

Antimicrobial Efficacy of *Cassia occidentalis* (Kasmard) against Some Human Pathogenic Bacteria and Fungi

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Summary

The occurrence of side effects after a long term use of synthetic drugs is always feared during the treatment of chronic diseases. Such possibilities are experienced to be of negligible extent in the case of herbal drugs and thus are more efficient to treat infectious diseases than synthetic antibiotics. Therefore it is necessary to evaluate, in a scientific base, the potential use of natural medicine for the treatment of infectious diseases. In the present investigation decoction of seeds *Cassia occidentalis* was evaluated for its antimicrobial potential against nine different human pathogenic bacteria and five fungi by agar well diffusion method. The leaves, roots and seeds of *Cassia occidentalis* are purgative. They are useful in cough and whooping cough. Externally they are applied smeared with grease to slight sores, itch. The infusion of the root is considered as an antidote to various poisons. The root is considered diuretic and is used in incipient dropsy and fever. Leaves are used externally in scabies, ringworm and other skin diseases. Korku tribes use this plant for skin diseases and whooping cough. The decoction of *Cassia occidentalis* has shown potential antibacterial activity against four Gram-negative bacteria including *Escherichia coli*, *Salmonella typhimurium*, *Enterobacter aerogenes* and *Pseudomonas aeruginosa*. Among them *Pseudomonas aeruginosa* was found most sensitive. In case of Gram-positive bacteria tested, only *Staphylococcus aureus* was found sensitive. Among the fungi *Aspergillus flavus*, *Candida albicans*, *Candida tropicalis*, *Candida glabrata*, *Trychophyton rubrum* and *Epidermophyton floccosum* were found sensitive except *Microsporum gypseum*. Among them all *Aspergillus flavus* have shown highest sensitivity towards the decoction. The results indicate that *Cassia occidentalis* has broad spectrum antimicrobial potential against antibiotic resistant microorganisms.

Key words: Antimicrobial, Ayurveda, *Cassia occidentalis*, decoction, herbal.

Introduction

The revival of interest in natural drugs especially those derived from plants and animals, started in the last decades mainly because of the widespread belief that natural medicines are healthier and safer than synthetic ones. This is because herbal medicine has a type of chemical balance, also the plant has the complete knowledge of the know how of its own ecosystem therefore if the medicinal property gets changed to some extent due to some reasons the plant has its own defense mechanism so it will try to maintain all its important characteristics. Also the emergence of resistance to conventional antimicrobials is a serious problem that physicians face.

This necessitates constant development of newer agents, which can inhibit the growth of or kill resistant organisms. Therefore the study attempts to portray the potential role of Ayurveda. The objectives of the *in vitro* study are to validate efficacy of decoction of seeds of *Cassia occidentalis* as an antimicrobial agent. Drugs prepared according to the Ayurvedic methods are often found to be simply marvelous. And that is why the extraction method for the antimicrobial study is according to the Ayurvedic pharmacognosy, 'Bhaishjyakalpana'.^[1] The method of extraction of active ingredients used here is decoction method using water as a solvent. *Cassia occidentalis* is also known as Negro coffee (English), Kasmard (Sanskrit), Kasunda (Hindi), Chakwar (Korku). The leaves roots and seeds are purgative. They are useful in cough and whooping cough. Externally they are applied smeared with grease to slight sores, itch. The seeds are used in France and West Indies as a febrifuge in the form of vinous tincture. The infusion of the root is considered as an antidote to various poisons. The root is considered diuretic and is used in incipient dropsy and fever. Leaves are used externally in scabies, ringworm and other skin diseases.^{[2] [3]} Korku tribes use this plant for skin diseases and whooping cough. The chemical constituents of these plant are Emodin, tannic acid, achrosine, malic acid and chrysophanic acid, phytosterolin, emodin and betasitosterol.^{[3][4]}

Cassia occidentalis (Kasmard) is one of the constituents of some of the following formulations of reputed Ayurvedic pharmaceutical companies.

Himalaya Drug Company: *Liv 52 syrup* for liver disorders.

