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ISOLATION AND CHARACTERISATION OFANTIMICROBIAL COMPOUNDS FROM LEAF EXTRACT OF ABUTILN INDICUM

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ABSTRACT

Medicinal plants are gifts of nature to cure limitless number of diseases among human beings. The production of medicines and the pharmacological treant of diseases began with the use of herbs. The leaves extracts *Abutilon indicum* and *Morinda citrifolia* were were taken the antimicrobial activity from the extracts of the plants *Abutiln indicum* was carried out in crude (ethyl acetate, methanol, chloroform) and column fractionated extracts (ethyl acetate (F1), ethyl acetate: chloroform (F2), chloroform (F3), methanol: chloroform(F4) & methanol(F5) against six bacterial and two human fungal pathogens *Candida albicans* and *Aspergillus flaves* using disc diffusion technique. Crude ethyl acetate, chloroform extract of *A. indicum* showed highest activity against *S. flexneri* (6mm) and crude methanol extract showed 6mm against *V. cholerae*. Among the tested pathogens F1 fraction & F5 fractions were well in inhibiting most of the tested pathogens. Among the crude ethyl acetate, methanol and chloroform extracts, methanol extract of *A. indicum* and chloroform extract of exhibited maximum inhibition zone against *Candida albicans* (22 mm) and column fractionated extracts F1 (ethylacetate) and F5 (methanol) fractions exhibited maximum inhibitory zone against *C.albicans* (11 mm). This study validated the use of this plant in folkloric medicines and a potential source of new classes of antibiotics that could be useful on control of human infectious diseases.

KEYWORDS: Antifungal activity, inhibitory zone, GC-MS analysis, analgesic activity, anti-inflammatory activity.

1. INTRODUCTION

Medicinal plants are finding use as pharmaceuticals, nutraceuticals, cosmetics and food supplements.[1] Plant derived products have been used for medicinal purposes for centuries. In traditional Indian medicine or Ayurveda, Zingiber officinale and many other herbs have been used as medicine.[2] With an increase in the antibiotic-resistant strains of microorganisms, traditional plants are being investigated for their antibacterial and medicinal values. Traditional uses of plants have led to investigating their bioactive compounds, which have resulted in the detection of a significant number of therapeutic properties.[1] People all over the world are still affected by quite a large number of microbial infections with fungi causing a good number of them. It has since been discovered that active medicinal substances are present in plants and this has encouraged the inclusion of herbal remedies in the delivery of health care.[3] [4]

In the past few decades, a worldwide increase in the incidence of microbial infections has been observed. The majority of clinically used anti-microbial's have various drawbacks in terms of toxicity, efficacy and cost, and their frequent use has led to the emergence of resistant strains. The challenge has been to develop effective strategies for the treatment of candidiasis and other fungal diseases, considering the increase in opportunistic fungal infections in human immunodeficiency virus-positive patients and in others who are immune compromised due to cancer chemotherapy and the indiscriminate use of antibiotics.[7] Development of medication induced antibiotic resistance, has been evident in *E.coli* and other urinary tract bacteria. This situation forced scientists to search for new antimicrobial substances. Therefore there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants (Cordell, 2011). Hence the present study aimed to investigate the antimicrobial activity from the extracts of the plants *Abutilon indicum* against six bacterial and two fungal pathogens.

2. MATERIAL AND METHODS

2.1 Collection of Plant Material

The fresh plants of *Abutilon indicum* was collected in the month of September 2015. The plant was collected from Saveriyar Palayam at Dindigal District.

2.2 Preparation of Extract

The leaves of plants of *A. indicum* was thoroughly washed with distilled water and kept in the room temperature at 27°C for two weeks. The dried plant samples were ground well into a

fine powder in a mixer grinder and sieved to give particle size of 50 – 150micron. The plant powder was stored in air sealed polythene bags at room temperature before extraction. Each 150 gram of this fine powdered samples were soaked in 200ml AR grade of methanol, chloroform and ethyl acetate for 15 days and these extracts were filtered through What Mann No.1 filter paper and evaporated at room temperature for dryness. These residues were stored at 4^{o}C for further studies.

