

## Antimicrobial activity of some crude herbal drugs used for skin diseases by Pawra tribes of Nandurbar district

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Identification of traditional remedies for the skin is an important activity for the preservation of traditional knowledge and for the search of novel antimicrobial treatments against skin and soft tissue infections. A study was undertaken to document and authenticate treasured knowledge of Pawra tribe of Nandurbar district in terms of botanicals used for the topical applications on skin ailments. Antimicrobial activity of five herbal crude drugs, viz. stem of *Cryptolepis buchanani* Roem. & Schult., roots of *Curculigo orchiooides* Gaertn., stem bark of *Diospyros melanoxylon* Roxb., leaves of *Kleinia balsamica* (Dalz. & Gibs.) P. Halliday and stem bark of *Ougenia oojeinensis* (Roxb.) Hochr. was tested against different Gram positive and Gram negative bacteria and a yeast, using agar diffusion method. Extracts at higher concentration (60 and 80 mg/l) exhibited considerable antimicrobial activity but less or no inhibition was observed in case of lower concentration (20 and 40 mg/l).

**Keywords:** Antimicrobial, Herbal crude drugs, Maharashtra, Nandurbar district, Pawra tribes, Skin diseases.

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### Introduction

Infectious dermatological conditions are of common occurrence in rural settings and in the forest dwelling tribal populations. Skin abrasions, burns, inflammation and minor wounds occur frequently. Applications of herbal remedies, tattooing with herbo-mineral preparations are usual practices and have become part of several tribal rituals for skin problems including dermal inflammation, folliculitis (inflammation of follicles with infection by bacteria like *Staphylococcus*), etc. Other common skin problems include, skin abuses, acne, perioral dermatitis, rosacea, subaceous cysts, etc<sup>1</sup>. UV exposure and pollutants also add to the skin problems. Multidrug resistant bacteria including nosocomial pathogens have become important cause for higher skin care costs. A novel compound, with difference in mode of activity of antibiotics against microbes, is an attractive alternative against multidrug resistant bacteria. Immuno compromised individuals are frequently found suffering from skin infections that are difficult to cure<sup>2</sup>. A wide array of natural products

from botanicals are traditionally in use over several hundred years. Plant kingdom is a gold mine for novel and affordable skin care acting through novel mechanisms against skin pathogens.

Pawra tribes inhabit the Nandurbar district, a part of Western Satpura regions of Maharashtra and is a treasure toe of the ethnobotanical knowledge. During the Indigenous Traditional Knowledge (ITK) survey, it was found that these tribes possess very good knowledge of medicinal plants for the treatment of skin diseases, which are available locally and in their script-less Pawri language. This traditional knowledge is on the verge of extinction because of recent availability of primary health care (PHC) centre facility of State Government. In addition, there is remarkable depletion in the availability of medicinal plants. The tribes are largely unaware of the importance of documentation, conservation and scientific utilization of plants. To the best of our knowledge, this is the first ethno-botanical survey of this area focusing use of plants against skin ailments. Vaidoos are the medical practitioners in this area, who are known to be the primary repository of the traditional knowledge, apart from elder women who are having knowledge about the household remedies.

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The work on antimicrobial activity has been carried out since 1940's<sup>3</sup>. Many plants have been studied for their medicinal and antimicrobial properties<sup>4-7</sup> and some of them resulted in development of drugs<sup>8</sup>. The chemical constituents and formulation of a large number of plant based drugs have been documented including raw drugs sold in the village market<sup>9</sup>. During the ethnobotanical exploration in Nandurbar district of Maharashtra State, it is recorded that herbal crude drugs of five species (Plate 1), viz. stem of *Cryptolepis buchanani* Roem. & Schult. (Family — Periplocaceae), roots of *Curculigo orchioides* Gaertn. (Family — Hypoxidaceae), stem bark of *Diospyros melanoxylon* Roxb. (Family — Ebenaceae), leaves of *Kleinia balsamica* (Dalz. & Gibs.) P. Halliday (Family — Asteraceae) and stem bark of *Ougenia oojeinensis* (Roxb.) Hochr. (Family — Fabaceae) have been used extensively to cure skin diseases, wound infection, infection in hair glands and seborrheic dermatitis, etc. During the survey these plants were available throughout the year and data was compared with earlier reports<sup>10-12</sup>.