Charak Pharmaceuticals India Ltd.: *Lyvomyn syrup, tablets* and *drops* for infective hepatitis, cirrhosis, liver disorders and jaundice.

Vasu Healthcare Pvt. Ltd.: *Cutis cream* as fungicidal and antiallergic and it is used for other skin infections.

Materials and Methods

Preparation of decoction: The seeds of *Cassia occidentalis* were collected from Nagarjun Medicinal Plants Garden, (P.K.V.), Akola (MS). The seeds were dried in shade and powdered. 25 gm of this powder was soaked in thirty-two parts (800ml) of potable drinking water in a glass beaker and left this for overnight. The next day decoction as a general rule of Ayurveda was prepared by boiling in the glass beaker in a water bath over a slow fire till it reduces to one-fourth (200 ml) and the decoction was then strained through cloth first^[1] and then through Whatmann filter paper no.1 two times to remove suspended particles. Thus the final concentration of decoction was found to be 125 mg/ml of water. The actual concentration of active principle is being represented by the concentration of powder in water.

Test microorganisms: Some of the Gram-positive and Gram-negative bacteria, yeast and fungi responsible for gastrointestinal diseases, respiratory tract diseases, urinary tract infections, enteric and other type of fever, septicemia, pyogenic infections and wound infections, infections of ear and eye, skin diseases etc. were selected for this study. They are:

Gram-negative bacteria: *Escherichia coli* ATCC 10536, *Salmonella typhimurium* ATCC 23564, *Salmonella typhi* ATCC 531, *Klebsiella pneumoniae* MTCC 109, *Enterobacter aerogenes* ATCC 13048, *Proteus vulgaris* NCIM 2027 and *Pseudomonas aeruginosa* ATCC 27853.

Gram-positive bacteria: *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212.

Fungi: *Aspergillus flavus* MTCC 277, *Candida albicans* MTCC 3017, *Candida tropicalis* MTCC 184, *Candida glabrata* MTCC 3019, *Trichophyton rubrum* MTCC 3272, *Microsporum gypseum* MTCC 4479 and *Epidermophyton floccosum* MTCC 613.

Preparation of inoculums: All microorganisms were obtained from NCIM (National Collection of Industrial Microorganisms) Pune, India and IMTECH (Institute of Microbial Technology) Chandigarh, India. Four to five colonies from pure growth of each test organism were transferred to 10 ml of Nutrient broth, MRS broth and Sabouraud's dextrose broth depending upon the microorganisms and then incubated at 37 °C for 18-24 hours for bacteria and at room temperature (25-30°C) for different time intervals, according to the fungus.

Screening for antimicrobial activity: The antibacterial and antifungal activity was determined by using agar well diffusion method.^[5] The media used for antibacterial study was Nutrient Agar Hi-Media (M 244) with composition of Peptone (10 gm/lit), Beef extract (10 gm/lit), NaCl (5 gm/lit) and pH was adjusted to 7.4 ± 0.2 (at 25 °C). Nutrient agar plates were inoculated with eighteen hour old broth culture of each test bacterium. The media used for antifungal activity was Sabouraud's Dextrose Agar Hi-Media (M 033) with composition of Mycological peptone (10 gm/lit), Dextrose (40 gm/lit), and pH was adjusted to 5.6 ± 0.2 (at 25 °C). Sabouraud's Dextrose agar plates were inoculated with the spore suspension of the each test fungus. The inoculum (0.1 ml) was spread evenly over the plate with sterile glass spreader. A sterile cork borer of standard 8 mm diameter was used to cut the well on agar surface and 500 ul of the decoction was poured in the well. The control was kept using sterile distilled water. Three replica plates were prepared for each microorganism. The plates were incubated at 37 °C for 24 hours for bacteria and at room temperature (25-30 °C) for different time intervals according to the fungus. The results were observed and the zones of inhibition (ZOI) were measured from the three replica plates and the mean was recorded. The minimum inhibition concentration (MIC) was also determined by making the dilutions of the decoction as 75% (93.75 mg/ml), 50% (62.5 mg/ml), 25% (31.25 mg/ml), 10% (12.5 mg/ml) and 5% (6.25mg/ml). MIC was recorded as the lowest concentration of test sample that prevented the growth of the test bacteria and fungi. The results of *in vitro* antibacterial and antifungal activities were analyzed statistically and are presented in terms of mean zone of inhibition \pm standard deviation.

