

## ANTIFUNGAL ACTIVITY OF *Sebastiania chamaelea* Muell. Arg. LEAF CRUDE AND FRACTIONAL EXTRACTS

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### ABSTRACT

**Objective:** Most of the Euphorbiaceae members like *Acalypha*, *Croton*, *Jatropha* and *Phyllanthus* species are reported as best antifungal herbal drugs. *Sebastiania chamaelea* leaf extracts also possess herbal remedies against Syphilis and diarrhoea. Hence screening for antifungal activity is necessitated.

**Materials and Methods:** Collection of leaf material, authentication of the specimen and preservation of the specimen were followed as per Jain and Rao method. Preparation of aqueous and methanol, ethyl acetate and alcohol extracts, and isolation of fraction A and B compounds through column chromatography as per the method from Harbone and Kokate book. Anti fungal activity by well diffusion method of Perez *et al*, Minimum Inhibitory Concentrations Vollekova method modified by Usman *et al*.

**Results:** The most effective results with ethyl acetate followed by methanol, aqueous and fraction A and fraction extracts at 10mg/well zone of inhibition ranges from 16-26mm than the control drug *Nystatin* only 10-12 mm of zone of inhibition with chloroform extracts no activity observed. *Candida albicans* is most susceptible than *A.niger*. MIC values ranges from 1.42 to 3.31 mg/ml.

**Conclusion:** Antifungal activity of *S.chamaelea* leaf extracts showed one of the effective antifungal drugs of Euphorbiaceae to that of *Acalypha indica*, *Croton zambesicus* bark, *Jatropha curcus* roots and *Phyllanthus acidus* leaf extracts at lower concentrations of 10 mg/well with an 20-36mm of zone of inhibition.

**Keywords:** *Aspergillus niger*, *Candida albicans*, Astringent, Diarrhoea, Antifungal activity, Minimum Inhibitory Concentration

### INTRODUCTION

Herbal usage of *S.chamaelea* whole plant decoction in ghee is given as tonic and applied to the head in vertigo. The juice of the plant is astringent and is used as a remedy for syphilis and diarrhoea [1-2]. Leaf Methanolic and aqueous extracts showed the presence of flavonoids, glycosides, phenols, tannins and sterols. Phenolic compounds like caffeic acid, melilotic acid, aesculetin, p-hydroxy benzoic acid, coumarin, cinnamic acid, salicylic acid and scopoletin; flavonoids like myrecetin, quercetin, kaempferol, luteolin, and apigenin were reported. Antibacterial activity of leaf, methanol extracts on *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* revealed their efficiency against all bacterial strains. Leaf methanol, ethanol and chloroform extracts were also reported more efficient anthelmintic activity at 50mg/ml in time taken for paralysis 14-23min and 30-56min for death compare to that of the control drug Albendazole 25mg/ml [3-4].

### MATERIALS AND METHODS

#### Collection and identification of Plant material

The plant material *Sebastiania chamaelea* (Euphorbiaceae) was collected from the fields of S.V.Veterinary College, Tirupati, Andhra Pradesh, India during the month of June.2010 and preserved as per the standard method [5]. The taxonomic identity was confirmed by Prof. N. Yasodamma, Department of Botany, Sri Venkateswara University, Tirupati. The voucher specimen KS711 was preserved in the Herbarium of Department of Botany. The leaves were thoroughly washed and then dried under shade for one week. The dried leaves were ground in a mixer grinder and sieved. The powder was stored in air sealed polythene bags at room temperature until further use.

#### Preparation of crude extracts

Dried leaf powder (70 g) was extracted with water after 72 hr the filtrate was dried on water bath. The dried powder (40 g) was extracted in a soxhlet apparatus using 200 ml of each solvent of methanol, ethanol and chloroform. The filtrates were concentrated on rotavapour and dried. All the extracts were stored at 4°C in refrigerator until further use.

#### Fractionation by Silica Gel Column Chromatography [6]

Dried crude leaf aqueous extract was dissolved in minimum amount of distilled water and absorbed on silica gel (100 to 200 mesh size) to form slurry. The slurry was dried under reduced pressure in rota-vapor. Column was prepared using silica gel (100-200 mesh size) ten to twenty times to that of the weight of dry crude extract in ethyl acetate by dry packing method. Now the dry slurry was subjected to the prepared column (100 cm × 3.5 cm i.d). The column was eluted initially with hexane, then followed by Ethyl acetate, methanol on polarity gradient finally exhausted by using 100% methanol. Each fraction of 75 ml was collected, concentrated and left for crystallization. Purification of isolated compounds was carried out by washing and recrystallization technique.

