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STUDY OF ANTIMICROBIAL ACTIVITY OF *CASSIA OCCIDENTALIS* ALONG WITH ANTIBIOTICS

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ABSTRACT

Cassia occidentalis is an ayurvedic plant with huge medicinal importance. The microbial strains 2-bacterial & 1-fungal were used for detection of antimicrobial activity for *Cassia occidentalis.* Well diffusion and disc diffusion methods with the leaves extracts of *Cassia occidentalis* is having ability of antimicrobial activity so, it can further used as an antibiotics to treat diseases caused by bacteria and fungi. These work shows that it can used against *E. coli, Staphylococcus aureus* and *Candida albicans*. For bacteria ciprofloxacin tablets and for fungus co-trimoxazole tablets were as a antibiotics against microorganisms and the leaves extracts of plant were used as a medicine. Now a day's increasing the demands of natural and herbal products because of its safeness and effectiveness. production of antibiotics by using plant leaf shown very effective result against microbe. The extracts of *Cassia occidentalis* is possible source of effective herbal medicine to treat microbial infection caused by microorganisms.

KEYWORDS: *Cassia occidentalis,* medicinal importance, antibiotics.

INTRODUCTION

Cassia occidentalis is an aromatic plant that achieves a height of about 2- 4 feet. It attains this height in rainy season. Leaves are about 6-12 inch in length that has a leathery appearance. Flowers are of yellow color and are of ½ - ¾ inch in diameter. It has 4-5 inch long pod that is flattened. It is of dark brown color and is slightly curved upwards at the edges. It contains 10-30 seeds. This seeds are olive brown and flattened at the both ends. It bear flower in autumn and fruits in winter (Pierre Saotoing *et.al*., 2011).

Cassia occidentalis is an ayurvedic plant with huge medicinal importance (Chatterjee *et. al*., 2010). Leaves of *Cassia occidentalis* plant have ethnomedical property like paste of leaves is externally applied on healing wounds, sores, itch, cutaneous diseases, bone fracture, fever, ringworm, skin diseases and throat infection. They can also show different biological activity as antimicrobial activity, anti-carcinogenicity, antiproliferation and antioxidant properties (Chukwujekwu JC *et. al*., 2006; Cushnie, 2005; Huang, 2004; Nanasombat, 2009).

Some species of *Cassia* contain special substances as antidiabetic, antimalarial, anticarcinogenic and hepatoprotective (Nishteswar K. IRJP, 2011) studies of plant showed that the nature and amount of the phytochemicals depends on the seasons and geographical location. All the results encouraged us to deepen the studies on antimicrobial properties of the leaves of *C. occidentalis* by evaluating the inhibition zone by using agar disk diffusion method. In the present study, an attempt was made to investigate the antimicrobial activity from leaves of *C. occidentalis* collected from Chhattisgarh region of India.

It is found in tropical and temperate area and is very commonly seen all over. It is growing abundantly immediately after rain. It is found everywhere in India. It is also found in Pakistan, Burma, Nepal, Afghanistan and southern parts of Asia.

MEDICINAL USES

The *Cassia* genus comprises 600 species of trees, shrub, vines and herbs, with most of the species growing in the South American rainforest and tropics. Since from ninth or tenth century many *Cassia* plants known as a purgative and laxative as well as anti-neuralgic and vermifuge. In Mexico, the leaves and stems have been found to be hypersensitive in dogs. In Nigeria, ijo people used leaves for the treatment of malaria and body aches. In Yoruba land, the preparation with palm oil is used to cure convulsion in children (Yadav JP,2009).

The root is believed to have depurative properties. In Gabon, infusion and decoction is used to cleanse the blood and Trinidad citizens, used to clean body after parturition. The ability of the plant extracts as antimicrobial agent is due to of their antioxidant properties which are correlated with their phenolic content (Arya Vedpriya et. al. 2010). The phenolic compounds are common in many plants and exhibited antioxidant properties due to their high redox potential (Abo *et. al*.1998).

METHODOLOGY

PLANT SAMPLE: Leaves of *Cassia occidentalis* were collected from Indira Gandhi Agricultural institute Bilaspur, Chhattisgarh.

MICROORGANISM: The microbial strains (2-bacterial & 1-fungal) i.e. *E.Coli*, Staphylococcus aureus and Candida albicans were used in this research work was locally collected from the Department of Microbiology, Chhatisgarh Institute of Medical Science, Bilaspur (C.G.).