Results and Discussion

Table 1 presents the results of antimicrobial activities of *Cassia occidentalis*. Among the tested Gram-negative bacteria *Pseudomonas aeruginosa* ATCC 27853 was found most sensitive. The diameter of ZOI was 25 mm at 125 mg/ml concentration of the decoction. The minimum inhibition concentration was found to be 6.25 mg/ml. Among the other tested Gram negative bacteria, *Escherichia coli* ATCC 10536, was sensitive showing a ZOI of 22 mm at 125 mg/ml concentration. The MIC was found to be 6.25 mg/ml. Other sensitive bacteria were *Enterobacter*

aerogenes ATCC 13048 (ZOI 20 mm at 125 mg/ml, MIC 31.25 mg/ml). The ZOI of *Salmonella typhimurium* ATCC 23564 at 125 mg/ml concentration of the extract was found to be 13 mm and MIC was also found at 125 mg/ml. The only Gram-positive bacteria found sensitive were *Staphylococcus aureus* ATCC 25923 (ZOI 16 mm at 125 mg/ml and MIC 6.25 mg/ml). Among the fungi, almost all fungi were found sensitive except for *Microsporium gypseum* MTCC 4479. The highly sensitive was *Aspergillus flavus* MTCC 277 (ZOI 25 mm at 125 mg/ml, MIC 6.25 mg/ml) followed by *Candida albicans* MTCC 3017 (ZOI 22 mm at 125 mg/ml, MIC 12.5 mg/ml), *Trychophyton rubrum* MTCC 3272 (ZOI 22 mm at 125 mg/ml, MIC 62.5 mg/ml), *Epidermophyton floccosum* MTCC 613 (ZOI 20 mm at 125 mg/ml, MIC 62.5 mg/ml), *Candida glabrata* MTCC 3019 (ZOI 18 mm at 125 mg/ml, MIC 31.25 mg/ml) and *Candida tropicalis* MTCC 184 (ZOI 12 mm at 125 mg/ml, MIC 125 mg/ml) (Plate 1A and 1B). The results shows variation in sensitivity of the susceptible microorganisms as depicted in figure 1.

The ethanol extracts of different parts and the calli of *Cassia occidentalis* and sequential extracts (petroleum ether, benzene, chloroform, ethanol and water) of whole plant and metabolite-rich fractions (anthraquinones, sennosides and flavonoids) of leaves, pods, flowers and callus have tested against indicator human pathogenic bacteria and fungi. The anthraquinones were found to be more active against *Escherichia coli* and *Staphylococcus aureus* while sennosides were more active against *Aspergillus flavus*.^[6] The present investigation is in fair correlation with these findings (Plate 1A and 1B). Also the present investigation strongly supports the view of Hussain who found the antimicrobial properties of *Cassia occidentalis*.^[7] The leaves, seeds and seeds of the plant are useful in cough and whooping cough and can be applied externally to slight sores and itch. The root is considered diuretic and is used in incipient dropsy, skin diseases and fever.^{[2] [3]} Korku tribes use this plant for skin diseases and whooping cough. *Cassia occidentalis* (Kasmard) is one of the constituents of some of the formulations of Ayurvedic pharmaceutical companies like, *Liv 52 syrup* for liver disorders (Himalaya Drug Company), *Lyvomyn syrup, tablets* and *drops* for infective hepatitis, cirrhosis, liver disorders and jaundice (Charak Pharmaceuticals India Ltd.). The present investigation supports the above mentioned uses of the plant. The present findings depict the use of *Cassia occidentalis* as broad spectrum antibacterial as well as antifungal agent.