2.3 Microorganisms Used

The test pathogens were supplied by the Basic Biomedical Science, Bharathidasan University, Trichy. Six bacterial pathogens viz. *V. cholerae Ogawa, S. typhi, S. flexneri, E. coli, M. tuberculosis and P. fluorescens* bacterial strains and two fungal strains *C. albicans* and *A. flavus* were used in the present study. The organisms were sub cultured on Muller Agar medium and Czapex Dox solidified nutrient agar respectively. All the cultures were incubated at 37°C for 24 hrs and stored at 4°C in the refrigerator to maintain stock culture.

2.4 Preparation of Media

The medium was prepared by dissolving nutrient agar (Hi Media Laboratories Pvt. Ltd.,) in distilled water and autoclaving at 121°C for 15 minutes. It is used for preliminary antibacterial study.

Active culture for experiment were prepared by transferring a loopful of microorganisms from stock cultures to test tubes of nutrient broth and incubated for 24 hours at 37°C.

2.5 Antifungal Susceptibility Assay

Pathogenic fungal strains were inoculated in potato dextrose agar medium and incubated at 48 hrs. In vitro antifungal activity of plant extracts were determined against *C. albicans* and *A. flavus* respectively. Fungal strains were gently swabbed on the surface of the sterile petridishs containing 20 ml and Czapex Dox solidified nutrient agar with the help of a sterile cotton swab. Same procedure was followed for crude as well as for the fractions. Areas of inhibited fungal growth were observed after 48 hrs.

2.6 Identification of Compounds

The most potent crude ethyl acetate extract of the test plant was subjected to GC-MS study which was carried out on a GC Clarus 500 Perkin Elmer system for the identification of different compounds responsible for inhibition of pathogens tested.

Interpretation on mass spectrum GC-MS was conducted using the data base of National Institute Standard and Technology (NIST) having more than 62,000 patterns of the mass spectra of crude extract test plant unknown components found were matched with the spectrum of the known components stored those in Library used NIST Version – year 2005 National Institute of Standard technology, the mass spectra library.

3. Pharmacological Qualities of the Identified Compounds

3.1 Selection of Experimental Plants

Adult Wistar albino rats of either sex weighing between 150 and 180 gm maintained in Sankaralingam Bhuvaneswari College of Pharmacy animal house, Sivakasi were used for the study. The selected animals were housed under standard environmental conditions (temperature of 22 ± 1°C) maintained by giving uniform pellet diet, water ad libitum with an alternating 12 hrs light dark cycle and relative humidity of 60 ± 5%. (Prior approval of Institutional Animal Ethics Committee (IAEC) was obtained).

3.2 Acute Toxicity of Column Fractionated Extract of Test Plants

Acute toxicity study was performed in Albino rats divided into different groups of 5 each. After an overnight starving, the suspension of column fractionated extract in 0.5% (W/v) of Sodium Hydroxide was administered orally in graded doses (100 mg to 200 mg/kg body weight) to albino rats. They were observed continuously for the first 2 hours for toxic symptoms and up to 24 hours for mortality.

3.3 Analgesic Testing (Narcotic Type of Analgesic Activity

Wistar albino rats were screened for its sensitivity by placing the tip of the tail (last 1 – 2 cm) gently in warm water maintained at 55°C ± 2°C. Any albino rats flicking the tail within 5 sec. were selected for the study. The selected rats were divided into four groups of four animals each. Group I and Group II received distilled water 1ml/kg (Control) and Pentazocine 4 mg/kg p.o. (Standard) respectively. Group III received F1 fraction of *A. indicum* extract with 100 mg/kg and 200mg/kg p.o. After drug treatment, the basal reaction time of all groups of animals was noted at different time intervals like 1 hr, 2 hrs, 3 hrs and 4 hrs.

