

PHYTOCHEMICALS AND ANTIMICROBIAL STUDIES ON COSTUS SPECIOSUS

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Abstract: The aim of the present study was to investigate antimicrobial activity of methanolic extract of *Costus Speciosus* (CS). The percentage yield and physical status of the methanolic leaf extract (MECS) and chloroform (CSC) and aqueous fractions *Costus Speciosus* (CS) are showed. The antibacterial activity of a compound is expressed in terms of its ability to inhibit the growth of bacteria in nutrient broth or agar media. The bacterial growth inhibition can be measured by two methods (Cup plate method and Serial dilution method). Nutrient broth has been used for the preparation of inoculum of the bacteria and nutrient agar is used for the evaluation of antibacterial activity. All the Extracted compounds (BMC & BMQ), were found to be effective antibacterial agents. Antifungal activity evaluation against *C. Albicans* showed that compound BMH also did not possess significant antifungal properties. The study confirms that *Costus Speciosus* (CS) and all possess antimicrobial activity with different potency against variety of selected microorganisms. The differentiating activities of these extracts encourage developing an over broad spectrum antimicrobial herbal formulation in future.

Keyword: *Costus Speciosus* (CS), Antimicrobial Activity, Antifungal Activity, MECS, BMC & BMQ, BMH, *Albicans*.

INTRODUCTION

Medicinal plants are part and parcel of human society to combat diseases, from the dawn of civilization [1, 2]. They usually contain many biologically active ingredients and are used primarily for treating mild or chronic ailments. According to World Health Organization (WHO), about 80% of the world population relies chiefly on the plant based traditional medicine especially for their primary healthcare needs. Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological and medicinal activities, higher safety margins and lesser costs [3,4]. Infectious diseases are a major cause of morbidity and mortality worldwide.

The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics,

immunosuppressive agents, intravenous catheters, organ transplantation and ongoing epidemics of human immune deficiency virus (HIV) infections [5, 6]. This situation provided the impetus to the search for new antimicrobial substances from various sources like medicinal plants. Synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with adulterations and side effects. Therefore, there is a need to search for new infection-fighting strategies to control microbial infections [7].

Plant medicines are used on a worldwide scale to prevent and treat infectious diseases [8]. Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids, terpenoids and flavonoids having been found in vitro since they have antimicrobial properties and may serve as an alternative, effective, cheap and safe antimicrobial for the treatment of microbial infections [9].

Plant based antimicrobial compounds have great therapeutic potential as they have lesser side effects as compared with synthetic drugs and also little chance of development of resistance. Therefore an attempt has been made to study the antibacterial activity of ten medicinally important plants viz. *Artocarpus heterophyllus*, *Berberis aristata*, *Chromolaena odorata*, *Embelia ribes*, *Jasminum angustifolia*, *Mahonia leschenaultii*, *Pluchea lanceolata*, *Plumbago indica*, *Terminalia chebula*, *Vitex negundo* [10]

MATERIALS AND METHODS

Collection of Plant Material

The leaves of *Costus speciosus* (CS) were collected from the Puri, Odisha, India, during month of December and authenticated by botanist Dr. N.K Dhal, Department of Botany, IMMT BBSR, Odisha India.

Methanolic Extraction

The collected leaves of *Costus speciosus* (CS) were shade dried, powdered and extracted with Methanolic solvent by using Soxhlet

apparatus. Further, the methanolic extracts of leaves/root/bark of *Costus speciosus* (CS) were fractionated with ethyl acetate, n-Hexane, chloroform and water as solvents. The extract of MEBM, BME, BMC, BMH, and BMQ were dispersed in 1 L of distilled water separately and fractionated with ethyl acetate, n-Hexane, chloroform and water as solvent in succession. The obtained fractions were concentrated under reduced pressure to yield corresponding extracts. The obtained extracts and fractions were kept in a desiccator to remove moisture and stored properly until used. Yield of each extract and fraction is noted in terms of w/w of dry material.