Figure 1: Antimicrobial activity of *Cassia occidentalis* (Kasmard)

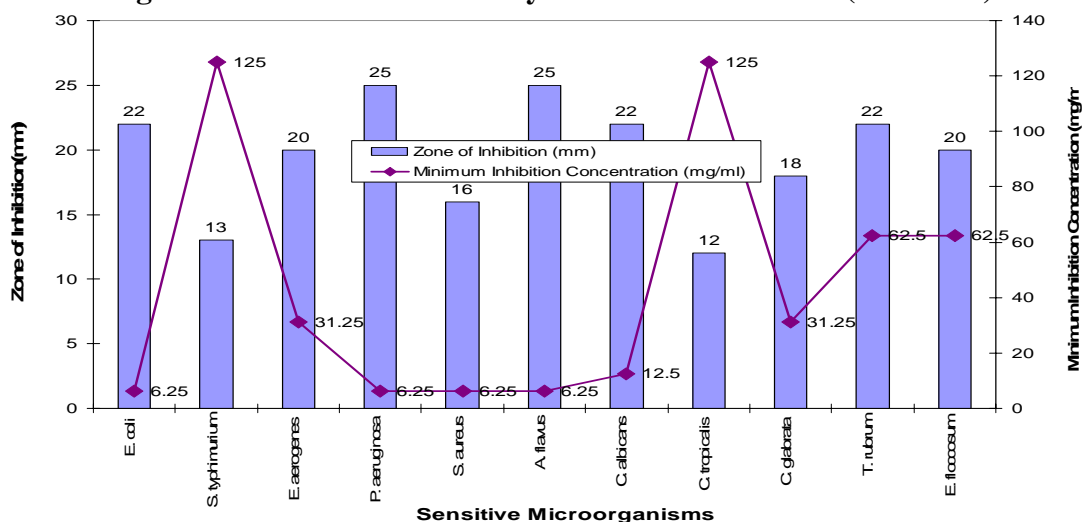


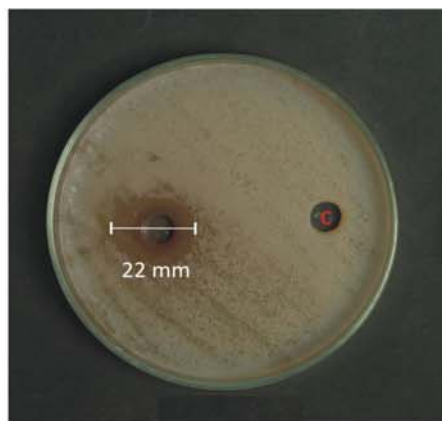
Table 1: Antimicrobial activity of decoction of *Cassia occidentalis* (Kasmard)

Sr. No.	Name of microorganism	Zone of inhibition (ZOI) in millimeter at different concentrations of decoction.						Mean (ZOI) ± standard deviation
		125 mg/ml	93.75 mg/ml	62.5 Mg/ml	31.25 mg/ml	12.5 Mg/ml	6.25 mg/ml	
1	Escherichia coli ATCC 10536	22 mm	20mm	18 mm	16 mm	12 mm	11mm*	22 ± 2.16
2	Salmonella typhimurium ATCC 23564	13mm*	-----	-----	-----	-----	-----	13 ± 1.41
3	Salmonella typhi ATCC 531	-----	-----	-----	-----	-----	-----	
4	Klebsiella pneumoniae MTCC 109	-----	-----	-----	-----	-----	-----	
5	Enterobacter aerogenes ATCC 13048	20 mm	18mm	16 mm	15mm*	-----	-----	20 ± 2.44
6	Proteus vulgaris NCIM 2027	-----	-----	-----	-----	-----	-----	
7	Pseudomonas aeruginosa ATCC 27853	25 mm	20mm	16 mm	15 mm	11 mm	10mm*	25 ± 0.82
8	Bacillus subtilis ATCC 6633	-----	-----	-----	-----	-----	-----	
9	Staphylococcus aureus ATCC 25923	16 mm	14mm	13 mm	12 mm	11 mm	10mm*	16 ± 1.92
10	Enterococcus faecalis ATCC 29212	-----	-----	-----	-----	-----	-----	
11	Aspergillus flavus MTCC 277	25 mm	18mm	16 mm	15 mm	13 mm	10mm*	25 ± 1.63
12	Candida albicans MTCC 3017	22 mm	18mm	15 mm	12 mm	10mm*	-----	22 ± 0.82
13	Candida tropicalis MTCC 184	12mm*	-----	-----				12 ± 2.64
14	Candida glabrata MTCC 3019	18 mm	15mm	13 mm	10mm*			18 ± 2
15	Trychophyton rubrum MTCC 3272	22 mm	17mm	10mm*	-----	-----	-----	22 ± 1.63
16	Microsporium gypseum MTCC 4479	-----						
17	Epidermophyton floccosum MTCC 613	20 mm	14mm	11mm*	-----	-----	-----	20 ± 0.82