ANTIBIOTIC: For bacteria ciprofloxacin tablets and for fungus co-trimoxazole tablets were used in powdered form (1 g) as antibiotics against microorganisms.

MEDIA: Nutrient agar media and Sabouraud dextrose agar media.

INSTRUMENTS: Soxhlet apparatus, Incubator**,** Laminar air flow, Autoclave, Electronic weighing machine, Hot air oven.

METHODS

Preparation of the leaf extracts: The leaves were collected, cleaned with deionized water, dried at room temperature for 48 hours and crushed into a fine powder with the help of a grinder. The powdered were stored in airtight container for further studies.

Purified Extraction

10 g of dried powder were placed in soxhlet tube and kept in round flask filled with organic solvent (ethanol and chloroform).Kept in soxhlet apparatus for 24 hrs. After 24 hrs the solution were filtered and centrifuge at 5000 x for 15 min. the supernatant were collected and the solvent were evaporated to make a final volume one-fourth of the original volume. The extract were dried and stored in air tight bottle at 4^{o}C for further studies.

Preparation of test samples

Test samples of the plant extracts were prepared in 10 ml solvents (ethanol and chloroform). It is further diluted as 1, 2 and 3ml concentration of plant extracts in 4, 3 and 2ml concentration of solvents (ethanol and chloroform) and stored in refrigerator for further use.

Preparation of antibiotics: Antibiotic were prepared by dissolving 10 g of powdered antibiotic in 100 ml of sterilized distilled water.

Preparation of inoculums: Bacterial and Fungal strains were isolated from pure cultures and a loop full of cultures were diluted in distilled water(10 ml). It is stored in refrigerator for further use.

Antimicrobial Bioassay: Antimicrobial activity of plant extracts were determined by three methods. Nutrient agar media and Sabauroud agar media were used for bacteria and fungi respectively. Under aseptic condition, in laminar air flow the Nutrient agar medium and Sabauroud agar medium pour into presterilized petriplate and inoculated by bacterial and fungal strain respectively and kept for 10-15 minutes for drying. It is used in different methods.

Agar well diffusion method: In inoculated plate 3 wells were prepared with the help of gel borer. Ciprofloxacin (bacteria) and co-trimoxazole (fungus) were used as a negative control. Solvents (ethanol and chloroform) were used as a positive control. Wells were pouring with negative control, positive control and plant extract. Plates were incubated for 24 hrs (bacteria) and for 48 hrs (fungus). After incubation inhibition zones were measured.

Agar disc diffusion method: The discs were prepared by impregnated the sterile disc of particular size into plant extracts, solvents (positive control) and antibiotics (negative control). The disc were dried and suspended into Nutrient agar media (bacteria) and Sabauroud agar media (fungus). Plates were incubated for 24 hrs (bacteria) and for 48 hrs (fungus). After incubation inhibition zones were measured.

RESULT

Table1.1 Inhibition Zone Of Plant Extract By Well Diffusion Method.

PLANT EXTRACT	***E.COLI***	***S.AUREUS***	***C.ALBICANS***
ETHANOL			
1ml	10mm	5mm	8mm
2ml	7.5mm	7mm	7mm
3ml	5mm	10mm	9mm
CHLOROFORM			
1ml	5mm	3mm	5.6mm
2ml	8mm	6.5mm	7mm
3ml	9mm	8mm	7.5mm

Table: 2 Inhibition Zone Of Plant Extract By Disc Diffusion Method

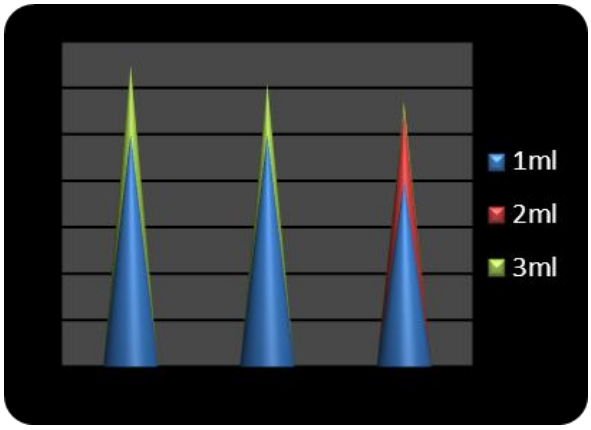
PLANT EXTRACT	*E.COLI*	*S.AUREUS*	*C.ALBICANS*
ETHANOL			
1ml	8.7mm	5mm	8.1mm
2ml	7mm	7.5mm	8mm
3ml	5.5mm	9.7mm	12mm
CHLOROFORM			
1ml	7mm	4mm	4.2mm
2ml	8mm	7mm	7mm
3ml	8.5mm	8.2mm	8.2mm

Table: 3 inhibition zone of antibiotic (ethanol extract) by disc and well diffusion method.