Studies on Extract and Fractions of *Costus Speciosus*

Physical nature and percentage yield of extracts. The percentage yield and physical status of them ethanolic leaf extract (MECS) and chloroform (CSC) and aqueous fractions *Costus speciosus* (CS) are showed in the Table 1. Yield of the extracts calculated with reference of the weight raw material used. For fractions, the yield was calculated with respect to corresponding methanolic extract.

Table 1: Percentage yield and physical status of the methanolic extract and fractions of *Costus Speciosus* (CS)

Extract/Fraction	Code	Yield (%)
Methanolic	MEBM	12.5
Ethylacetate	BME	6.5
Chloroform	BMC	5.3
n-hexane	BMH	6.1
Aqueous	BMQ	3.3

ANTIBACTERIAL AND ANTIFUNGAL ACTION

Antibacterial Activity: Generally the antibacterial activity of a compound is expressed in terms of its ability to inhibit the growth of bacteria in nutrient broth or agar media. The bacterial growth inhibition can be measured by two methods.

- Cup plate Method
- Serial dilution Method

Bacterial Culture Medium

Nutrient broth has been used for the preparation of inoculum of the bacteria and nutrient

agar is used for the evaluation of antibacterial activity.

ANTIBACTERIAL ACTIVITY

Antibacterial activity by Cup plate Agar Diffusion method by measuring the zone of inhibition in mm.

Materials

- Nutrient Broth Media
- Nutrient Agar Media
- Streptomycin (Standard)
- Dimethyl Sulfoxide (DMSO)
- Distilled Water

Required Equipment

- Laminar airflow cabin
- Incubator
- Refrigerator
- Micropipettes
- Culture Plates
- Boiling Tube
- Eppendorf Tubes

Evaluation for Antimicrobial Activity

The antibacterial activity of substituted chalcones (3a-3e) and their derivatives 4, 6 diaryl oxazines (4a-4d) had been assayed against four different strains of bacteria by cup-plate agar diffusion method by measuring the zone of inhibition.

Table 2: Composition of the nutrient agar medium

Nutrient	Medium (in gm)
Peptone	5
Sodium chloride	5
Beef extract	1.5
Yeast extract	1.5
Agar	1.5
Distilled water upto	1000 ml
pH	7.4 $\pm$ 0.2

The test organisms were sub cultured using nutrient broth medium. The tubes containing sterilized medium were inoculated with respective bacterial strains. After incubation at 37 $\pm$ 1 $^{\circ}$ C for 24 hours they were stored in refrigerator. The stock cultures were maintained. Bacterial inoculum was prepared by transferring a loopful of culture to nutrient broth in conical flask.

The flasks were incubated at 37 $\pm$ 1 $^{\circ}$ C for 48 hours before the experimentation. Solution of test compound was prepared by dissolving the sample in DMSO. A reference standard for both Gram positive and Gram negative bacteria was made by dissolving accurately weighed quantity of Moxifloxacin and Streptomycin in sterile distilled water.

The nutrient agar medium was sterilized by autoclaving at 121 $^{\circ}$ C for 15min. The Petri plate, tubes and flasks plugged with cotton were sterilized in hot air oven at 160 $^{\circ}$ C for two hours. Into each sterilized Petri plate,

about 25 mL of molten nutrient agar medium inoculated with the respective strains of bacteria was transferred aseptically. The plates were left at room temperature to allow solidification. In each plate, cups of 5 mm diameter were made with sterile borer. Then 100 μ l of the test solution was added to the respective cups aseptically and labelled accordingly.

The plates were kept undisturbed for at least 2 hours in refrigerator to allow diffusion of the solution properly into the nutrient agar medium. After the incubation of the plates at 37 $\pm$ 1 $^{\circ}$ C for 24 hours, the diameter of the zone of inhibition surrounding each of the cups was measured with the help of the scale and tabulated.