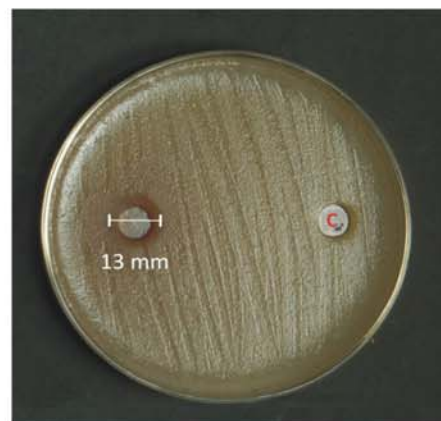
mm- millimeter; mg- milligram; ml- milliliter and * indicates minimum inhibition concentration.

Plate 3 A

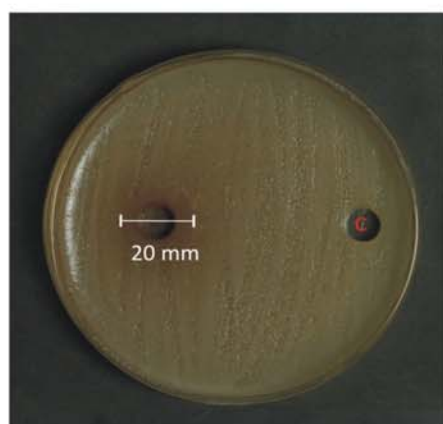
Antimicrobial Activity of *Cassia occidentalis*