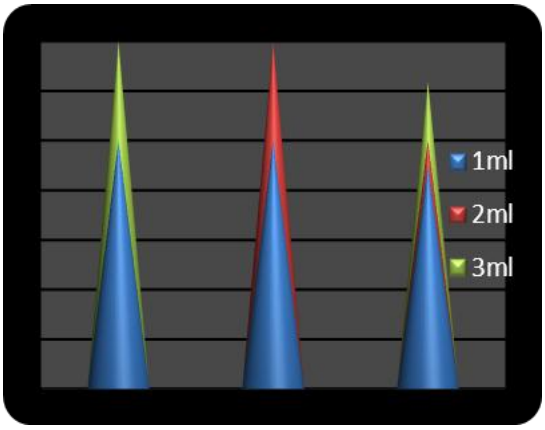
ANTIBIOTIC (ETHANOL EXTRACT)	*E.COLI*	*S.AUREUS*	*C.ALBICANS*
WELL DIFFUSION METHOD			
1ml	4mm	5mm	1mm
2ml	6mm	9mm	2.5mm
3ml	7mm	7.5mm	3mm
DISC DIFFUSION METHOD			
1ml	5mm	4.5mm	2.5mm
2ml	7.5mm	7mm	2.5mm
3ml	9.7mm	5mm	4mm

Table 4 Inhibition Zone Of Antibiotics (Chloroform Extract) By Disc And Well Diffusion Method

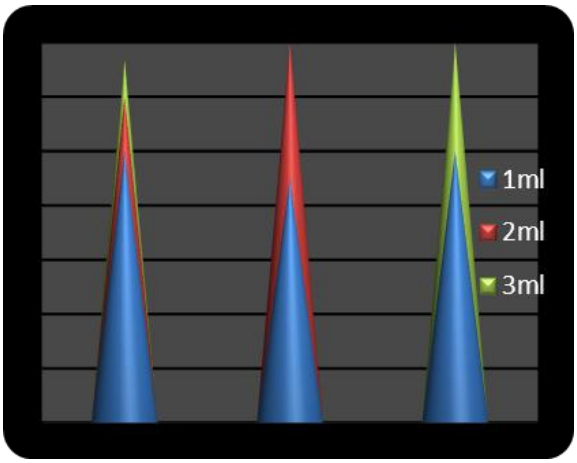
ANTIBIOTIC (CHLOROFORM EXTRACT)	*E.COLI*	*S.AUREUS*	*C.ALBICANS*
WELL DIFFUSION METHOD			
1ml	5mm	5mm	4.5mm
2ml	5.7mm	7mm	5mm
3ml	7mm	7mm	6.2mm
DISC DIFFUSION METHOD			
1ml	5mm	5mm	4mm
2ml	4.5mm	5mm	5.5mm
3ml	6.5mm	6.1mm	5.7mm



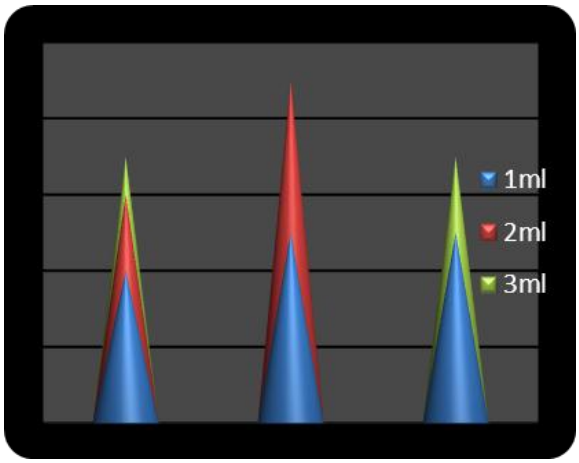
Inhibition zone of antibiotics by disc diffusion method (Chloroform)