Standard Drugs Used

Streptomycin, Moxifloxacin

RESULTS AND DISCUSSIONS**Table 3: Zone of inhibition of solvent extract of CSM against E. Coli**

Compounds	50 μ g/ml (mm)	100 μ g/ml (mm)	1000 μ g/ml (mm)
MEBM	6	10	12
BME	10	12	16
BMC	11	15	18
BMH	8	10	14
BMQ	12	18	20
CPN	12	16	20
MXN	14	18	24

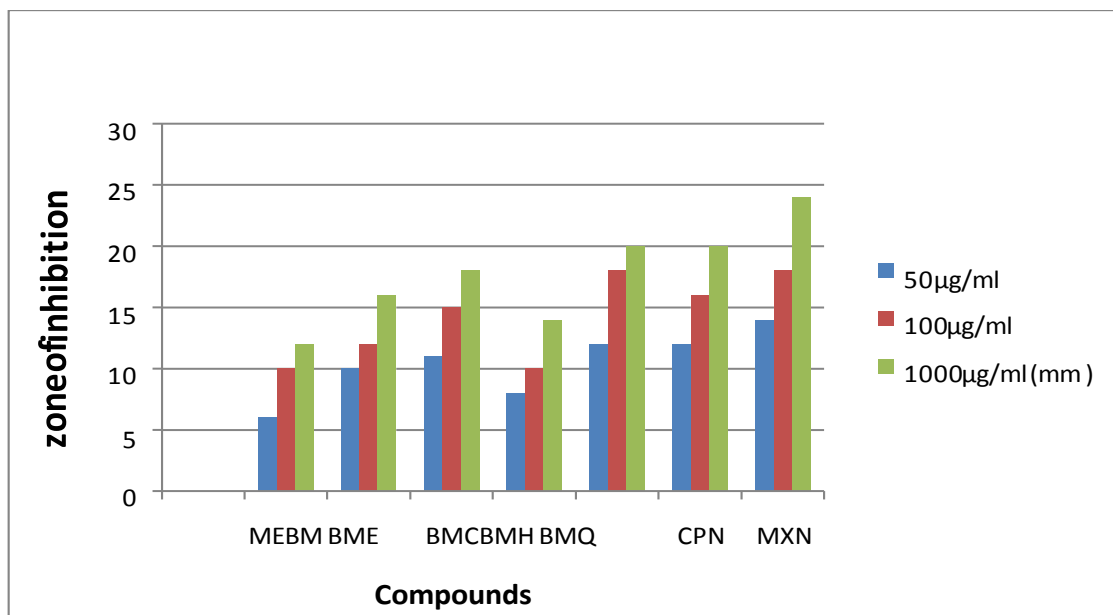


Figure 1: Zone of inhibition of solvent extract of CSM against E. Coli

Antibacterial activity of compounds against *E.coli*

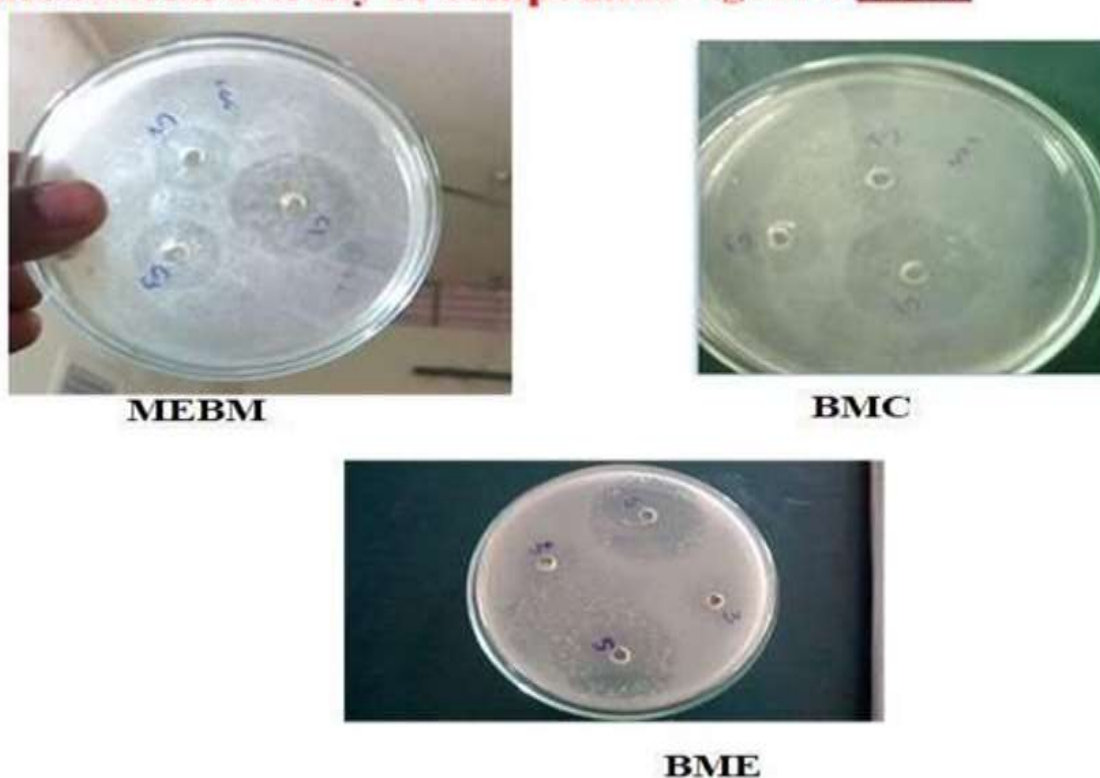


Figure 2: Zone of inhibition of solvent extract of CSM against E. Coli

Table 4: Zone of inhibition of CSM against on streptococcus aureus

Compounds	50µg/ml	100µg/ml	1000µg/ml
MEBM	5mm	9mm	14mm
BME	10mm	12mm	18mm
BMC	6mm	10mm	15mm
BMH	10mm	12mm	14mm
BMQ	6mm	10mm	16mm
CPN	14mm	18mm	22mm
MXN	16mm	20mm	24mm

