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ANTIFUNGAL ACTIVITY OF ROOT OF KASAMARDA (CASSIA OCCIDENTALIS LINN.)

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ABSTRACT

Kasamarda (Cassia occidentalis) is a flowering plant of Leguminosae Family. It has been used in the indigenous system of medicine since long time. It increases digestive power and clears the obstruction of the throat. Antifungal activity was tested against three fungi. The various Concentrations (3.12%, 6.25%, 12.5%, 25%, 50%, 100% mg/ml) of the water, ethanol, methanol, chloroform and benzene extracts were tested. Evaluations were based on the zone of inhibition using Agar disc diffusion assay. The inhibitory activity was found to be dose dependent. fluconazole used as standard for antifungal activity. From this study, it was evident that water extract and ethanol extracts of Cassia occidentalis Linn. has antifungal activity**.** Among different extracts from root of Cassia occidentalis, i e. water, ethanol, methanol, chloroform & benzene ethanol exhibited highest antifungal activity.

KEYWORDS: *Kasamarda*, Antifungal, Candida albicans, Rhodoturola glutinis, Aspergillus niger.

INTRODUCTION

Kasamarda (Cassia occidentalis) is a flowering plant of Leguminosae Family. It has been used in the indigenous system of medicine since long time. It increases digestive power and clears the obstruction of the throat. It cures cough, indigestion, toxicosis and impurity of

blood (Chopara et al., 1956). Cassia species are rich in anthraquinones and anthrones (Shah and Shinde, 1969; Acharya and Chatterjee, 1975). In the present work, we have studied the antifungal activity of different extracts of root of Cassia occidentalis.[1]

Kasamarda plant

Kasamarda seeds

Kasamarda flower

Kasamarda pods

Kasamarda root powder

MATERIALS AND METHODS

The ethanol, methanol, water, chloroform, and hexane extracts of kasamarda (Cassia occidentalis Linn.) root were investigated against Candida albicans, Rhodotorula glutinis, and Aspergillus niger. Antifungal activity was studied by using agar diffusion method. The minimum inhibitory concentration (MIC) was determined using broth microdilution method.

Plant material - Root of Cassia occidentalis were collected from the Campus of the herbal garden, Podar medical (Ayu) college, Mumbai on Mach-2018. The plant specimen was botanically identified and authenticated by botanist Alarsin pharmacy, Mumbai. The plant part was washed with water then dried under shade and ground into fine powder using electric mixer - grinder. Preparation of extracts Plant extracts were prepared by Soxhlet extraction method.

METHODOLOGY FOR ANTI-MICROBIAL ACTIVITY

- **Material**

1. **Drug-** The Root of Kasamarda was collected properly.

- Chloroform Water
- Distilled Water
- Ethanol
- Benzene
- Methanol

Extracts of the samples were studied for Anti-fungal Activity by Disc Diffusion Method.

2. **Microorganisms**

- In Ayurveda, Kasamarda is widely used in skin disorders so accordingly following fungus were selected.

- Rhodotorula glutinis (NCIM 3353)
- Asperagillus niger (NCIM 1272)
- Candida albicans (NCIM 3100).

- Strains of fungi were obtained from National Chemical Laboratory, Pune.

Drugs Used for In-Vitro Study

1. **Test Drug:** Root of *Kasamarda (Cassia occidentalis Linn.)*

Control Drug: Fluconazole

Chemical & solvents used during In-Vitro Study

1. M.H.A. (Muller Hilton Agar)
2. Distilled water
3. Buffer Pepton Water
4. Surgical Spirit

Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration is defined as the lowest concentration of test samples that results in complete inhibition of visible growth of micro-organisms.

Media used for MIC

Nutrient Borth– Nutrient Borth was used to test the MIC of samples.[2],[4]

Ingredients of Nutrient Borth- Table(1)

Ingredients of Nutrient Borth	Gm/litre
Beef extract	1.5g
Peptone	5.0g
Nacl	5.0g
Distilled Water	1 lit.

Nutrient Agar - Nutrient Agar was used to confirm the bacterial and fungal growth on plates.

Ph was adjusted to 7.2.