3.4 Anti Inflammatory Testing

Anti - inflammatory activity was assessed by the method suggested by Winters *et al*., (1962) using Carrageenan as phlogestic agent. The selected Wistar albino rats of either sex weighing between 150 and 180 gm/kg were housed in groups of eight. They were starved overnight

during the experiment but had free access to water. The volume of paw of each animal was determined before giving any drugs.

Group I and II were given orally the distilled water 1ml/kg (Control) and suspension of Diclofenac Sodium 10 mg/kg b.w (Standard) respectively. III, IV and V group of animals 30 minutes prior to the injection of Carrageenan (0.1 ml of 1% W/v solution) in normal saline into sub planter region of left hind paw of each rat, received 100 and 200 mg/kg of the column fractionated test drugs of *A. indicum* (F1), dissolved in 0.5% W/v Sodium Hydroxide and administered orally (Ocete *et al.*, 1989).

The degree of oedema formation at the hind paw volume was measured by plethysnographically at each hour, for 4 hours after Carrageenan was injected.

The percentage inhibition of edema has been calculated by the following formula.

$$A - B = \frac{C}{A} \times 100$$

Where A represents the average increase in paw volume of control and B represents the average increase in paw volume after the administration of drug.

3.5 Statistical Analysis

All the data's were expressed as mean ± S.E. Statistical significance of the difference between control and treated groups were accessed by the method of analysis of one way ANOVA followed by Dunnett's t-test. $P<0.1$, $P<0.05$ and $P<0.001$ were considered as statistically significant.

Table 1: Activities of compounds identified by GC – MS in ethyl acetate extract of *A. indicum*.

No.	RT	Name of the compound	Molecular Formulae	Peak Area %	Compound Nature	**Activity
1.	6.88	Phenol, 2-methyl-5-(1-methylethyl)-	$C_{10}H_{14}O$	0.56	Phenolic compound	Antimicrobial Antioxidant, Anti-inflammatory Analgesic
2.	11.40	Cyclohexane carboxamide, N-hydroxy-2(E)-2,4-pentadienyl-	$C_{12}H_{19}NO_2$	0.02	Amino compound	Antimicrobial
3.	13.98	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	12.54	Terpene alcohol	Antimicrobial Anti-inflammatory

4. RESULTS AND DISCUSSION

Plant has long been a very important source of drug and many plants have been screened if they contain compounds with therapeutic activity. In vitro evaluation of plants for antimicrobial properly is the first step towards achieving the goal for developing eco-friendly management of infectious disease of humans by search for new bio-molecules of plant origin. Therefore to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants the antimicrobial activity from the extracts of the plant *A. indicum* was screened in vitro against six human pathogenic bacteria and two fungal pathogens known to cause diseases in humans were carried out based on traditional medicine knowledge. In our study, the antibacterial activity of the various solvent extract of the herbal plants *A.indicum* was investigated against bacterial isolates viz., *V. cholerae Ogawa, S. typhi, S. flexneri, E. coli, M. tuberculosis and P. fluorescens* andfungal pathogens such as *C. albicans* and *A. flavus.* Both crude and Silica gel column fractionated extracts of *A.indicum* exhibited varying degree of antibacterial activities against the test organisms.

In the present study the antibacterial activity of crude ethyl acetate extract of *A. indicum* showed the highest activity against *S. flexneri* (6mm), in the crude chloroform extract the potent zone of inhibition ranged from 3mm to 6mm and in the crude methanol extract the variation obtained between 2mm to 6mm respectively.