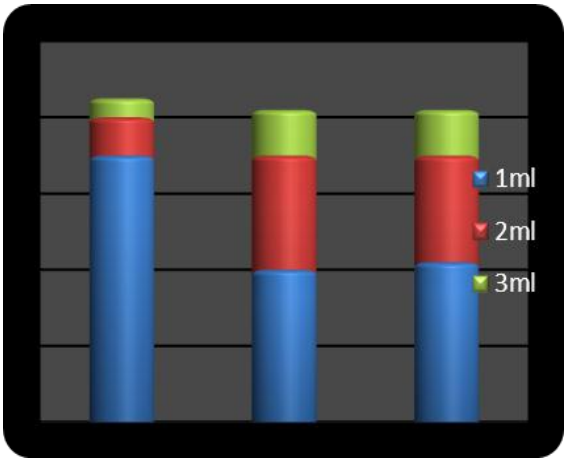
Inhibition zone of antibiotics by well diffusion method (Chloroform).



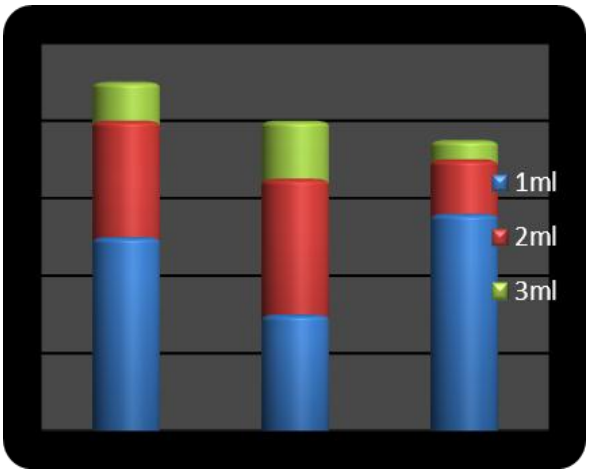
Inhibition zone of antibiotics by disc diffusion method (Ethanol)



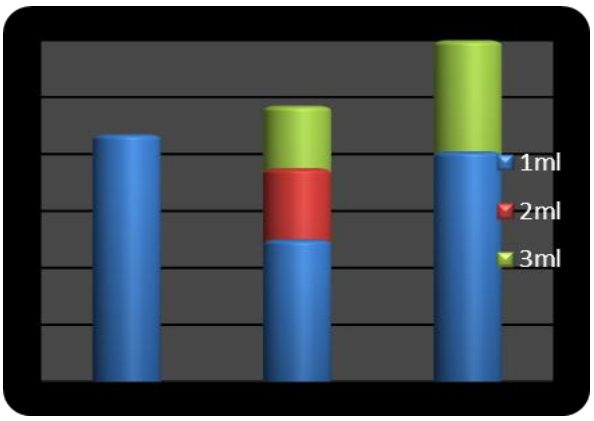
Inhibition zone of antibiotics by well diffusion method (Ethanol).



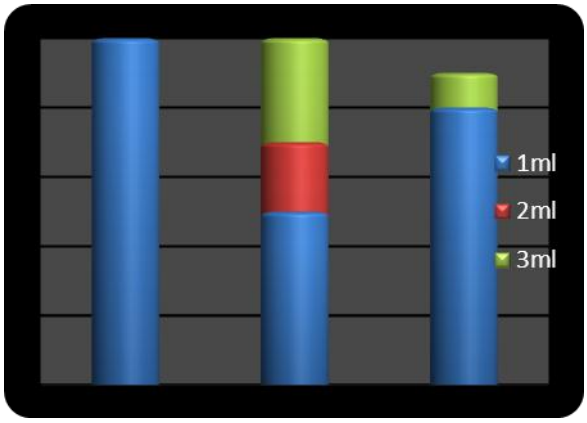
Inhibition zone of plant extract by disc diffusion method (Chloroform)



Inhibition zone of plant extract by well diffusion method (Chloroform).