Method conducted to observe antifungal activity (In-Vitro Study) of sample drugs

Research drug being incompletely soluble in water, it was necessary to detect the proper solvent to dissolve it to check the sensitivity on various micro-organisms at various concentrations. According to previous research done on anti-antifungal activity of extracts of *Kasamarda (cassia occidentalis Linn.)* against different fungus. DMSO was selected as a solvent for test drug. So, by taking the scientific support of this previous research, on trial basis research drug was dissolved at various concentrations 3.12%, 6.25%, 12.5%, 25%, 50%, 100% separately in 1ml of DMSO.[3]

To observe the anti-microbial activity of drug, muller Hilton's agar media was selected for the study. As perthe textual reference of microbiology the selected media is suitable for

growing of fungi like Rhodoturola glutinis, Aspergillus niger, and Candida albicans.

Method conducted to observe minimum inhibitory concentration (MIC) of drug

Here the series of dilutions with 3.12%, 6.25%, 12.5%, 25%, 50% and 100% of the test sample were poured in Muller-Hilton agar media tubes at room temperature which has been previously inoculated with the selected test fungi. Now above test tubes were inoculated at 37^0 c for bacteria for 24 hours.[3],[4]

RESULTS AND DISCUSSION

Results For Anti-Microbial Activity

Anti-fungal study of Kasamarda root powder -Water, Ethanol, Methanol, Chloroform extract and Benzene extract.

Table showing Zone of inhibition of Candida albicans, R.glutinis, Aspergillus niger in different extract of root of *Kasamarda* Table *(2)*

No.	Sample name	Zone of Inhibition in (mm) Microorganisms studied					
		A*	mean	B*	mean	C*	mean
1	Water	9,9,11,9,11	9.8	9,10,10,10,10	9.8	8,8,9,10,10	9
2	Ethanol	9,9,9,9,9	9	9,10,9,9,9		12,11,11,11,10	11
3	Methanol	9,9,9,9,9	9	10,9,11,10,10		9,10,10	9.6
4	Chloroform	10,8,8,8,10	8.8	-	-	10,9,8,10,9	9.2
5	Benzene	9,9,9,9,9	9	-	-	10,8,8,9	8.75

A* - Candida albicans B* - Rhodotorula glutinis C* - Asperigillus niger

Graph showing Zone of inhibition of extract of root of *Kasamarda* against different microorganisms. (Graph 1)

ZONE OF INHIBITION (mm)

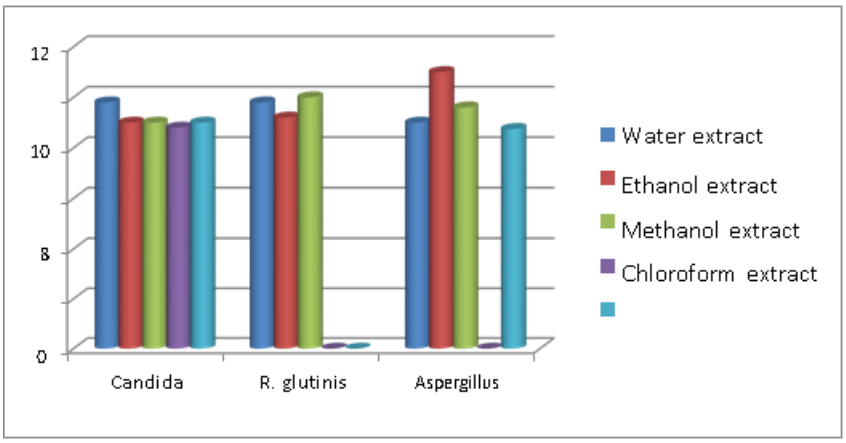
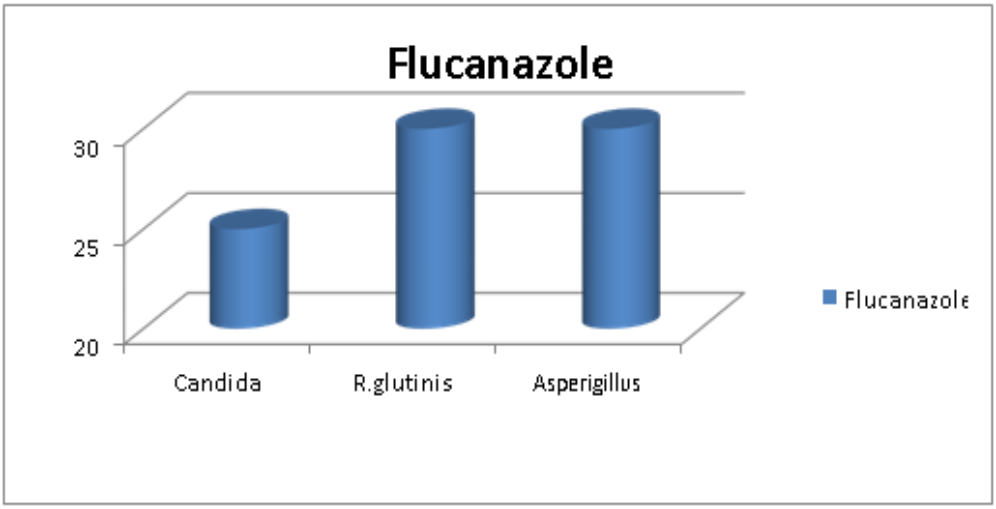


