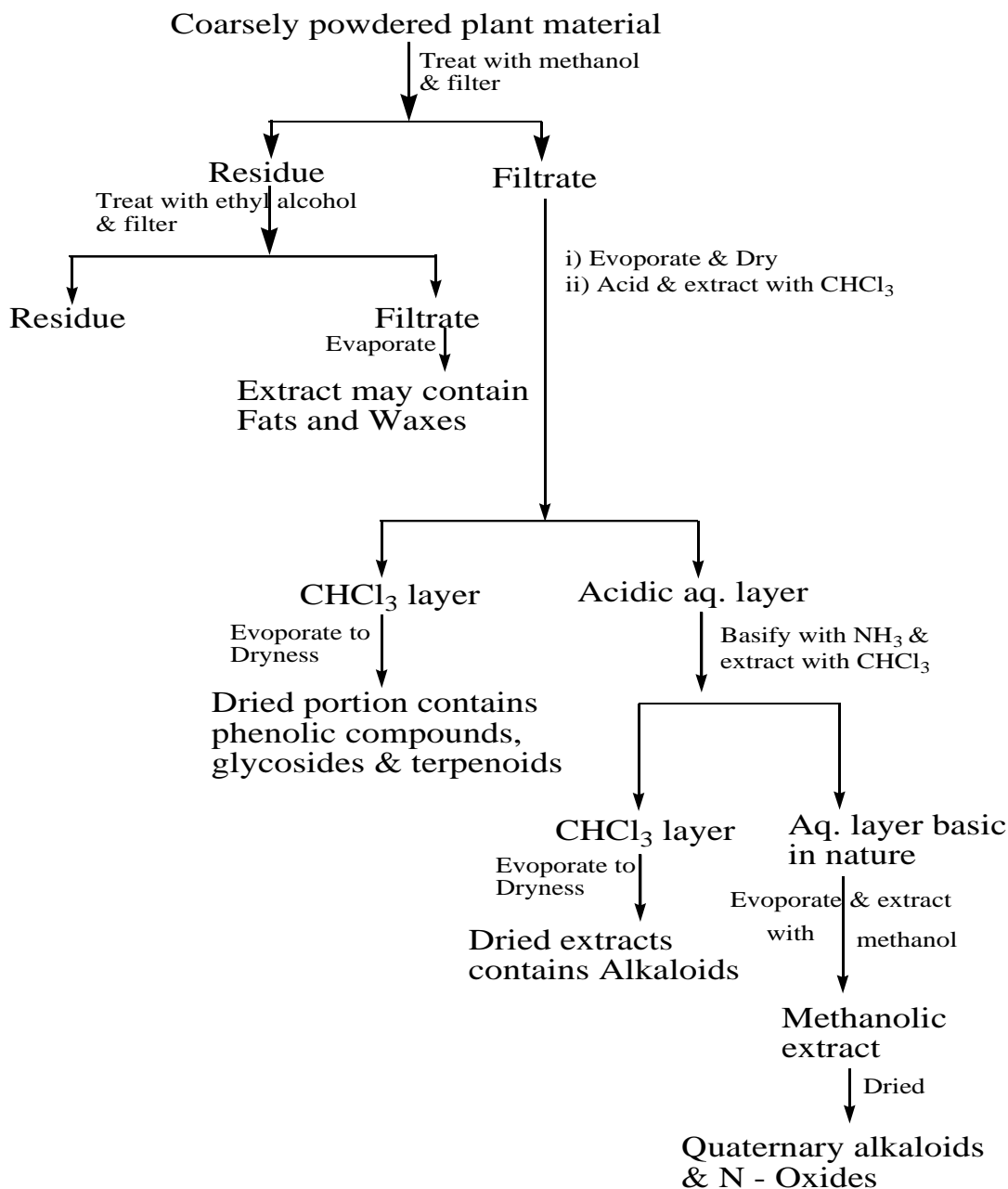
CHEMICALS:

All the chemicals used in the study were obtained commercially and of analytical grade.

MICROORGANISMS:

The test organisms *Streptococcus pyogenes*, *Nocardia calcarea*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*, were procured from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune 411 008.

Fig. 1: Tree Diagram showing extraction and isolation method



ANTIBACTERIAL ASSAY:

The Fats & Waxes, Terpenoids & Phenolics, Alkaloids, Quaternary alkaloids & N-oxides, were examined for their antibacterial potency by Cup plate agar method [3, 4] against bacterial species viz., *S. pyogenes*, *N. calcareo*, *B. subtilis*, *P. aeruginosa* which generally related to skin diseases. The petriplates were prepared with 25ml sterile Mueller Hinton Agar. A sterile cork borer (8 mm) was used to make wells in each plate. 1 ml inoculum's suspension was swabbed uniformly over the agar medium to get uniform distribution of bacteria. After labelling the plates 100µl of each test compound (at concentration of 0.01 mol) was added aseptically into the wells. The petriplates were then incubated at 37°C for 24 hrs during which the activity was evidenced by the presence of zone of inhibition surrounding the well. The negative control was prepared using respective solvent. *Ampicilin disc* (10 mcg/disc) and *Vancomycin disc* (30 mcg/disc) were used as positive control. The zones of inhibition were recorded in millimetres by using Himedia Zone Reader Scale¹⁰.

Table No. 1:- Antibacterial activity of different extract of the plant *Ceasalpinina bonduc*

S.N.	Test material	Concentration	Zones of inhibition in mm			
			S. <i>Pyogenes</i>	N. <i>calcareo</i>	B. <i>subtilis</i>	P. <i>aeruginosa</i>
1.	Fats & Waxes	0.01 mol	08	10	11	09
2.	Terpenoids & Phenolics	0.01 mol	12	10	12	11
3.	Alkaloids	0.01 mol	11	11	13	12
4.	Quaternary alkaloids & N-oxides	0.01 mol	10	10	12	13
5.	<i>Ampicilin disc</i>	10 mcg/disc	16	20	17	16
6.	<i>Vancomycin disc</i>	30 mcg/disc	16	20	17	18

RESULTS AND DISCUSSION

The results obtained for the antibacterial assay performed for various isolated ingredients of *Ceasalpinina bonduc* plants are presented (Table 1). Among the various isolated ingredients, Fats & Waxes set of extract was found to be more effective against *N. calcareo* and *B. subtilis*. Terpenoids & Phenolics group extract was effective against *S. pyogenes* and *B. subtilis*. Alkaloids, Quaternary alkaloids & N-oxides were found to be more effective against *B. subtilis*, and *P. aeruginosa*. Activities of the various extracts were comparable to those of standard antibacterial agent *Ampicilin* and *Vancomycin disc*. In the present study four different bacterial strains were used to screen possible antibacterial activity related to skin diseases of *C. bonduc* extracts. A result clearly indicates that, extracts showed significant antibacterial activity. However, further detailed study in the light of medicinal science is advised to get correct insight of the treatment.

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