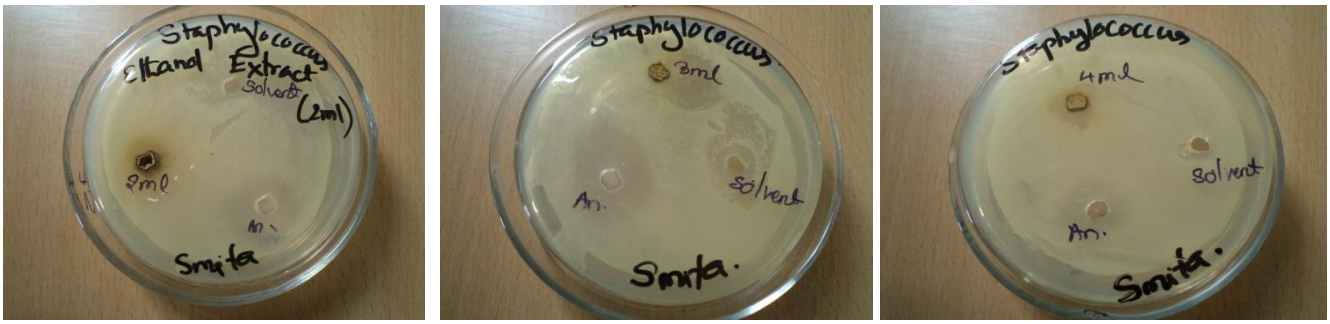
Inhibition zone of plant extract by disc diffusion method (Ethanol)



Inhibition zone of plant extract by well diffusion method (Ethanol).

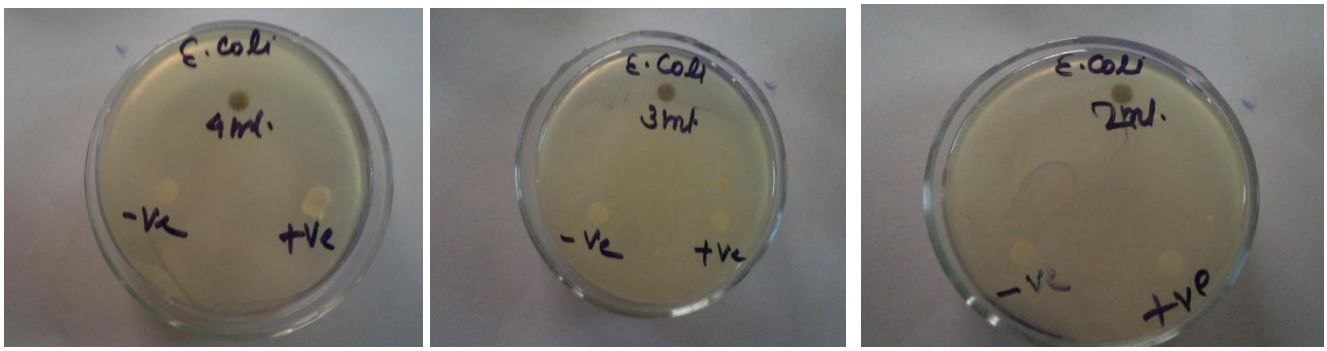
Observation

ETHANOL EXTRACT IN WELL DIFFUSION METHOD (*E. Coli)*

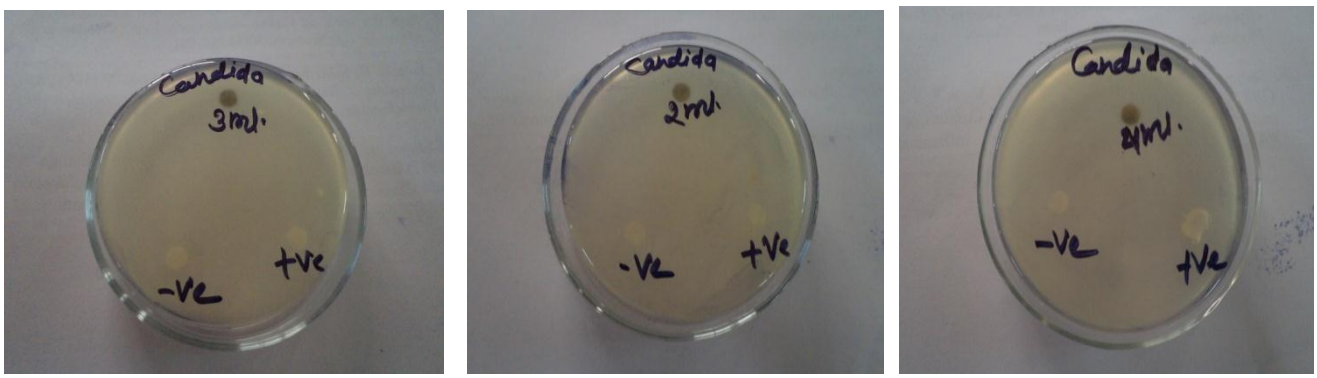


ETHANOL EXTRACT IN WELL DIFFUSION METHOD *(Staphylococcus aureus)*

CHLOROFORM EXTRACT IN DISC DIFFUSION METHOD (*Staphylococcus aureus)*



CHLOROFORM EXTRACT IN DISC DIFFUSION METHOD (*E. coli)*



CHLOROFORM EXTRACT IN DISC DIFFUSION METHOD (*Candida albicans*)