#### Thin layer Chromatography

The glass plates of (20 × 20) were washed thoroughly under running tap water followed by distilled water and was kept ready for silica gel application. Silica gel-G, 25 g (Acme) was dissolved in 50 ml of double distilled water, stirred well and the slurry was then passed on the spreader. The spreader was gently pulled along the plates, (2-3 mm thickness) to get an even application. The plates were activated at 100°C for about 45 minutes, allowed to cool, removed from the oven and subjected to use. The fractions were applied with micropipette, 2 cm above the base of the plate. The material was applied in the form of spot and was allowed to dry with hair dryer. Then the plates were developed in ethyl acetate: methanol 9:1 (v/v). The solvent was allowed to move up to 15 cm, then were removed, allowed to dry. The plates were sprayed with 8% methanolic sulphuric acid, then subjected to heating on hot air oven at 100°C for 5 minutes the compounds were visualized. Fractions collected were monitored on silica gel (G) TLC. The visualization of spots on TLC plates was carried out either in UV light or exposing TLC plates to iodine vapors. Similar fractions were combined and the results borne out in the chromatography are recorded. The fractions with similar spots with same R<sub>f</sub> values are mixed together and distilled. Phytochemical screening of these fractions was carried out using standard methods. And antifungal activities of these fractions were performed.

### Separation of Fraction A & B

The ethyl acetate: methanol (6:4) eluates on concentration gave fraction A, which was showing three compounds when spotted on a TLC plate and when phytochemically screened, this fraction was giving positive test for phenolic compounds; Ethyl acetate and methanol (3:7) eluates on concentration gave brown colored viscous fraction B, which was giving positive test for tannins. Fractions A&B were further tested for antifungal activities.

### Antifungal activity

#### Test organisms

Fungal cultures of *Candida albicans* (ATCC-10231) and *Aspergillus niger* (ATCC-16404) were procured from the department of Microbiology, S.V. University. These were further maintained on nutrient agar slants at 4°C until further use.

#### Preparation of Inoculum

Active cultures were prepared by transferring a loop full of cells from the stock cultures to test tubes of Potato Dextrose agar medium and were incubated without agitation for 24hrs at 37°C.

#### Well diffusion method

Antifungal activity of plant extracts was determined using agar well diffusion method with slight modifications [7]. Potato dextrose agar was inoculated by spreading the selected fungal inoculums on the media. Wells (9 mm diameter) were punched in the agar and filled with plant crude extracts and fraction A & fraction B extracts of 10mg/well; control wells containing pure solvents (negative

control) or standard antibiotic (positive control) *Nystatin* (10mg/well). The plates were incubated at 25+2°C for 48 hours for fungal growth. The antifungal activity was assessed by measuring the diameter of the zone of inhibition for the respective drug. The data of crude drugs activity is given the mean of quadruplicates along with the standard error.

### Minimum inhibitory concentration (MIC)

MIC is defined as the lowest concentration where no visible turbidity is observed in the test tube (fungal concentration). The Vollekova method [8] modified by Usman [9] was employed. In this method the broth dilution technique was used where plant extracts were prepared to the highest concentration of 10mg/ml (stock concentration). By adding sterile distilled water and serially diluted (two fold dilution) using the potato dextrose broth and later inoculated with 0.2ml standardized suspension of the test organisms. After 18hrs of incubation at 37°C., the test tubes were observed for turbidity. The lowest concentration of the tube that did not show any visible growth can be considered as the MIC.

### RESULTS

#### Antifungal activity of crude and Fraction A and B Extracts :(Plate- 1&2) (Table.1).

Aqueous, methanol and ethanol extracts have shown significant antifungal activity except with chloroform extract. The zone of inhibition ranges from 19.2 to 26.2 mm with crude extracts and with fraction A & B at 10mg/well, which are more effective when compared with control drug Nystatin at 10 mg/well with 10 to 12 mm. However *Calbicans* is more susceptible in comparison to *Aniger*.

Table 1: Antifungal activity

Organisms	Crude Extracts 10mg/well				Fractions 10mg/well		Control - Nystatin 10 mg/well
	AQ	ME	ET	Cl	F.A	F.B	
<i>C. albicans</i>	21.5± 0.06	24.5± 0.13	26.2± 0.06	0.0± 0.0	20 ± 0.07	18 ± 0.12	10±0.56
<i>A. niger</i>	19.2± 0.04	21.7± 0.20	21.7± 0.20	0.0± 0.0	17 ± 0.05	16 ± 0.07	12±0.59

