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Antimicrobial activity of *Celosia argentea* L. in the Hyderabad Karnataka region

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Abstract
Celosia argentea is a herbaceous plant and belongs to Amaranthaceae family that grow in a terrestrial habitat. Plant show simple and spirally arranged leaves, flowers are often pinkish or white colour, fruits are in globular shape and seeds are black. The *C. argentea* has great medicinal value, used in the treatment of fatigue, leucorrhoea, atherosclerosis and osteoporosis and the seeds have been used for reducing the "liver heat", improving the eye sight, clearing wind heat and as an anti- inflammatory agent. In the present study the antibacterial and antifungal activity of *C. argentea* stem and root using the chloroform and methanol extracts were evaluated from the *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans* and *A. Niger*. A total of 4 microorganisms (2 bacteria and 2 fungal strains) were used for the antimicrobial activity. The results shows that, antimicrobial activity of *C. argentea* reported to confer resistance against microbial pathogens and thus explains the manifestation of antibacterial activity by the stem and root extracts. The extract obtained acts as a potential source for biological antibacterial activity against selective bacteria stains *S. aureus* and *E. coli.* Significant antibacterial and antifungal activity was obtained by comparing with the standard Ciprofloxacin and Amphotericin respectively.

Keywords: *C. argentea, E. coli, S. aureus, C. albicans, A. Niger,* Antimicrobial activity activity

Introduction
Understanding of weed biology is essential for the expansionion of economic and environmentally acceptable weed management systems (Bhowmik, 1997). Thus, weeds grow as an integral component with crop plants and enjoy the benefits which crop plants receive and at the same time release some organic compounds which interfere with the metabolism of crop plants thereby reducing their yield. Jethro (1731) was the first man who used the word 'weed' in literature in his famous writing on 'Horse Hoeing Husbandry.

C. argentea L. (Amaranthaceae) is one of the most dominating herbaceous annual weed found in all semiarid land crops such as Groundnut (*Arachis hypogaea* L.*)*, Finger Millet (*Eleusine coracana* L.) Maize (*Zea mays* L.) Radish (*Raphanus sativus)*, Jowar (*Sorghum bicolor*), hyacinth bean (*Dolichos lablab*), Cowpea (*Vigna unguiculata)*, Red gram (*Cajanus cajan*), Green gram (*Phaseolous aureus)*. The economic importance of these plants have been documented (Ayensu, 1978; Nwalozie, 1984). *C. argentea* is an erect plant and grows to a height of 1.0 to1.6 m under favorable condition (Gogga, 2008). Weeds have enormous reproductive capacity, huge seed banks in the soil, viability and dormancy of seeds, synchronizing the biological clock with that of the crop, sociability with crops, ecological races within the weed populations, *etc.* (Robert, 2008). In addition to the above, this weed species have allelopathic effects.

C. argentea is herbaceous plant and grow locally in various regions in Karnataka, India. Plant bears simple and spirally arranged leaves, often pinkish or white flowers, while fruits are globular and seeds are black (Fig. 1&2). Literature indicated that *C. argentea* used for treatment of fatigue, atherosclerosis, leucorrhoea and osteoporosis. The plant is also used as antidiarrhoeal agent and its other parts also used in the Ayurveda medicine (Wiart). Sequential extraction was carried out by using solvents such as petroleum ether, ethanol and aqueous from leaf, root and stem of the plant were investigated for preliminary phytochemical analysis and exhibiting antimicrobial activity. Aqueous extract showed moderate inhibitory activity against bacteria and fungi. Phytochemical analysis showed the presence of Alkaloids, Phytosterols, Fixed oils, Saponins and Phenolic compounds (Doddabasawa and Ravikumar, 2014) [12].

Taxonomical hierarch

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Carypphyllales
Family: Amaranthaceae
Genus: *Celosia*
Species: *C. argentea*

Cockscomb plants *Celosia cristata* are named for the striking resemblance of their flowers to a rooster's comb. Their large, flat flower heads form a curving crest with a ruffled edge and are usually bright red. Cockscomb is part of the Amaranth or Celosia family, and several other plants in that family have growth patterns similar to cockscomb's patterns**.**

Some plants in the Celosia genus, called the "Plumosa" variety *Celosia plumosa*, produce fluffy, colorful flower heads that resemble feathery plumes. Their plumes actually are made up of hundreds of tiny flowers that are quite similar to those on cockscomb, but they are grouped tightly along slim, upright stems. Grown as sun-loving annuals, they add flowers throughout summer and don't require deadheading. Depending on the cultivar, the plants reach a mature height of 24 to 40 inches. Varieties include "Forest Fire Improved," which has fiery orange to scarlet plumes and bronze-red leaves, "Golden Triumph," with deep-yellow plumes, and "Sparkler Mix," a group that has especially stiff yellow, orange or red plumes.