RESULT

Depending on bacterial/ fungal strain and concentration of antibiotics and plant extracts, well diffusion and disc diffusion methods show different inhibition zones. The ethanol extract (1ml) is more effective in E. coli (10mm). The chloroform extract (1ml) is more effective in *Candida albicans*. Ethanol and chloroform extracts (2ml) is more in *E. coli*. Ethanol and chloroform extracts (3ml) is more in *Staphylococcus aureus* and *E. coli* respectively.

In *E. coli* ethanol extract (1ml) is more effective, chloroform extract (2ml) shows more inhibition zone, chloroform extract shows clear zone. In *Staphylococcus aureus* ethanol extract (1, 2 and 3ml) is more effective. In *Candida albicans* ethanol extract (1ml) is more effective, ethanol and chloroform extract (2ml) shows equal inhibition zone, ethanol extract shows clear zone. So, ethanol extract is more effective as compared to chloroform extract.

Antibiotic is (1ml), in ethanol extract in Staphylococcus aureus and Candida albicans and in chloroform extract *E. coli* and *Staphylococcus aureus* are more effective. In 2ml, in ethanol extract in *E. coli* and *Staphylococcus aureus* and in chloroform extract in *E. coli* and *Staphylococcus aureus* are more effective. In 3ml, in ethanol and chloroform extract *E. coli* and *Staphylococcus aureus* are more effective.

Ethanol 3ml is more effective in *E. coli* (10.5mm), in 2ml it is effective in *Staphylococcus aureus* (7.5mm) and in 1ml *E.coli* is more effective (9mm). Therefore, ethanol is more effective in *E. coli.* Chloroform 3ml is more effective in *Staphylococcus aureus* (6.8mm), in 2ml it is effective in *Staphylococcus aureus* (6mm) and in 1ml it is effective in *Staphylococcus aureus*. Therefore, chloroform is more effective in *Staphylococcus aureus.* . Ethanol extract (12mm-5mm) is more effective than chloroform extract (9mm-3mm). As a solvent also ethanol (10.5mm-1mm) is more effective than chloroform (6.8mm-3mm). In 1ml concentration ethanol extract is more effective, in 2ml concentration ethanol extract is more effective, in 3ml also ethanol extract is more effective. Therefore, ethanol extract is more effective in all aspects as compared to chloroform extract.

DISSCUSSION

The effectiveness of the extracts depends on the type of solvents used. Through study different plant extracts were found to be active against bacterial and fungal strains and such preparation with different solvent may serve as a treatment against infections. It has been shows that the mechanism of antimicrobial effects involves various cellular processes, followed by an increase in plasma membrane permeability and ion leakage from the cells (Oladunmoyem M K et.al,2006).

Due to the emergence of antibiotics against microbial diseases, plants are being looked upon as a good alternate to control over the further spread of microbial diseases caused by microorganisms. Plants are included several chemical compounds also like tannins, flavonoids, saponins, essential oils and phenolic compounds due to these compounds only plants having capability to shows antimicrobial activity (Tutomu H *et.al.*,1999;Mahida Yogesh *et.al*., 2007; Yadava R N,2011)

This is the first time to find inhibition zone in chloroform extract of leaves of *Cassia occidentalis* in *E. coli*, *Staphylococcus aureus* and *Candida albicans*. The zone of inhibition found in chloroform is less as compared to ethanol extract. Therefore, in future antibiotics can be made by using chloroform plant extract.

CONCLUSION

All plant extracts showed the antimicrobial activity, hence it is proved and accepted by well diffusion and disc diffusion methods that leaves extracts of *Cassia occidentalis* is having ability of antimicrobial activity so, it can further used as an antibiotics to treat diseases caused

by bacteria and fungi. Study indicates the antimicrobial properties of leaves extracts of *Cassia occidentalis* shows antimicrobial activity against *E. coli*, *Staphylococcus aureus and Candida albicans.* It is also rich in secondary metabolites.

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