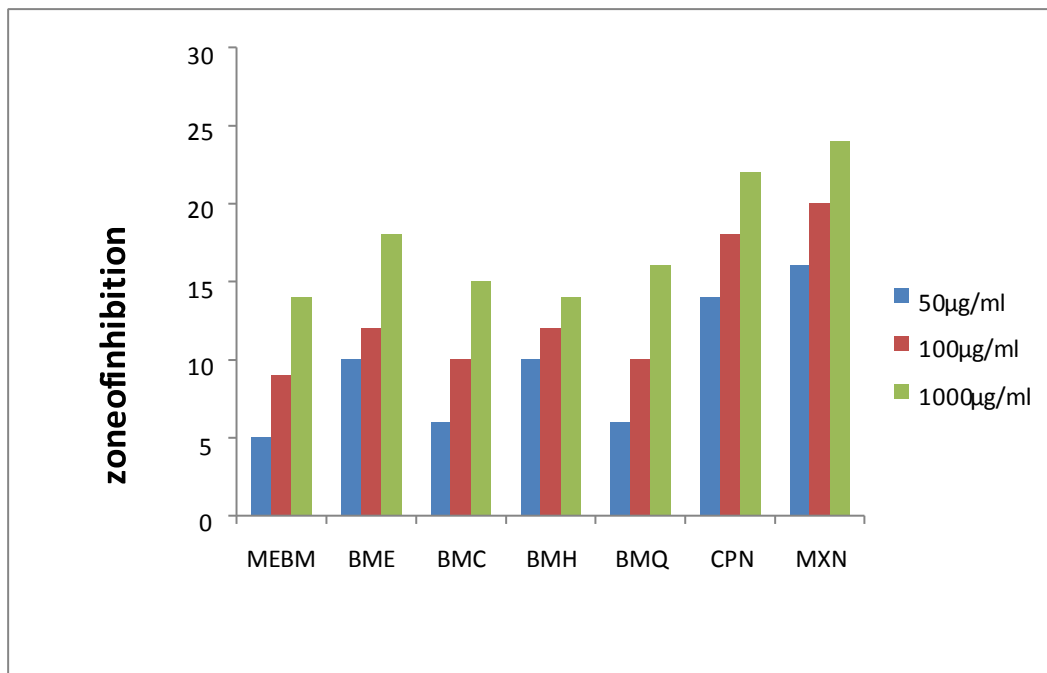


Figure 3: Zone of inhibition of CSM against on *Streptococcus aureus*

Antibacterial activity against *staphylococcus aureus*

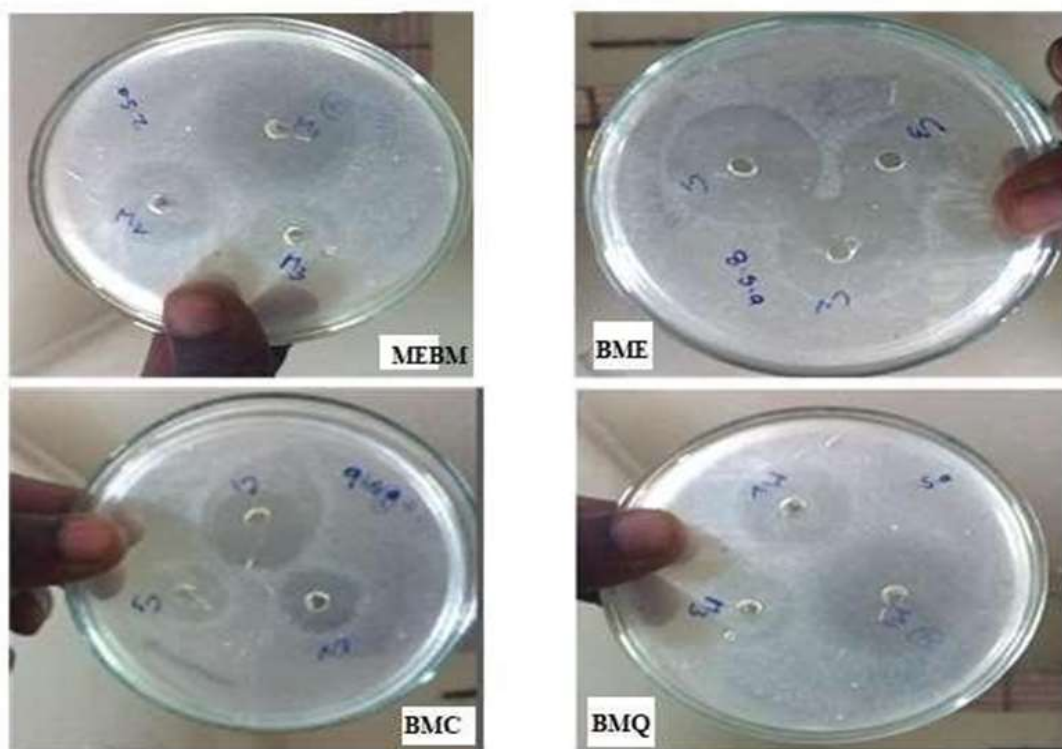


Figure 4: Zone of inhibition of CSM against on *Streptococcus aureus*

Results and Discussions of Antimicrobial Activity

The Extracted compounds MEBM, BME, BMC, BMH, BMQ has been evaluated for their antimicrobial activity against Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and

Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria by measuring the zone of inhibition. The results have been compared with a broad spectrum antibacterial agent ciprofloxacin as standard drug.

- All the Extracted compounds (BMC & BMQ), were found to be effective antibacterial agents.
- The compounds MEBM showed minimum activity against E.coli with zone of inhibition 6mm, 10mm, 12 mm.
- The compounds BMC showed maximum activity against Gram positive bacteria E. Coli at 100µg/ml with zone of inhibition 15mm. Among all those compounds MEBM, BMC and BMQ showed Poor activity against Gram-positive (Staphylococcus aureus) with zone of inhibition of 5mm, 6mm and 5mm.

ANTI-FUNGAL ACTIVITY

Using a broth micro dilution experiment, the extracted chemicals were evaluated against *Candida albicans* (ATCC10231) and *Saccharomyces cerevisiae* (PTCC 5052) fungal strains. NCCLS rules were followed when

using the broth micro dilution procedure. Sabouraud Maltose Agar (DIFCO, Becton) was used to subculture both cultures. In a sterile 0.9 percent NaCl solution, *C. albicans* and *S. cerevisiae* were suspended. A spectrophotometer set to 530 nm was used to adjust the turbidity of fungal suspensions to 75–77 percent transmittance (T).