Another plant related to cockscomb is the wheat-type celosia *Celosia spicata*, also called spiked cockscomb. Varieties of this plant produce narrow, spike-shaped flower heads that resemble stalks of wheat. They are generally tall plants, reaching a height up to 4 feet, and produce abundant flower heads that give the plants a shrub like appearance. Varieties include "Flamingo Feather," which features burgundy, pink and white flower heads, "Tassle Deep Rose," with pink to purple flowers, and "Flamingo Purple," which has purple flowers that are considered excellent for use as dried flowers. A dwarf variety called "Kosmo Purple Red" is only 12 inches tall, has green and purple leaves and produces narrow, red flower heads that mature to resemble small cockscombs.

Weeds are said to be the harmful agents in agriculture, because of their powerful rate of growth and quick dispersal and quick distribution, Apart from their weedy character weeds have some special features like exhibiting novel phytochemicals, antimicrobial properties, etc. Because of this many of medicinal system like Ayurveda, Unani, and many other use these weeds as constituent in the medication (Ghayal *et al.* 2004) [15].

After listing out many of the local available weeds and come to know that *Celosia argenteta* is the ridiculous and sever weed of all time. According to the literature these plants showed many other exclusive properties like Antidiaherrol, Anti-inflammatory, Antioxidant, Anti-diabetic Anti-hepatotoxic (Shirish *et al.* 2002) with high nutritive value; it is a problematic weed of resistant to the hyper climatic conditions. Moreover its high seedling, dispersal and high severity of spreading made to select *C. argentea* as topics.

Materials and methods

1. Identification of Plant

The present study is the outcome of work undertaken under the Department of Botany, Vijayanagara Sri Krishnadevaraya University, Ballari district of Karnataka, India. The plant species was identified with the help of standard floras (Gamble, 1990).

2. Plant collection

The leaves, stem and root of *C. argentea* L. were collected from uncultivated land located in Kustagi, Koppal dist, Karnataka-India. The stems and roots were washed with water and dried in open air for about 7 days. They were then ground and stored in air tight containers for further studies.

3. Soxhlet extraction

50 gm powder samples was extracted using 350 ml of chloroform and methanol for both stem and roots for 24 hours respectively with three replicas. The extract obtained was dried at room temperature and used for the further studies.

4. Anti-bacterial assay

Solvent Used: Dimethyl sulfoxide **(**DMSO)
Antibiotic used: Ciprofloxacin
Concentrations screened: 25, 50, 100, 250, 500 &1000 µg
Sample preparation: 10 mg in 1 ml Of DMSO
Stock Sample Concentration: 10 mg/ml
Name of the analysis method: Agar diffusion method
Bacteria Analyzed: *Staphylococcus aureus*, *Escherichia coli.*

Initially, the stock cultures of bacteria were revived by inoculating in broth media (Media composition-Peptone-10 g, NaCl-10g and Yeast extract 5g, Agar 20g in 1000 ml of distilled water) and grown at 37ºC for 18 hrs. The agar media were prepared, poured in petriplates and wells were made in the plate. Each plate was inoculated with 18 hrs old cultures (100 µl, 10^{-4} cfu) and spread evenly on the plate. After 20 min, the wells were filled with of compound and antibiotic at different concentrations. All the plates were incubated at 37ºC for 24 h and the diameter of inhibition zone (mm) were noted.

5. Anti-fungal assay

Solvent Used: DMSO
Std. Antibiotic used: Amphotericin
Concentrations screened: 25, 50, 100, 250, 500 and 1000 µg
Sample preparation: 10mg/ml sample in solvent
Stock Sample Concentration: 10mg/ml
Remarks: Nil
Name of the analysis method: Agar diffusion method
Fungi Analysed: *Candida albicans, Aspergillus niger*

Initially, the stock cultures of were revived by inoculating in broth media (Media composition: Czapek-Dox Agar: Composition (g/l) Sucrose-30.0; Sodium nitrate 2.0; K_2HPO_4-1.0, $MgSO_4$. $7H_2O$-0.5; KCl-0.5; $FeSO_4$-0.01; Agar-20;)and grown at 27ºC for 48 hrs. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 48 hrs old cultures (100 µl 10^4 CFU) and spread evenly on the plate. After 20 min, the wells were filled with different volumes of samples. All the plates were incubated at 27°C for 96 hrs and the diameter of inhibition zone were noted.