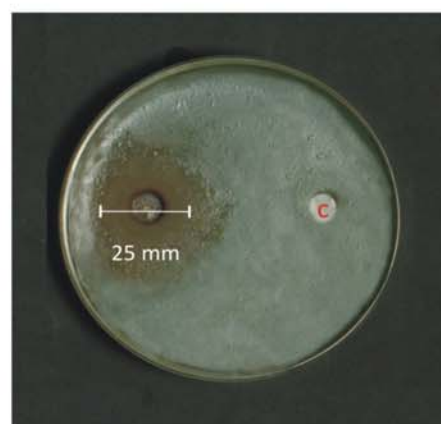
Escherichia coli



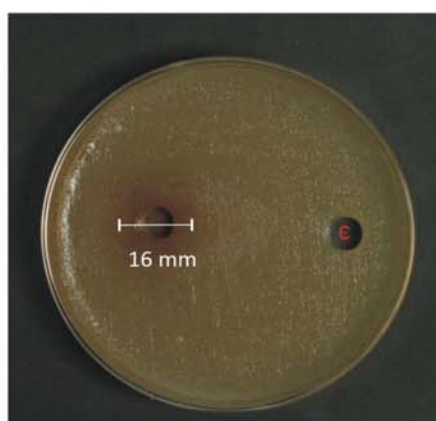
Salmonella typhimurium



Enterobacter aerogenes



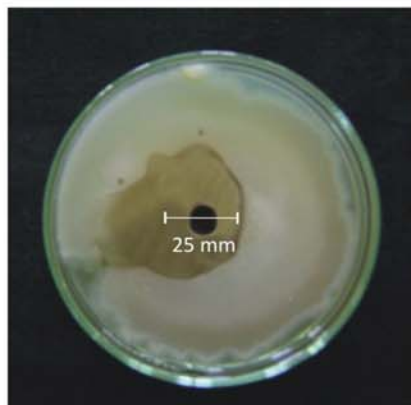
Pseudomonas aeruginosa



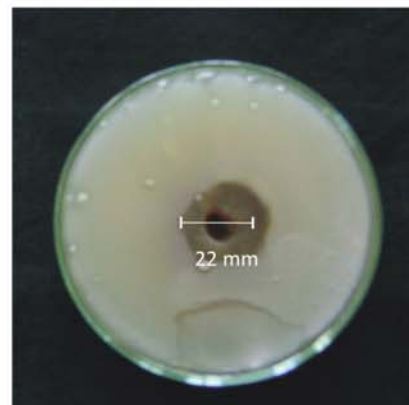
Staphylococcus aureus

Plate 3 B

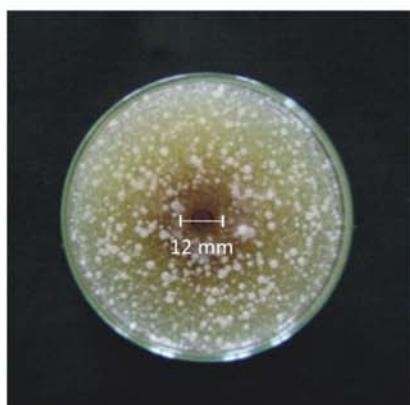
Antimicrobial Activity of *Cassia occidentalis*



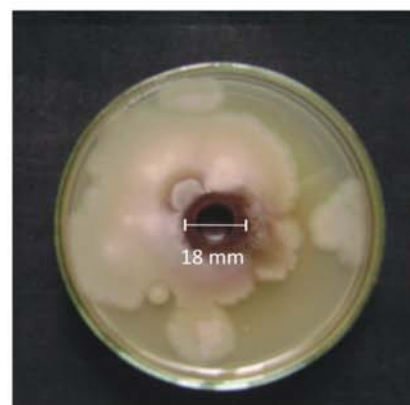
Aspergillus flavus



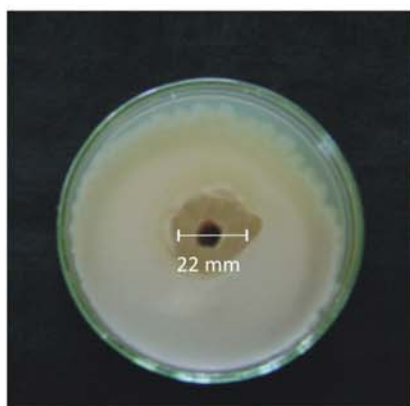
Candida albicans



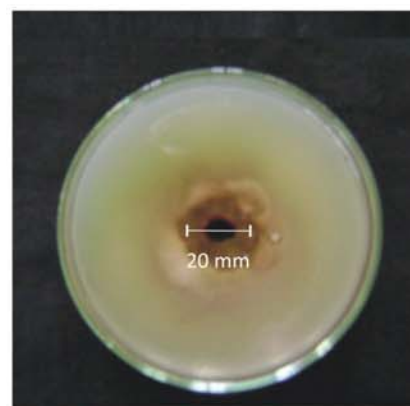
Candida tropicalis



Candida glabrata



Trychophyton rubrum



Epidermophyton floccosum

Acknowledgements

It gives us immense pleasure to express our special appreciation to Shri S. G. Koparkar Guruji for providing information on Ayurveda, Ethnomedicine and Naturopathy. The authors gratefully acknowledge Dr. Archana Nikam, a reputed Ayurvedic practitioner and Dr. V. G. Thakare, Principal, Shri Shivaji Science College, Amravati, for providing the laboratory and library facilities during the course of the research work.

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