Table showing zone of inhibition of Fluconazole (control drug) in different

microorganisms. Table (3)

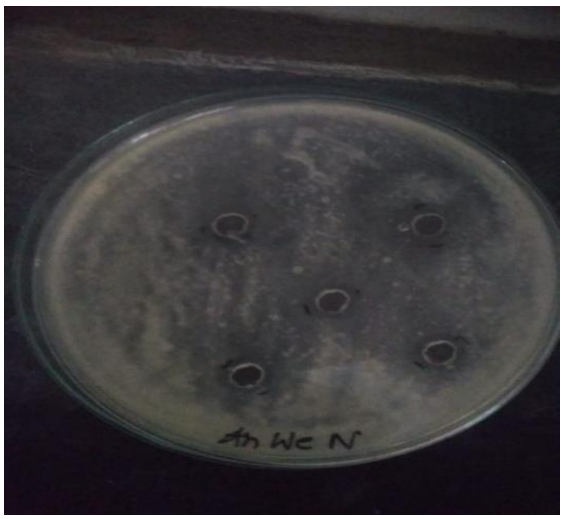
Sr no.	Name of the micro-organism	Mean zone of inhibition
1	Candida albicans	25
2	Rhodotorula glutinis	30
3	Asperigillus niger	30

Graph showing zone of inhibition of Fluconazole cream (control drug) in different microorganisms (Graph 2)



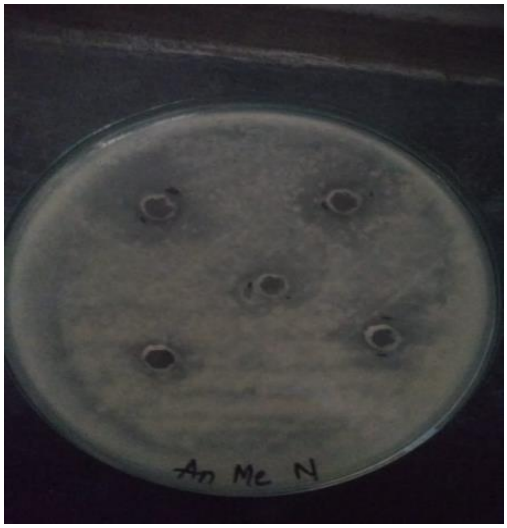
COMPARATIVELY SHOWING THE ANTIFUNGAL SCREENING WITH ZONE OF INHIBITION (WATER, ETHANOL, METHANOL, CHLOROFORM AND BENZENE EXTRACT OF SAMPLE DRUG)