Results and Discussion

Soxhlet extraction

The extract obtained from the Soxhlet extraction process of *C. argentea* stem using methanol solvent was found to be 1.32±0.15 and for chloroform was 0.99±0.11 and from the root using methanol solvent was found to be 2.11±0.14 and for chloroform was 1.06±0.31 respectively. The statistical data was analyzed by one way ANOVA (online software http://www.physics.csbsju.edu/stats/anova.html).

Antibacterial Activity

The study showed that the antimicrobial activity of *C. argentea* reported to confer resistance against microbial

pathogens and thus explains the manifestation of antibacterial activity by the stem and root extracts. The extract obtained acts as a potential source for biological antibacterial activity against selective bacteria stains *S. arulaus* and *E. coli* (Table 1 & 2).

Antibacterial activity of *C. argentea* against *Staphylococcus aureus* using ciprofloxacin as antibiotic. In the root and stem extracts were taken by using chloroform and methanol. In chloroform extract of root and stem 25µg, 50µg, 100µg, 250µg and 500µg, there is no such bacterial inhibition were found. But especially in the 1000µg, about 5mm and 6 mm inhabitation were found in root and stem extract respectively, which were taken from chloroform, Meanwhile in the other two methanol extraction *C. argentea* root and stem of various concentration 25µg, 50µg, 100µg, 250µg 500µg, and 1000 µg, there is no inhibition zones found in the analysis.

Table 1: Antibacterial activity of C. argentea against Staphylococcus aureus

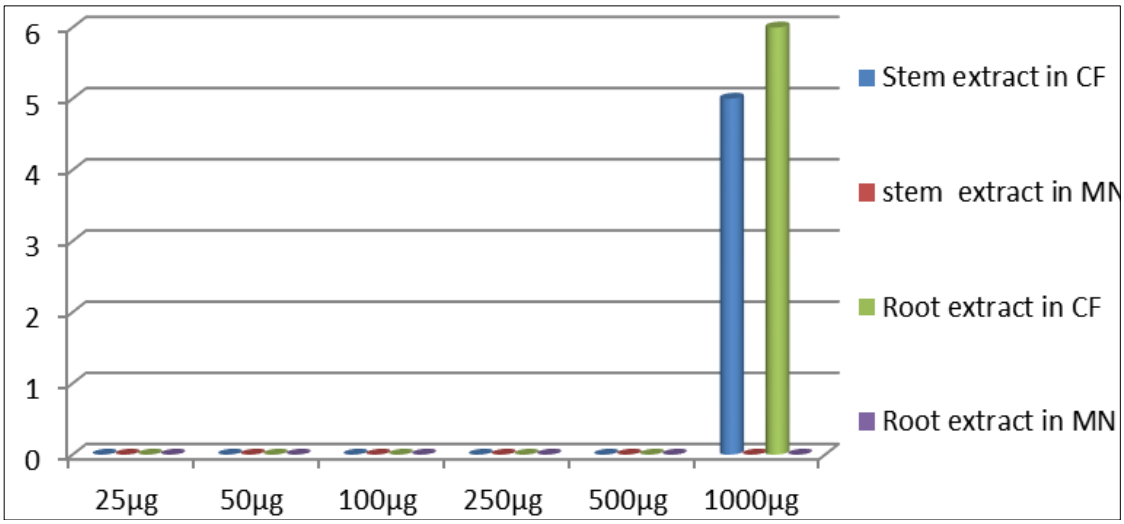
Sample	25µg	50µg	100µg	250µg	500µg	1000µg	MIC µg
Stem extract in CF	0	0	0	0	0	5	1000
Stem extract in MN	0	0	0	0	0	0	NF
Root extract in CF	0	0	0	0	0	6	1000
Root extract in MN	0	0	0	0	0	0	NF
Ciprofloxacin	13	18	21	25	27	*	25

Note: NF is **MIC** not found in the concentrations screened; **MN**: methanol; **CF**: chloroform; ***zones could not be measured due to merging. **Zones ≥3** mm considered for **MIC**