For testing, the suspensions were diluted 1:1000 in Sabouraud maltose broth. In micro dilution wells, serial dilutions of the test compounds with growth medium were made, and 100 L of the diluted suspension was added to 100 L of each test compound solution. To determine MIC (minimum inhibitory concentration) values, micro dilution trays were incubated at 35 °C for 24 and 48 hours and then analysed. As medium positive and negative controls, amphotericin B, fluconazole, ketoconazole solutions, and DMSO (Merck) 2.5 % were employed, respectively.

Table 5: Antifungal activity of extracted compounds against *S. cerevisiae* PTCC505

Compounds	MIC, µM	
	24h	48h
MEBM	85.32	85.32
BME	1087	1087
BMC	235	235
BMH	>2086	>2086
BMQ	1142	1142
Ketoconazole	65.41	65.41
DMSO	10%v/v	10%v/v

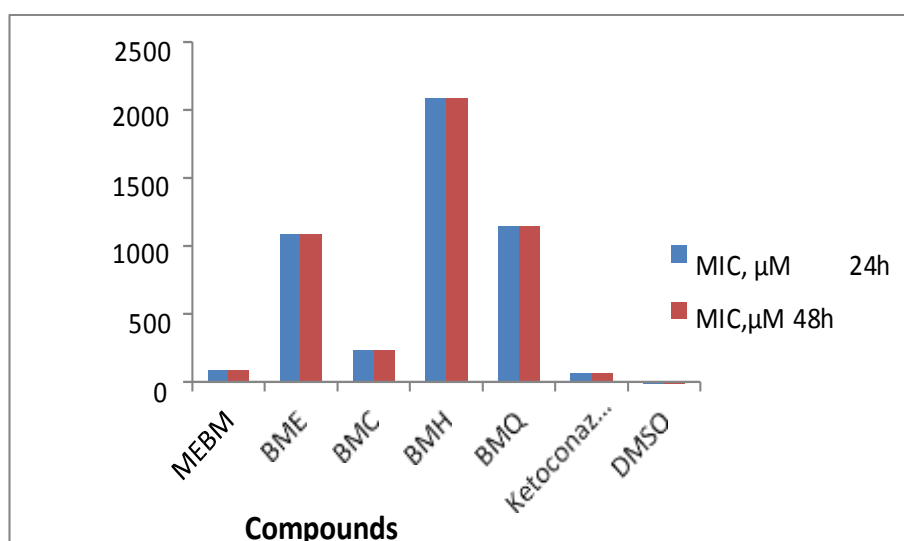
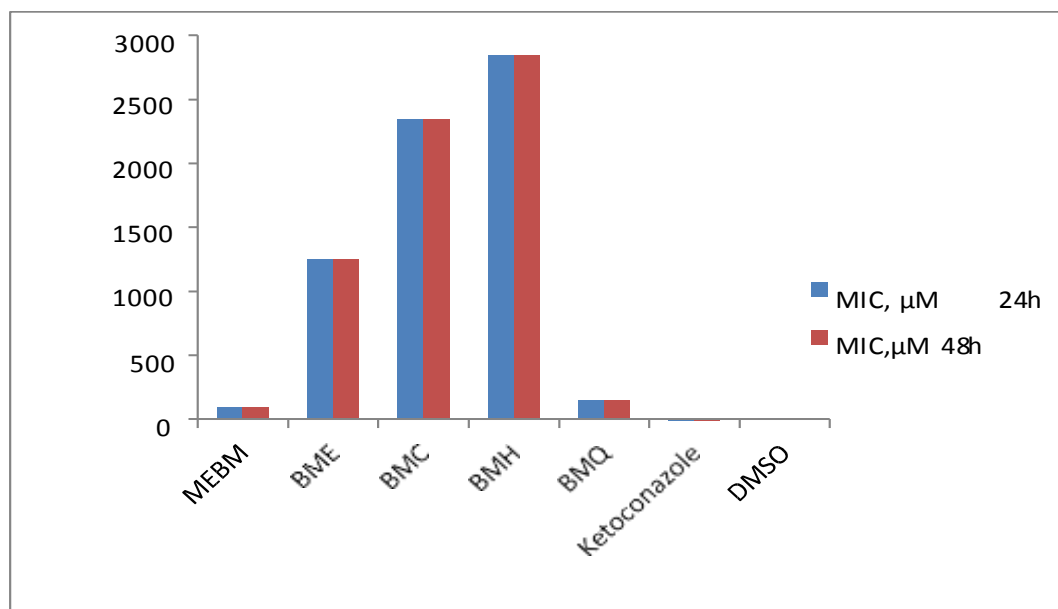