I. Asperigillus niger

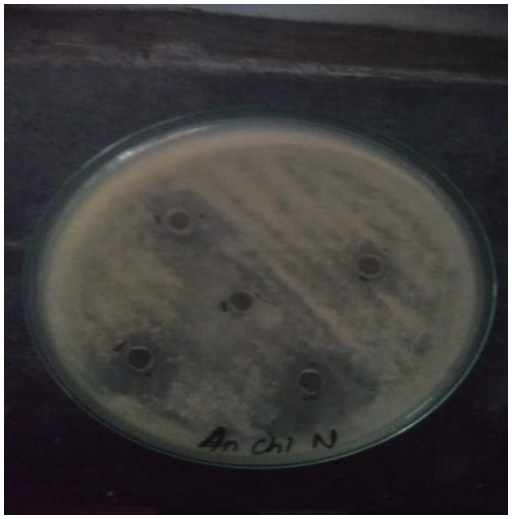


Water extract

Ethanol extract



Methanol extract



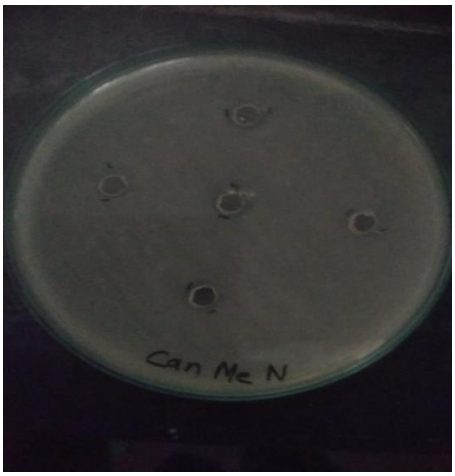
Chloroform extract

Benzene extract

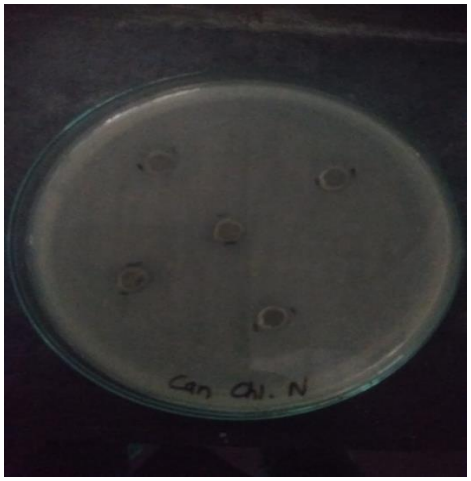
II. Candida albicans

Water extract

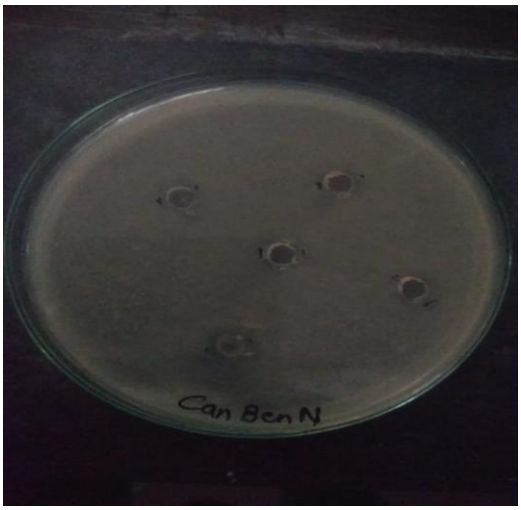
Ethanol extract



Methanol extract

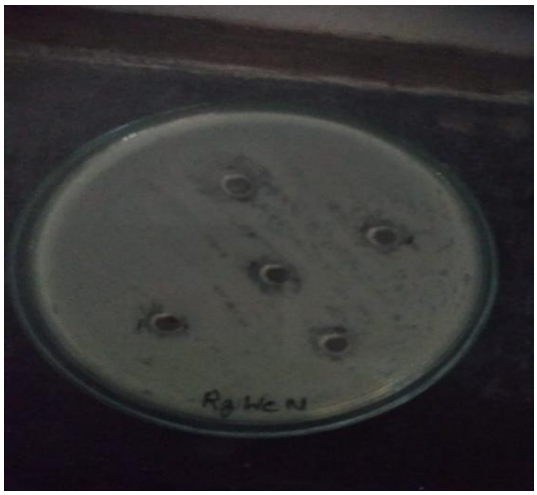


Chloroform extract



Benzene extract

III. Rhodotorula glutinis

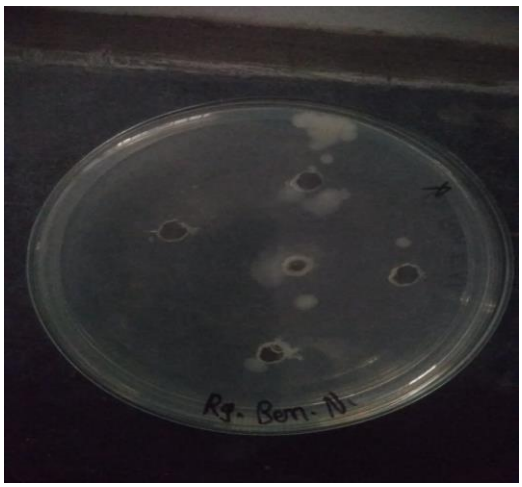


Water extract

Ethanol extract

Methanol extract

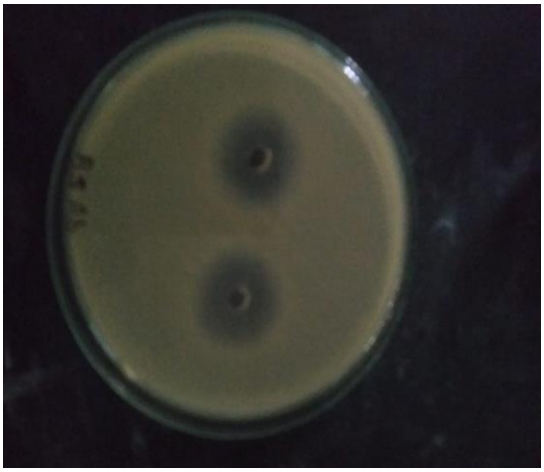
Chloroform extract



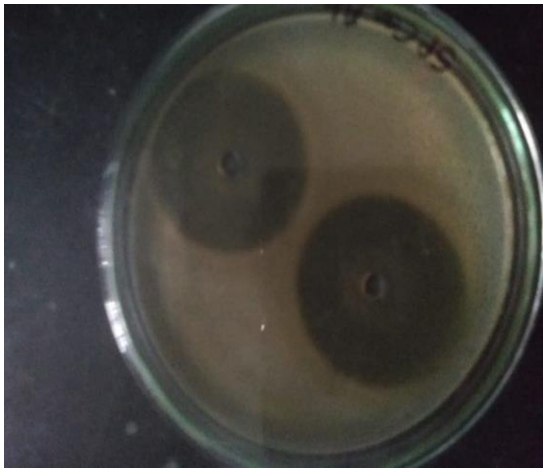
Benzene extract

SCREENING WITH ZONE OF INHIBITION (BY USING CONTROL DRUG)

Asperigillus niger



Rhodotorula glutinis



Candida albicans

To evaluate antifungal activity, Root of *Kasamarda* powder was taken in different solvents like water, methanol, chloroform, benzene and ethanol. FLUCONAZOLE was taken as a control drug for all microorganisms. In this method 1gm of *Kasamarda* powder was dissolved in DMSO solution. Before preparation of solution, antifungal activity of DMSO was checked. It didn't show any antifungal activity. The concentration used was 1mg/ml organisms were Rhodotorula glutinis, Aspergillus niger, and candida albicans. The mean zone of inhibition on Rhodotorula glutinis by water extract, methanol extract, ethanol extract, chloroform extract, benzene extract of *Kasamarda* sample was 9.8mm, 10mm, 9.2mm, 0mm, & 0mm respectively. And that of standard drug FLUCONAZOLE was 30 mm. The mean zone of inhibition on Aspergillus niger by *Kasamarda* sample was 9, 9.6, 11, 9.2 and 8.75. And that of standard drug standard drug was 30mm. [Table 2 and 3].