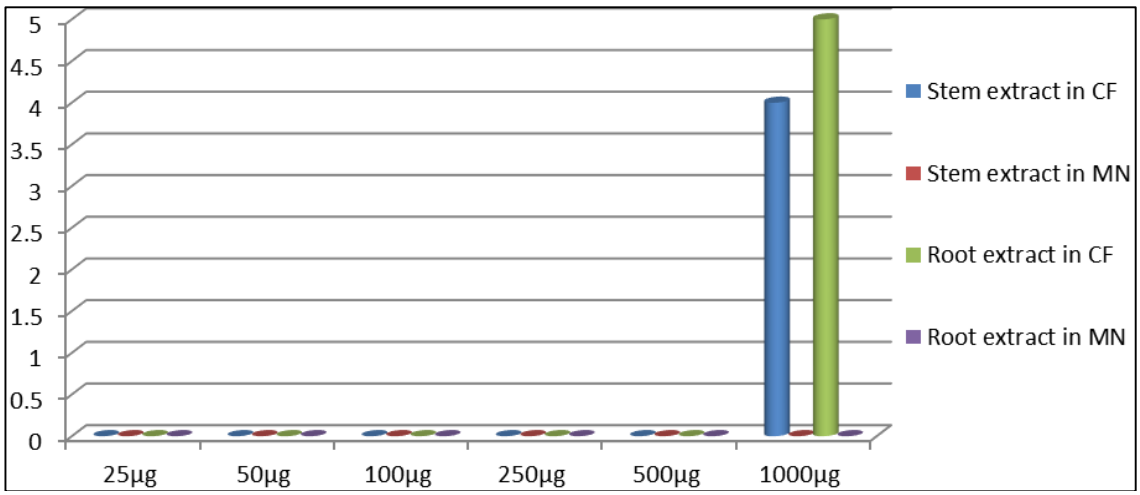
Table 2: Antibacterial activity of *C. argentea* against *E. coli*

Sample	25µg	50µg	100µg	250µg	500µg	1000µg	MIC µg
Stem extract in CF	0	0	0	0	0	4	NF
Stem extract in MN	0	0	0	0	0	0	NF
Root extract in CF	0	0	0	0	0	5	1000
Root extract in MN	0	0	0	0	0	0	NF
Ciprofloxacin	18	20	23	26	28	*	25

Note: NF is **MIC** not found in the concentrations screened; **MN**: methanol; **CF**: chloroform; ***zones could not be measured due to merging. **Zones ≥3** mm considered for **MIC**



Graph 1: Antibacterial activity of *C. argentea against Staphylococcus aureus*



Graph 2: Antibacterial activity of *C. argentea against E. coli*

Antibacterial activity of *C. argentea* against bacteria *E. coli.* using ciprofloxacin as antibiotic. In the root and stem extracts were taken by using chloroform and methanol. In chloroform extract of root and stem 25µg, 50µg, 100µg, 250µg 500µg, there is no such bacterial inhibition were found, but specially in the 1000µg. About 4mm and mm inhabitation were found in stem and root extract which were taken from chloroform, Meanwhile in the other two methanol extraction of various 25µg, 50µg, 100µg, 250µg 500µg, and 1000 µg, there is no inhibition zones found in the analysis as previous bacterial strain (Fig. 3-7).

Antifungal Activity

In the antifungal analysis, agar diffusion method is used. Here also the root and stem extract is prepared using soxhlet extractar in two different solvent namely chloroform, methanol and antibiotic used was and fungi analyzed are *Candida albicans* and *Aspergillus niger*. In various proportions of 25µg, 50µg, 100µg, 250µg 500µg, and 1000 µg, unfortunately there is no remarkable positive results in the antifungal analysis. The sample has not shown any inhibition zone (Fig. 8-12).

Discussion

The root and stem parts of *C. argentea* showed the antimicrobial activity against the microorganism's namely *E. coli S. aureaus, C. albicans* and *A. niger*. The chloroform and methanol extracts which was obtained from the solxhlet extraction had antimicrobila activity against the bacterial strains and two fungal strains namely *E. coli S. aureaus, C. albicans* and *A. niger* respectively. They were not as effective as Ciprofloxin, the standard drug. However the effect of the chloroform root and stem extracts of *C. argentea* against *Staphylococcus aureus* were better than that of methanol stem and root extracts and Ciprofloxin respectively.