AQ: Aqueous, Me: Methanol, ET: Ethyl acetate, Cl: Chloroform,

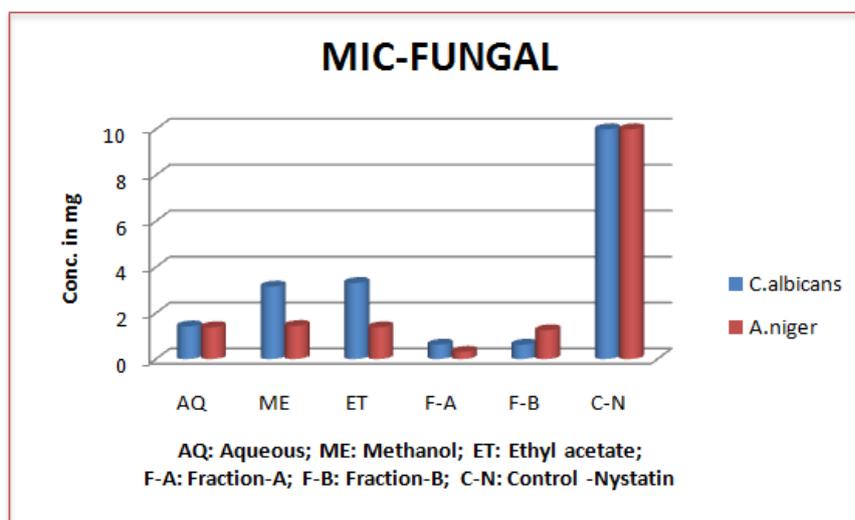
F.A: Fraction A, F.B: Fraction B

Values are mean Inhibition zone (mm) ± S.D of quadruplicate.

#### Minimum inhibitory concentration (Graph: 1)

Minimum inhibitory concentration (MIC) against *Candida albicans* with aqueous, methanol and ethanol extracts was 1.42, 3.15, and

3.31 mg/ml; against *Aspergillus niger* 1.38, 1.44 and 1.38 mg/ml. Chloroform extract was not showed any activity. When compared with standard Nystatin 10 mg/ml these concentrations are very low and effective.



Graph I: Minimum Inhibitory Concentration

Plate-1. Antifungal Activity  
Aqueous

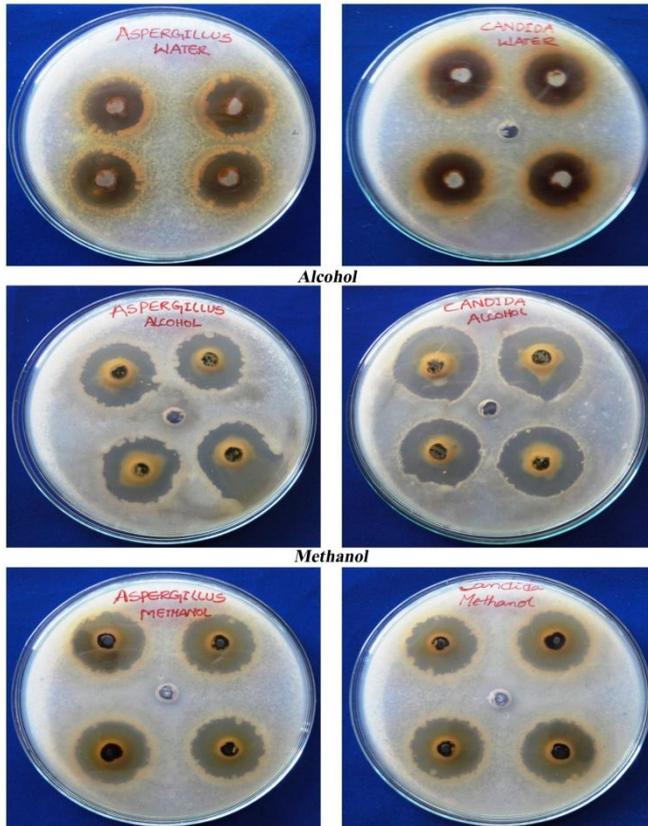
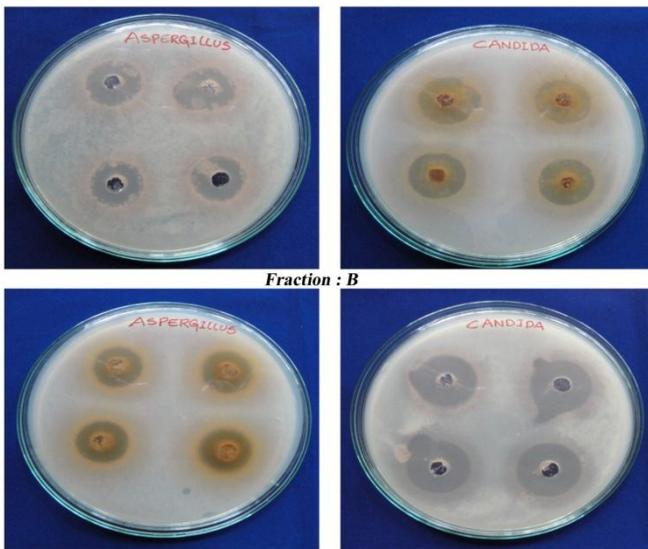
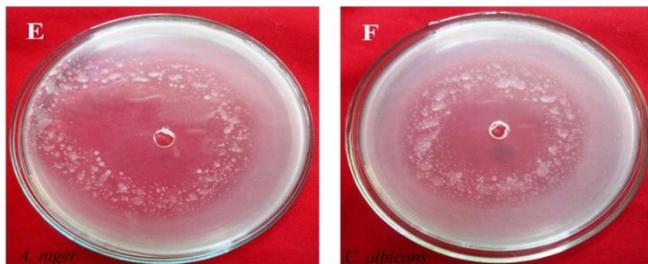