The antifungal activity of all three crude extracts of *M. citrifolia* showed highest activity against *C. albicans* (20mm), (22mm) and (21mm). The crude extract obtained from ethyl acetate extract of *A. indicum* F1 fraction, maximum inhibition zone was obtained against *S. flexneri* (11mm), F2 and F3 fraction exhibited very little activity, in F4 fraction maximum inhibition zone was obtained against *V. cholerae* (6mm) and in F5 fraction against *S. flexneri* and *S. typhi* (5mm) respectively and in chloroform fractionated extract F1 fraction exhibited maximum inhibition zone against *S. flexneri* (7mm), in F2 fraction against *E. coli* (4mm), in F3 fraction against *V. cholerae* and *S. typhi* (4mm), in F4 against *S. flexneri* and *E. coli* (6mm) and in F5 fraction against *S. flexneri*(4mm) respectively. The antifungal activity of crude ethyl acetate, chloroform and methanol extract of *A. indicum* exhibited very good activity against *C. albicans* (20mm), *A. flaves* (15mm); *C. albicans* (22mm) *A. flaves* (13mm); *C. albicans* (22mm), *A. flaves* (15mm) respectively.

In the F1 fraction of column chromatography extracts of six pathogens tested maximum inhibition zone was obtained against *S. typhi* (9mm) followed by *V. cholerae* (8mm), *P.*

fluorescens (7mm) and in F2 fraction against *S. typhi,* (7mm). F3, F4 and F5 fractions and the crude chloroform fractions also exhibited very little activity. The crude methanol column chromatography extract of F1 fraction *S. typhi exhibited* to13mm followed by *V. cholerae* (11mm), *S. flexneri* (9mm), *E. coli* (7mm). F2, F3 and F5 exhibited very little activities among the tested pathogens and the only pathogen inhibited by F4 fraction was *S. typhi*(7mm).

The fractionated extract obtained from methanol of *A. indicum* showed minimum inhibition zone by F1, F2, F3 fractions of the tested pathogens. Very good activity was obtained in F4 and F5 fraction against *V. cholerae* (6mm & 8mm).

Comparable result was obtained by Mahesh and Satish (2008) in *Acacia nilotica, Sida cordifolia, Tinospora cordifolia, withania somnifer* and *Ziziphus mauritiana* in methanol leaf extracts against Bacillus subtilis, *E. coli* and *P. fluorescens.* Similar findings are reported by Sumathi and Puspha, (2007), In the present study the crude ethyl acetate, chloroform and methanol extract of *A. indicum* the highest antifungal activity was exhibited against *C. albicans* (20mm), (22mm) (16mm) respectively. Among the silica gel fractions in the test plants, maximum antifungal activity was observed in fraction F1 and F5 against C. *albicans.*

Since the F1 fraction of ethyl acetate extract of *A. indicum* was exhibited maximum activity among the tested bacterial and fungal pathogens was subjected to GC-MS analysis to find out the responsible compound for the inhibitory activity of the tested human pathogens, The following compound were identified from mass spectra analysis, and the activity of compounds identified in *A. indicum*fractionated ethyl acetate extractwere Phenol, 2-methyl-5-(1-methylethyl), Cyclohexane carboxamide, N-hydroxy-2(E)-2,4-pentadienyl-3,7,11,15-Tetramethyl-2-hexadecen-1-ol,Z,E-3,13-Octadecadien-1-ol, 1,2-Benzenedicarboxylic acid, butyl octyl ester, Phytol, Bis(2-ethylhexyl) phthalate, Squalene.

Most of the components identified from GC-MS analysis were proved to be the antimicrobial activity. Similar result was obtained by Gaud Elizabeth Thomas (2013) in concentrated ethanol extract *Nervilia aragoana* which contains a variety of fatty acids. The compound 5-hydroxy-2-(hydroxyl methyl)-4H-pyran-4-one is having antibacterial antifungal properties and it inhibits melanin production and anti inflammatory compounds like Hexadecanoic acid, fragrance and flavouring agents such as 2-octenoic acid, pentadecanoic acid etc are also identified.