Conclusion

Antimicrobial properties of medicinal plants are increasingly reported from different parts of the world, antimicrobials therefore, may have a significant clinical value in treatment of resistant microbial strains; In particular, the antimicrobial activity of plant oils and extracts have formed the basis of many applications including pharmaceuticals, alternative medicine and natural therapies. It has been reported that the higher plants have shown to be a potential source for new antimicrobial agents, therefore it is concluded that plants can be used as antimicrobial agent. From the results it is concluded that the chloroform extracts from *C. argentea* stem and root showed to be more effective against the *S. aureus, E. coli, C. albicans* and *A. niger* when compared with the methanol extracts of *C. argentea* stem and root.

Acknowledgement

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Fig 1: Habitat of plant *C. argentea*

Fig 2: Collected roots and stem of plant *C. argentea*

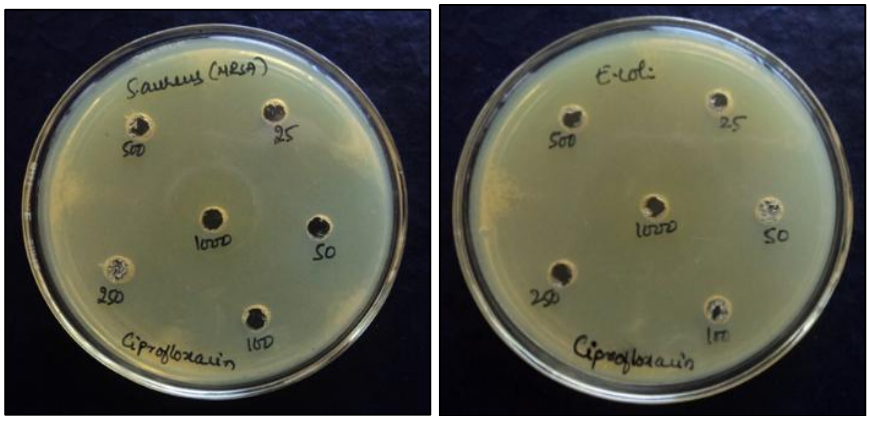

Fig 3: Petri plates before adding Chloroform extract for *S. aureus* (in left) and methanol stem extract for *E. coli* (in right)

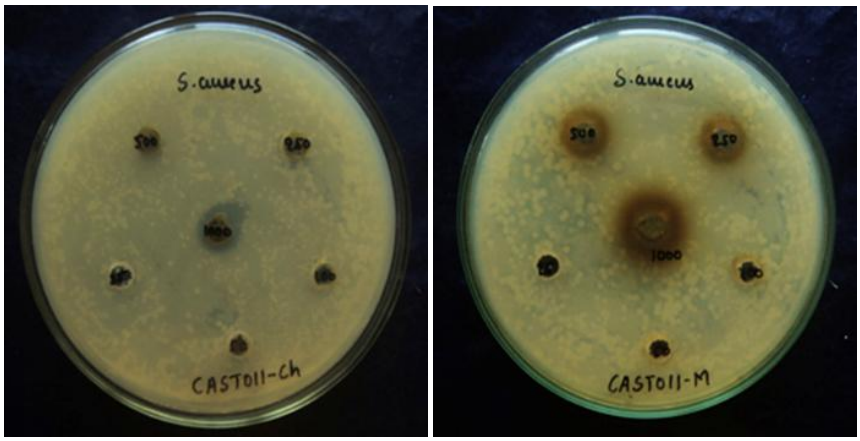

Fig 4: Petri plates after adding Chloroform extract for *S. aureus* (in left) and methanol stem extract for *E. coli* (in right)

Fig 5: Minimal inhibitory concentration (MIC) of *C. argentea* against *Staphylococcus aureus for* Chloroform stem extract (right) and methanol stem extract (left)

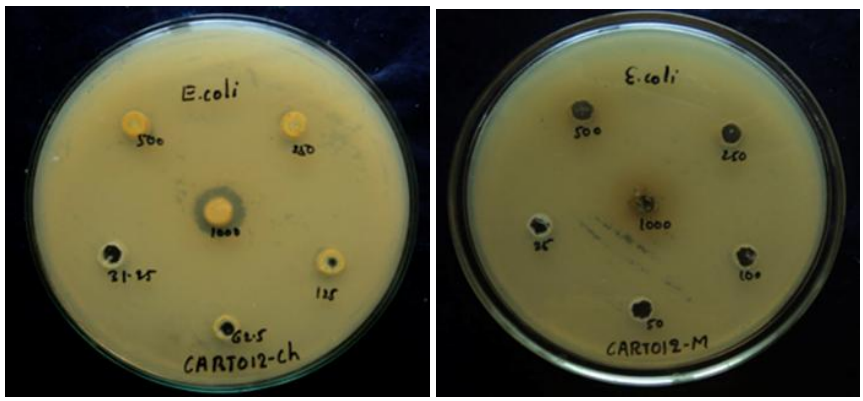

Fig 6: Minimal inhibitory concentration (MIC) of *C. argentea* against *E. coli for* Chloroform root extract (left) and methanol root extract (right)