Figure 5: Antifungal Activity of extracted Compounds against *S.cerevisiae* PTCC5052

Table 6: Antifungal Activity of extracted Compounds against C. albicans ATCC 10231

Compounds	MIC, μ M	
	24h	48h
MEBM	97.23	97.23
BME	1254	1254
BMC	2345	2345
BMH	2845	2845
BMQ	153.1	153.1
Ketoconazole	1.32	1.32
DMSO	10%v/v	10 % v/v

**Figure 6: Antifungal Activity of extracted Compounds against C. albicans ATCC 10231**

RESULTS AND DISCUSSIONS OF ANTIFUNGAL ACTIVITY

The in vitro antifungal activities of the extracted compounds **MEBM**, **BME**, **BMC**, **BMH**, **BMQ** tested against two representative pathogenic yeast fungi (*S.cerevisiae* and *C. albicans*) using broth micro dilution assay (Tables 5 and 6). The MIC values were determined by comparison to amphotericin B, ketoconazole as reference drugs. Antifungal activity evaluation against *S. cerevisiae* (Table-5) showed that compounds **BMH** and **BMC** did not possess significant antimicrobial properties against tested pathogenic fungi, while compound **BMH** exhibited significant antifungal effect. Compound **MEBM** is most active against *S. cerevisiae* (with a MIC value of 85.31M).

In addition, the activity of this compound against *S. cerevisiae* after 24 h is higher than that of reference drugs ketoconazole (with MIC values 65.41). Our antifungal evaluation results showed that compound **MEBM** was

active against *S. cerevisiae* after 24 and 48 h. However, all these compounds were less active than the reference drugs ketoconazole, and amphotericin B.

Antifungal activity evaluation against *C. albicans* (Table-6) showed that compound **BMH** also did not possess significant antifungal properties, while compound **MEBM** exhibited a more noticeable antifungal effect. However, all these compounds were less active than the reference drugs ketoconazole.

CONCLUSIONS

In the last few decades, there has been a significant surge in research into phytochemicals from foods for illness prevention. An ethnobotanical approach is a viable way for improving the results of phytochemical research. There has been little research into the health benefits of native Indian plants. The large number of edible plants utilized as food and medicine by the

Indian population opens the door to the discovery of novel physiologically active chemicals. They clearly justify research along modern methodological lines such as phytochemical analysis, biological assessment using experimental animal models, toxicity studies, molecular mechanism of action(s) of isolated phyto principles, and clinical trials. It's a tried-and-true method for finding new lead compounds for treating a variety of diseases.

Thorough the review of the literature on **Costus speciosus** (CS) shows that it is a popular medicine among diverse ethnic groups and Ayurveda practitioners for a variety of diseases. The scientific community, on the other hand, has made virtually little effort to investigate this plant's beneficial potential. It's interesting to see that crude organic extracts of *Costus speciosus* (CS) leaves have been tested for pharmacological activities and have shown anti- Alzheimer's, antioxidant, anti-aging, and antibacterial activity. Other plant parts, including as seeds, leaves, and seed oil, should also be studied scientifically for their therapeutic potential. The isolated plant principles will need to be tested using scientific experimental animal models in the future.

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