The most important of their bioactive principles are alkaloids, phenolic compounds, flavonoids and tannins that may be evolved in plants as self defense against pest and pathogens (Sukumaran *et al*., 2011). Sanjivani *et al.*, (2011) reported that the phytochemical analysis of methanol extract of leaf, stem, seed, and root sample of *Cassia auriculata* showed the presence of alkaloids, tannins, flavonoids. Kalaiselvi *et al.*, noticed the presence of anthroquionones, alkaloids, flavonoids, steroids, tannins and phenolic compounds in the root and leaf sample of *Cassia auriculata.* Sarayu *et al*., (2009) also reported the similar result in the methanolic extract of the leaf sample in *Coriander sativum*.

John De Britto and Herin Sheeba Gracelin, 2011, investigated the phytochemical present in leaves, stem, flowers and fruits of *Datura metal* which have some medicinal applications. It is clear that using organic solvents provides a higher efficiency in extracting compounds for antimicrobial activities compared to water based method (Lima-Filo *et.al*., 2002). The antimicrobial activity of *A. indicum* could also attributed to the presence of alkaloids, steroids and tannins. Alkaloids have anti microbial activity by precipitating protein content of the outer wall of the microbes. Alkaloids which are one of the largest groups of phytochemicals in plants have amazing effects on humans and this has led to the development of powerful pain killer medications.

The study revealed the presence of the most important antimicrobial and anti-inflammatory compounds (Sulfur, Phenolic, Monoterpene, Ester, Disaccharide, Myristic acid ester, Palmitic acid, Linoleic acid, Oleic acid, Diterpene, Steroid, Triterpene) which have many biological activities have justified the use of *A. indicum* in the treatment of many ailments in folk medicines. The findings of the present investigation suggests that the organic solvent extraction was suitable to verify the antimicrobial properties of medicinal plants and they supported by many investigation.

The present study justifies the claimed uses of herbal plants in the traditional system of medicine to treat infectious diseases caused by the microbes. This study also encourages cultivation of the highly valuable plant in large scale to increase the economic status of the cultivators in the country. The obtained results may provide a support to use of the plant in traditional medicine. Based on this, further chemical and pharmacological investigations can be done to isolate and identify minor chemical constituents in the leaves and to screen other potential bioactivities may be recommended. Commercial antibiotics are highly effective to kill the bacterial and fungal pathogens involved in significant antibacterial activity. It is

worthy to note that the product from natural source is good for health and devoid of side effects.

The investigation concluded that the stronger extraction capacity of ethanol and methanol could have been produced number of active constituents responsible for many biological activities. So that those might be utilized for the development of traditional medicines and further investigation needs to elute novel active compounds from the medicinal plants which may be created a new way to treat many incurable diseases. The presence of various bioactive compounds in the *A. indicum* justifies the use of leaves of test plants for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents and subjecting it to the biological activity will definitely give fruitful results. Antifungal activities of crude ethyl acetate extract of *A. indicum* against pathogens.

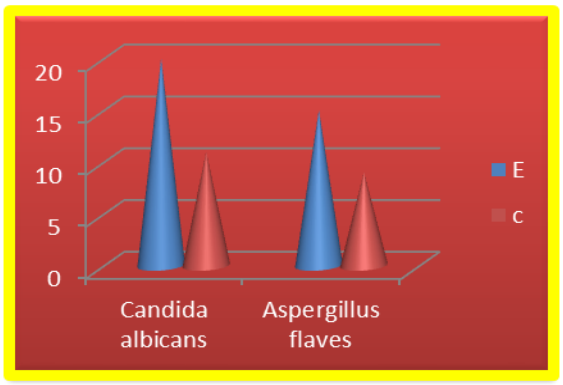


Figure 1: Antifungal activities of crude ethyl acetate extract chloroform extract of A. indicum against pathogens.