In the present investigation, the antimicrobial activity of these plant parts were tested, using standard procedures, against some reference strains of human pathogenic bacteria and yeast to assess the feasibility of applying crude preparations, as substitute for commercial products.

## Materials and Methods

### Plant material

Botanical names with herbarium number, family, local names, plant part used and their medicinal uses<sup>11, 12</sup> are summarized in Table 1. Five herbal crude drugs from stem of *Cryptolepis buchanani*, root of *Curculigo orchioides*, stem bark of *Diospyros melanoxylon*, leaves of *Kleinia balsamica* and stem bark of *Ougenia oojeinensis* were selected based on their frequent usage by the tribals to treat skin diseases and were screened to ascertain anti-microbial activity against human pathogens. Plants were selected for this study based on their medicinal use (Table 1). Fresh plant parts were collected from the Satpura forest of Maharashtra, India. The collected plant material was identified and the voucher specimens were deposited in the herbarium of Medicinal Plants Conservation Centre (MPCC), Pune (India).

### Microorganisms

The reference strains of three bacteria, viz. *Bacillus subtilis* (NCIM 2045), *Pseudomonas aeruginosa* (NCIM 5029), *Escherichia coli* (NCIM 2345) and a yeast *Candida albicans* (NCIM 3557) were obtained from Department of Microbiology, University of Pune, Pune. These were sub-cultured for further use. Bacterial cultures were maintained on nutrient agar whereas yeast was cultured on YEPD (Yeast extract-peptone-dextrose growth) medium.



*Cryptolepis buchanani*



*Curculigo orchioides*



*Diospyros melanoxylon*



*Kleinia balsamica*



*Ougenia oojeinensis*-Flowering and fruiting

Plate 1—Plants used for skin ailments

Table 1— Ethnomedicinal applications of plants selected for antimicrobial screening for skin diseases

S. No.	Botanical name/Voucher number	Family	Local name	Plant part used	Ethnomedicinal use
1	<i>Cryptolepis buchanani</i> Roem. & Schult (MPCC 1752)	Periplocaceae	<i>Dudhi</i>	Stem	Stem is crushed in water to make paste and this paste is applied locally.
2	<i>Curculigo orchioides</i> Gaertn. (MPCC 732)	Hypoxidaceae	<i>Kallo musulo</i>	Root	Root paste in water is applied locally and a piece of root is given orally.
3	<i>Diospyros melanoxyton</i> Roxb. (MPCC 2213)	Ebenaceae	<i>Tembrun</i>	Stem bark	Paste of stem bark in water is applied locally.
4	<i>Kleinia balsamica</i> (Dalz. & Gibs.) P. Halliday (MPCC 2524)	Asteraceae	<i>Thua</i>	Leaves	Leaves are crushed and applied locally.
5	<i>Ougenia oojenensis</i> (Roxb.) Hochr. (MPCC 2905)	Fabaceae	<i>Tivsyra</i>	Stem bark	Paste of stem bark in water is applied locally.

### Preparation of sample extract

The plant drug materials, collected from different localities were shade dried by cutting them into pieces and powdered in a blender and used for studying biological activities. Ten gram powder of each plant sample was weighed accurately and allowed to soak in 50 ml of methanol. These extracts were incubated at ambient temperature for 24 hours and filtered through Whatman filter paper no. 3<sup>(Ref. 13)</sup>. One ml of the extract was evaporated to dryness at 40°C and the concentration of the extract was determined for 50 ml. This stock solution was diluted accordingly.