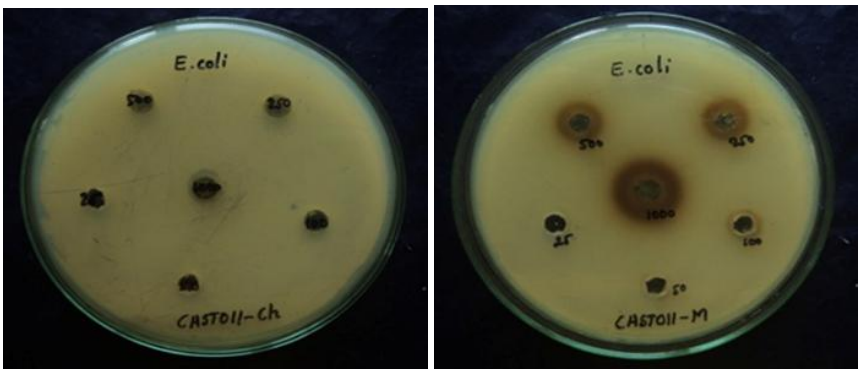

Fig 7: Minimal inhibitory concentration (MIC) of *C. argentea* against *E. coli for* Chloroform stem extract (left) and methanol stem extract (right)

Fig 8: Petri plate before adding extract for *A. niger antimicrobial activity*

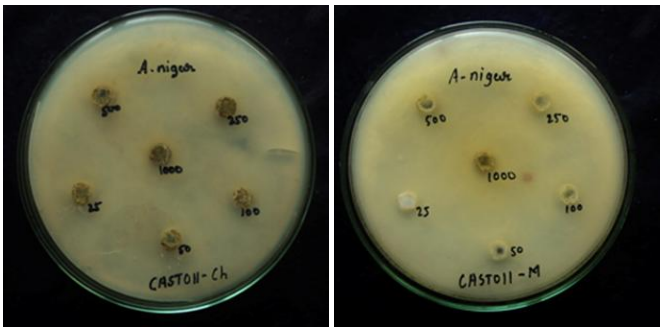

Fig 9: Minimal inhibitory concentration (MIC) of *C. argentea* against *A. niger* for Chloroform root extract (left) and methanol stem extract (right)

Fig 10: Petri plate before adding extract for *C. albicans* antimicrobial activity

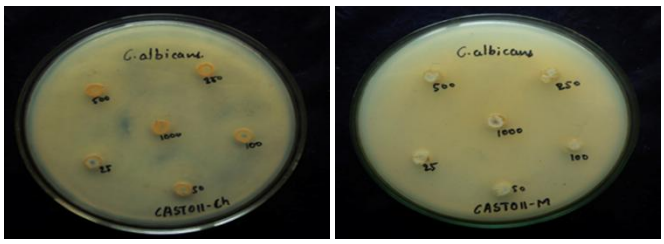

Fig 11: Minimal inhibitory concentration (MIC) of *C. argentea* against *C. albicans* for Chloroform stem extract (left) and methanol stem extract (right)

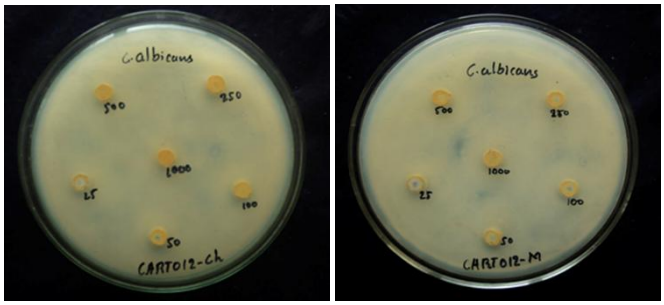

Fig 12: Minimal inhibitory concentration (MIC) of *C. argentea* against *C. albicans* for Chloroform root extract (left) and methanol root extract (right)

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