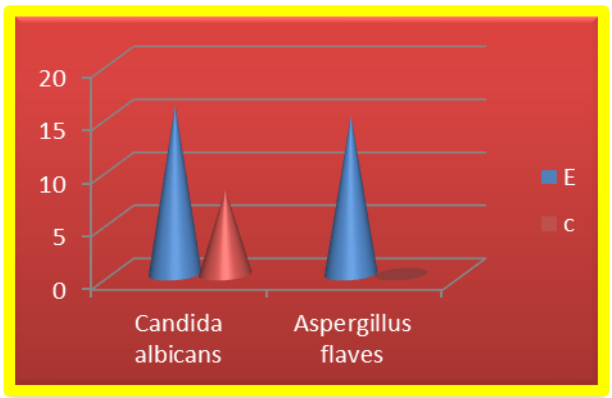


Figure 2: Antifungal activities of crude of *A. indicum* against pathogens.

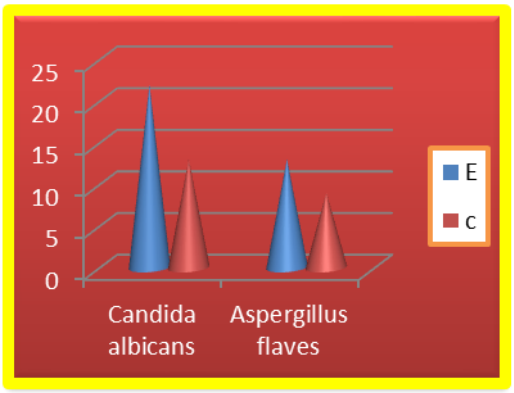


Figure 3: Antifungal activities of crude methonal extract ethyl acetate extract of *M. citrifolia* against pathogens.

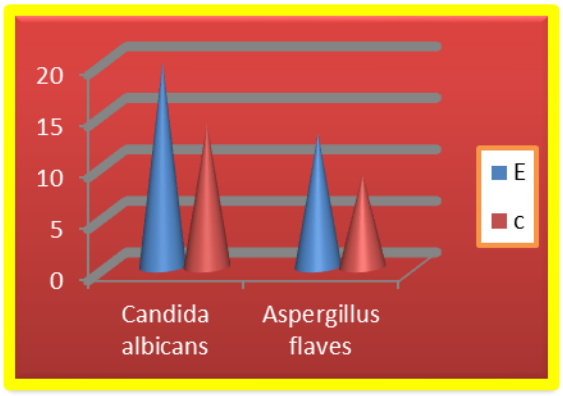


Figure 4: Antifungal activities of crude of *M. citrifolia* against pathogens.

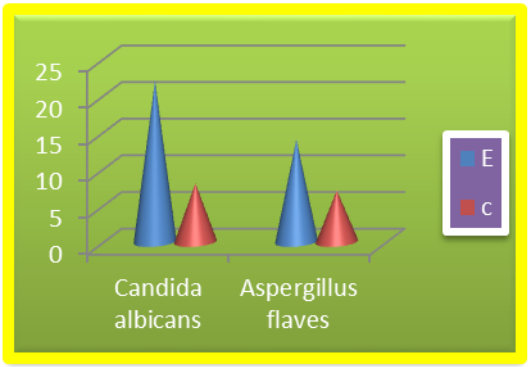


Figure 5: Antifungal activities of crude chloroform extract methanal extract of *M. citrifolia* against pathogens.