### Antimicrobial activity

The selected micro-organisms ( $2 \times 10^6$  colony forming units) were cultured on nutrient agar/YEPD by using spread plate technique and a well of 6-7 mm diameter was made at the center for loading the extract. Stock solutions in methanol were used to prepare desired dilutions with concentrations of 20, 40, 60 and 80 mg/l. 100 µl of each dilution was poured in to the well and the sample extracts were allowed to diffuse properly by keeping the petriplates in refrigerator at 4°C for 4 hours followed by incubation at 37°C for 24 hours. Solvent used for extraction (methanol) was used as a control in the same manner. The diameter of zone of inhibition (excluding well diameter) was taken as the measure of the antimicrobial activity of a particular extract. Each experiment was performed in triplicate and repeated twice.

### Results and Discussion

It was observed that most of the plant species showed significant antimicrobial activity at various concentrations except *O. oojenensis*, which did not exhibit anti-microbial activity against *P. aeruginosa* even at higher concentrations (Table 2, Fig. 1 A-D).

Table 2—Antimicrobial activity of selected herbal crude drugs

Plants and methanolic extract (mg/l)	Microbes and Zone of inhibition in mm			
	<i>Pseudomonas aeruginosa</i>	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Candida albicans</i>
<i>Cryptolepis buchanani</i> - stem				
20	9	15	0	6
40	18	16	0	12
60	10	18	13	15
80	12	24	14	16
<i>Curculigo orchioides</i> - root				
20	14	20	0	0
40	15	22	18	0
60	19	21	19	0
80	20	22	18	0
<i>Diospyros melanoxyton</i> – stem bark				
20	18	5	20	0
40	15	22	26	0
60	25	13	37	17
80	26	28	39	18
<i>Kleinia balsamica</i> - leaves				
20	0	0	0	0
40	10	2	4	10
60	16	8	10	12
80	14	10	14	14
<i>Ougenia oojenensis</i> – stem bark				
20	0	0	4	6
40	0	0	4	8
60	0	6	12	14
80	4	10	12	14

*C. orchioides* did not show any activity against *C. albicans*. (Fig 1D). The extracts of *C. buchanani* were found to be significantly active against *B. subtilis* and *P. aeruginosa*, however they did not show significant