The mean zone of inhibition on Candida albicans by water, Methanol, Ethanol, Chloroform and Benzene extract of *Kasamarda* sample was 9.8mm, 9mm, 9mm, 8.8mm, and 8.8mm respectively and that of standard drug standard drug was 25mm. The antifungal study reveals that among both the extracts of root of Kasamarda, the maximum zone of inhibition was noted for Aspergillus niger in Ethanol extract which was 11mm and that of control drug was 30mm. The results obtained were compared with the standard antifungals and it was observed that water, methanol and ethanol extracts showed pronounced anti-fungal activity against all selected microorganisms. Ethanol extracts of *Kasamarda* displayed remarkable activity against Aspergillus niger. The Methanol extract of sample exhibited appreciable activity against Candida albicans. There is no activity seen against Rhodotorula glutinis by chloroform and benzene extract and in the same fungus control drug has shown lowest

activity. The maximum activity seen in Ethanol extract of sample drug against Aspergillus niger whereas minimum activity seen in chloroform and benzene extract of Rhodotorula glutinis.

CONCLUSION

From this study, it was evident that the water extract and the ethanol extracts of Cassia occidentalis Linn. has antifungal activity**.** Among different extracts from root of Cassia occidentalis, i e. water, ethanol, methanol, chloroform & benzene. ethanol exhibited highest antifungal activity. Among the three test fungi Candida albicans is found to be the most sensitive. And this fungi is responsible for most common skin disorder, This study paves the way for further attention and research to identify the active compounds responsible for the plant biological activity.

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3. https://en.wikipedia.org/wiki/Nutrient_agar.