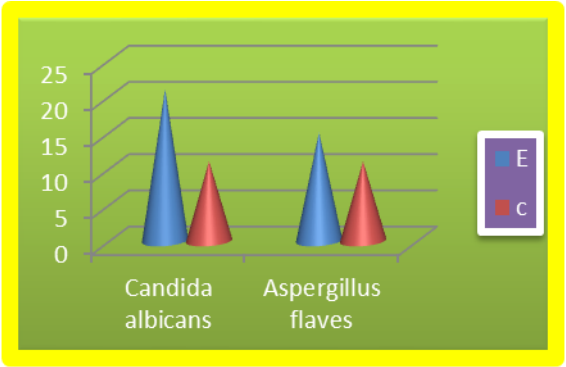
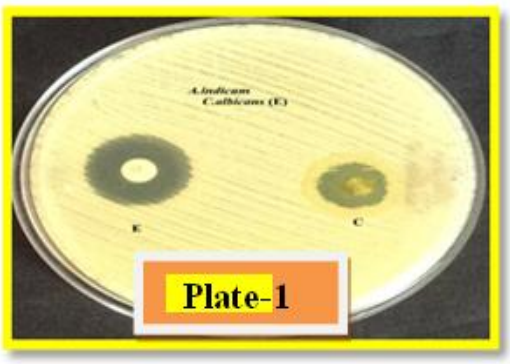
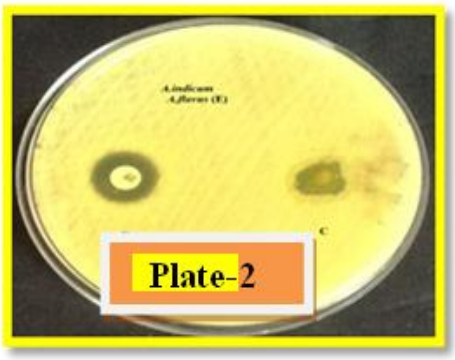


Figure 6: Antifungal activities of crude of *M. citrifolia* against pathogens.

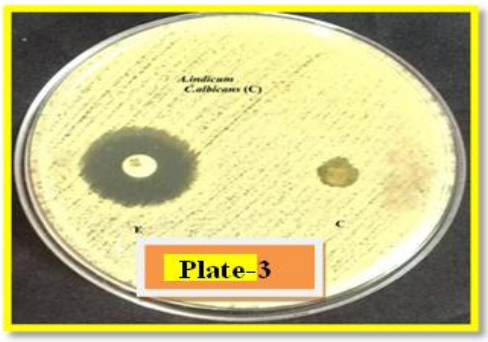
4.1 Antifungal Activities of Crude Ethyl Acetate Extracts of *A. Indicum*

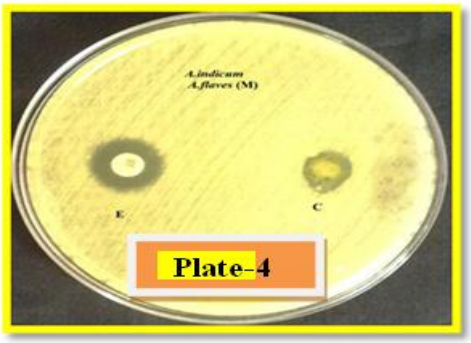
C. albicans ***A. flaves***

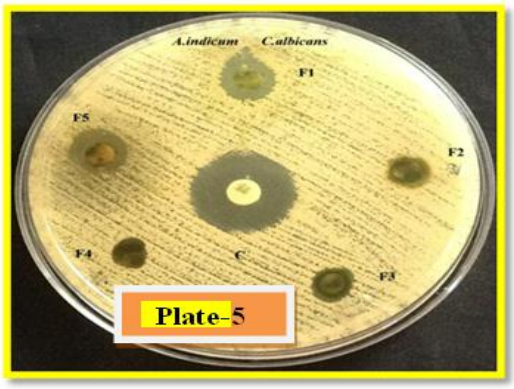


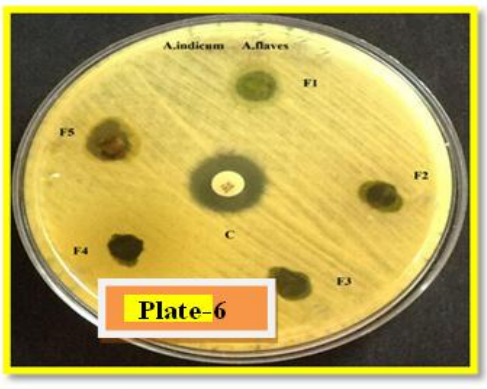


Albicans ***A.flaves***









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