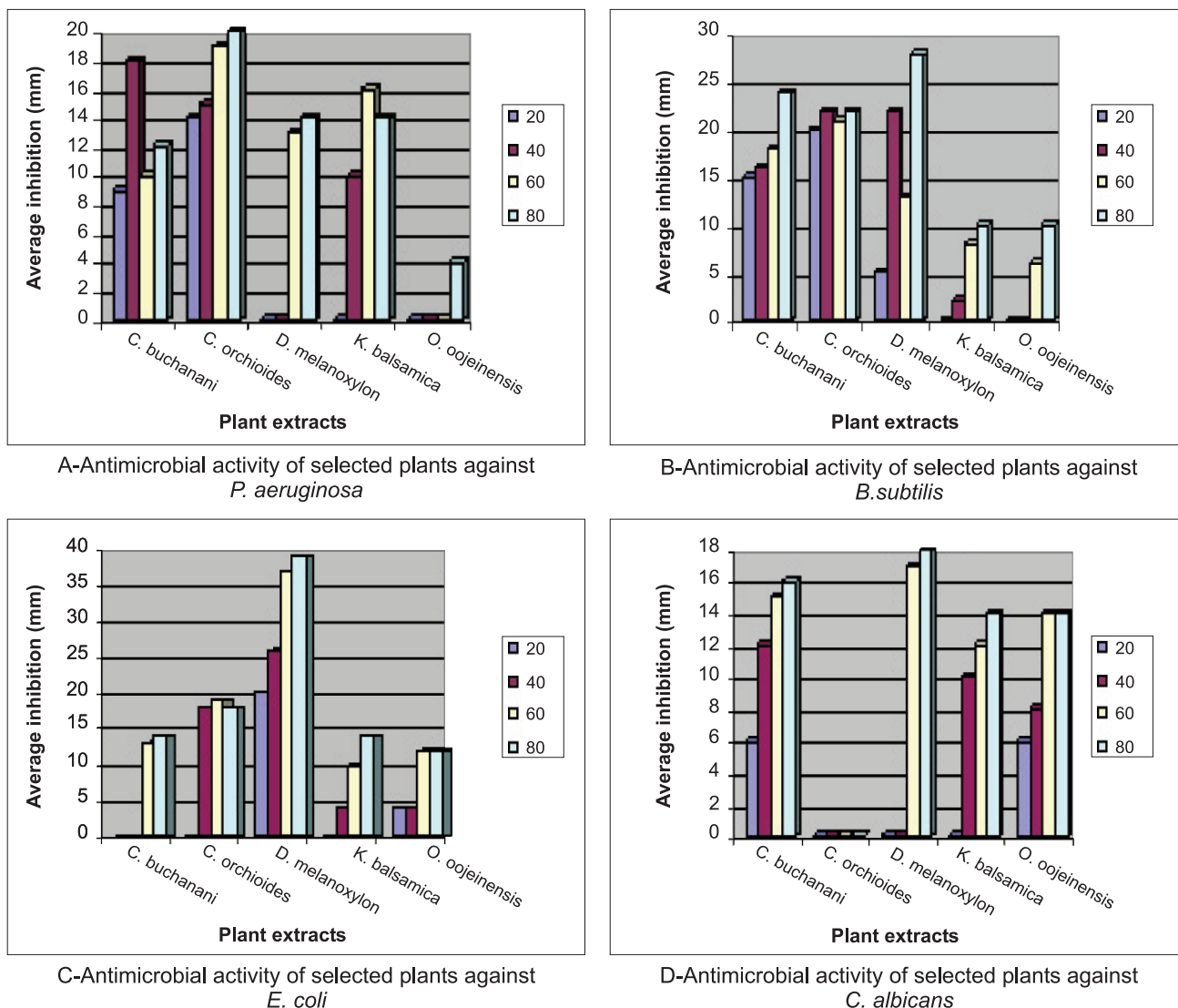


Fig. 1 A-D— Antimicrobial activities of selected plants

inhibitory activity against *E. coli* at lower concentration. *C. orchiooides* did not exhibit the inhibition for the fungus but showed significant inhibition for all three microbes under study. The maximum inhibition for these extracts was shown by the highest concentration (80 mg/l). The stem bark extract of *D. melanoxyylon* extracts showed inhibition to all the microbes at all concentrations except for the yeast except at low concentration did not inhibit yeast (Table 2). Extracts of *K. balsamica* showed activity only at a higher concentration for all microbes. This is in agreement with previous reports for *C. buchanani*, *C. orchiooides* and *D. melanoxyylon*<sup>14-16</sup>. There is no literature evidence for antimicrobial activity of *K. balsamica* and *O. oojeinensis*. All plant material

showed prominent activity against *E. coli*. *C. albicans* was inhibited maximum by *C. buchanani*, *O. oojeinensis* and *K. balsamica* (20, 40 mg/l) whereas no inhibition was observed by *C. orchiooides* at all concentration (Fig 1D). Almost same trend was followed by *B. subtilis* and *P. aeruginosa* with slight decrease in the activity. It clearly indicates that, the constituents extracted might be responsible for the promising activity as antibacterial agents (Fig. 1A-D).

The poor activity at lower concentrations may be attributed to the less solubility of its active compounds, which may improve with stringent extraction procedures. At higher concentration (40-80 mg/l) all the extracts showed significant antimicrobial activity.

## Conclusion

The antimicrobial activity of *K. balsamica* and *O. oojeinensis* are reported for the first time. This study clearly indicates that extracts of the plants studied possess potent antimicrobial activity. The use of crude drugs of such plants as an agent to control microbial pathogens needs further extensive research for their better economic and therapeutic utilization. The main focus of our work is on antimicrobial activities of herbs which we plan to take forward with the final objective of providing scientifically validated herbal remedies against microbes. Further, phytochemical studies are required to determine the types of compounds responsible for the antibacterial activities of these species.

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