Plate-2. Antifungal Activity  
Fraction : A



Control : Nystatin (10 mg/ Well)



## DISCUSSION

Reports on similar studies of antifungal activity were noticed in other species of the family Euphorbiaceae. Leaf methanol extracts of *Acalypha wilkesiana* displayed marked antifungal properties against *A. niger*, *C. albicans* with 20-20mm of zone of inhibition to that of Tiocanazole standard drug 12-16 mm. *A. indica* phenolic derivatives (gallicin, gallic, syringic and caffeic acids) and flavonoid compounds (Quercetin, kaempferol, isorhamnetin, isoquercetin) have potent antifungal activity against *Microsporum* Sps and *Trichophyton* Sps. [10]. *A. indica* chloroform and ethanol extracts showed effective antifungal activity against *C. albicans*, *C. tropicalis*, *Microsporum canis*, *A. fumigates* at 20-30 µg/ml 8.3 to 13.0 mm inhibition zone compared to that of Ketoconazole, Fluconazole and Itraconazole standard drugs at 30mg/ml with 11-25 mm of zone of inhibition [11]. Ethyl acetate, diethyl ether and methanol extracts of *A. indica* represented much better inhibitory activity against HIV associated 8 fungal pathogens *Aspergillus*, *Blastomyces*, *Candida*, *Cryptococcus*, *Fusarium*, *Histoplasma* and *Penicillium* [12].

*Croton zambesicus* stem bark methanolic extracts on *C. albicans* showed at 50mg/ml with 25-30mm of zone of inhibition and MIC values at 25mg/ml. [13]. Ethanol extracts of *Euphorbia hirta* showed effective activity against *Colletotrichum capsici*, *Fusarium pallidoroseum*, *Botryodiplodia theobromae*, *Alternaria alternata*, *Penicillium citrinum*, *Phomopsis caricae* and *Aspergillus niger*. *E. thymifolia* showed effective activity against *C. albicans* with 19mm of zone of inhibition at 50 mg/ml and 25mg/ml MIC Values [14].

Methanol extracts of *Jatropha curcas* was effective against *A. niger* [15]. *J. curcas* roots and seed extracts expressed effective inhibition against *C. albicans*, also rich in steroids, alkaloids and tannins [16-18]. *J. gumeri* root dichloromethane /methanol (2:1) extracts against *Candida albicans*; stem bark extracts of *J. podagrica* showed remarkable activity against *C. albicans* at 15mg/ml [19]. n-Hexane extracts of *J. pandurifolia* against *C. albicans*, *A. niger* and *Saccharomyces cerevisiae* with 14.43mm of zone of inhibition at 500µg/disc to that of Kanamycin 30 µg/disc. IC50 values with n-Hexane extracts 4.67µg/ml to that of vincristine sulfate 0.27 µg/ml against brine shrimp. [20]. Hexane extracts of *J. gossypifolia* fresh fruits exhibited effective activity against *C. albicans*, *A. terreus*, *Fusarium oxysporum* and *Pestalotiopsis palmarum* than with other extracts of the leaves and stem.

*Phyllanthus acidus* leaf ethyl acetate and ethyl alcohol extracts are shown more potential inhibitory activity with 75-84mm zone of inhibition against *C. albicans* at 0.35mg in 0.01L concentration to that of the Nistatin 0.2-0.6 ml with 50-51mm of zone of inhibition [21]. *P. amarus* showed good activity on selected 10 fungal strains of *Aspergillus*, *Candida*, *Cryptococcus* sps and *Issatchenkia orientalis* at 0.20 to 0.25mg/ml of acetone, ethanol and water extracts [22].

## CONCLUSION

Antifungal activity of *S. chamaelea* crude and aqueous fraction A & B extracts on *A. niger* and *C. albicans* with 10mg/well were proved as one of the best antifungal herbal drug which also possessed a large number of phenolic and flavonoid compounds in highest quantities. The same activity was observed as that of *Jatropha curcas*, *J. gossypifolia*, *Acalypha*, *Euphorbia*, *Phyllanthus* and *Croton* species of *Euphorbiaceae* with lowest concentrations at an effective inhibition zone (10mg/well 19-20mm with 1-3mg of MIC values).

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