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## ***Antifungal activity of Aegle marmelos, Calotropis procera and Solanum xanthocarpum extract against Aspergillus niger, Candida albicans and Phenerochaete chrysosporium***

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### **ABSTRACT**

*In our experiment three different types of plant species were selected (Aegle marmelos, Solanum xanthocarpum and Calotropis procera) against three different types of fungus (Aspergillus niger, Candida albicans and Phenerochaete chrysosporium) in five different organic solvents (Hexane, Benzene, Methanol, Ethanol, Petroleum ether). Only extract of Aegle marmelos and Solanum xanthocarpum in Hexane showed positive result against fungus Candida albicans and the remaining showed negative result. To screen the antifungal activity of plant, Pour plate and agar well diffusion technique is used. In the present study, if we increase the concentration, zone of inhibition also increases. This shows that in plant extract some compounds are present which have antifungal properties. Maximum growth inhibition was found in 500 ug/ml and minimum growth inhibition found in 100 ug/ml concentration of Hexane extract of S. xanthocarpum and A. marmelos against Candida albicans. As a positive control we used antifungal agent like fluconazole. In quantitative analysis fungal growth is inhibited if the time period is increased. In present study, LC-MS results of plant extract showed the presence of some chemical compounds which are responsible for antifungal property. Aegle marmelos leaves contained  $\gamma$ -sitosterol, aegelin, lupeol, rutin, marmesinin,  $\beta$ -sitosterol, flavone, glycoside, Oisopentenyl halfordiol, and marmeline and Phenylethyl cinnamamide. Solanum xanthocarpum Leaf contains different type of alkaloids (solasodine, solanine) and terpenoids, which are responsible for antifungal activity.*

**Keywords:** Antifungal activity, *Aegle marmelos*, *Aspergillus niger*, *Candida albicans*, *Calotropis procera*, *Phenerochaete chrysosporium*, *Solanum xanthocarpum* and Hexane

### **INTRODUCTION**

Medicinal plants represent a rich source of antimicrobial agent [1]. Many of plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine [2, 3]. Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides, fungicide and many pharmaceutical drugs. Plant products still remain the principal source of pharmaceutical agents used in traditional medicine [4, 5]. The effect of plant extract on fungus has not been much studied and hence requires further research. Much work has been done on ethno medicinal plants in India on bacterial species and little work on fungus species [6, 7].

*Aspergillus niger* is a fungus and one of the most common species of the genus *Aspergillus*. *A. niger* is less likely to cause human disease than some other *Aspergillus* species, but, if large amounts of spores are inhaled, a serious lung disease, aspergillosis can occur. *Candida albicans* is a diploid fungus (a form of yeast) and a causal agent of opportunistic oral and genital infections in humans. *C. albicans* is commensal and is among the gut flora, the many

organisms that live in the human mouth and gastrointestinal tract. Under normal circumstances, *C. albicans* lives in 80% of the human population with no harmful effects, although overgrowth results in candidiasis [8]. *Phanerochaete* is a genus of fungi. Several of the species in this genus are plant pathogens. This genus includes "white-rot" fungi that are able to degrade lignin to carbon dioxide.

Yellow Indian nightshade plant 'Kantkari' (*Solanum xanthocarpum*) is one of the members of the dasamula undertaken to find out the antifungal activity of herbal (ten roots) of the Ayurveda. Fruits are eaten as a drug against some human pathogenic fungus used in Ayurvedic medicine for cough, asthma, chest-pain, flatulence, sore throat and -toothache. The root is a pathogenic to animals, especially mammals. More than important ingredient of the well-known ayurvedic, 25% of the world cereals are contaminated with known medicine, Dasamula. Kant Kari is used in medicine in mycotoxin and more than 300 fungal metabolites are various forms, such as decoction, electuary, ghrita, etc [1]. *Aegle marmelos* (Linn) correa, commonly known as bael (or bel), belonging to the family Rutaceae, is a moderate-sized, slender and aromatic tree [9]. Fresh leaf juice is used in asthmatic complaints and jaundice. Broadly, *Aegle marmelos* leaves contained  $\gamma$ -sitosterol, aegelin, lupeol, rutin, marmesinin,  $\beta$ -sitosterol, favone, glycoside, O-isopentenyl halfordiol, marmeline and phenylethyl cinnamamides [10]. *Calotropis* is a genus of flowering plants in the dogbane family, Apocynaceae. They are commonly known as milkweeds because of the sap they produce. The plant is known as *aak* in Ayurveda and was used in cases of cutaneous diseases, intestinal worms, cough, ascites, asthma, bronchitis, dyspepsia, paralysis, swellings, intermittent fevers, anorexia, inflammations and tumors.

Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides, fungicide and many pharmaceutical drugs. Plant products still remain the principal source of pharmaceutical agents used in traditional medicine [4, 5]. The effect of plant extract on fungus has not been much studied and hence requires further research. There are a huge work has been carried out on ethno medicinal plants in India on bacterial species and little work on fungus species [6, 7]. After these facts were known, the present work was done to investigate the antifungal activity of the leaves of *Aegle marmelos*, *Solanum xanthocarpum* and *Calotropis procera*.

## MATERIALS AND METHODS

**Selected Fungal Species:** *Aspergillus niger*, *Candida albicans* and *Phanerochaete chrysosporium* Lyophilized culture were selected for present experiments.

**Selected Plant Species:** Leaf of *Aegle marmelous* (Beal), *Calotropis procera* (Aak) and *Solanum xanthocarpum* (Kantakari), was selected for test their antifungal activity. It was collected from the Ralamandal forest area of Indore, (M.P.) India.

**Selected Organic Solvent:** Hexane, Benzene, Methanol, Ethanol and Petroleum ether was selected.

### Extract Preparation

**Mother (Distil water) Extract:** Leaf of plant was taken. Wash with distilled water and blot dried kept in sun light for 2-3 days. There are a 5 gm Dry leaf was macerate with distilled water in a blender for 10 minutes to make the paste. It was filter with double layer muslin cloth make up volume up to 50 ml. The extract was centrifuge at 4000 rpm for 15 minute. The supernatant was filter and the prepared mother extract was stored at 4° C.

**Organic Extract:** Leaf sample was taken (washed& dried). Powder was prepared from the sample. Powder was dissolved in organic solvent. Preparation was kept in dark for 48 hours. After filtration supernatant was taken. Transferred to the dark bottle, Organic extract was stored at 4°C in dark bottle.

**Media:** Potato dextrose agar (PDA) and potato dextrose broth (PDB) media were used. PDA was used for well diffusion method and PDB was used for MIC method.

**Preparation of Pure Culture of Fungus:** PDA (500 ml) and PDB (200 ml) was prepared. There are 20 ml PDA was poured into Petri-plates and 5 ml of PDB in test tubes. Lyophilized foam of fungus was added in this plate and test tube. It was incubated for 2 to 3 days at 30°C. The culture plate and tube were stored at 4°C in freezer .we can also make sub culture by repeated this methods.

**Well Diffusion:** The susceptibility of fungus *Aspergillus niger*, *Candida albicans* and *Phanerochaete chrysosporium* Against *Aegle marmelos*, *Calotropis procera* and *Solanum xanthocarpum* was tested by well diffusion method [11].

**Determination of MIC (Maximum Inhibitory Concentration):** MIC was checked in Potato Dextrose Broth. In this method, various concentrations of *Aegle marmelos* and *S.xanthocarpum* extract were tested against *C.albicans* in PDB media. All the test tubes were incubated in a shaking incubator at 30°C. Reading was recorded after 24hr, 48hr and 72hr in spectrophotometer or calorimeter [12].

**LC-MS Analysis of compounds present in plant extracts:** LC-MS analysis was done by method recommended by Jordi Folch [13].

## RESULTS AND DISCUSSION

The antifungal activity of *Aegle marmelos*, *Calotropis procera* and *Solanum xanthocarpum* extract against *Aspergillus niger*, *Candida albicans* and *Phenerochaete chrysosporium* was assessed on the basis of Agar Well-diffusion method in PDA media and Growth inhibition in PDB media with Distil water and organic extract of plant leaf. Result of antifungal activity of *Aegle marmelos*, *Calotropis procera* and *Solanum xanthocarpum* extract against *Aspergillus niger*, *Candida albicans* and *Phenerochaete chrysosporium* are summarized in tables 1- 6 and presented by figures 1 & 2 and graph 1-8.

**Table 1: Screening of plant extracts showing antifungal activity (Agar well diffusion method) for *Aspergillus niger***

S. No.	Name of plant species	Name of fungus species	Solvent used	Conc. Used (in mg/ml)	Volume Used (in µl)	Zone of inhibition (P/A)	Diameter (in mm)
1.	P.C.(fluconazole)	<i>A.niger</i>	Distilled water	25	50	Present	4 mm
2.	N.C.	<i>A.niger</i>	Hexane	pure	50	Absent	None
3.	N.C.	<i>A.niger</i>	Methanol	Pure	50	Absent	None
4.	N.C.	<i>A.niger</i>	Ethanol	Pure	50	Absent	None
5.	N.C.	<i>A.niger</i>	Benzene	Pure	50	Absent	None
6.	N.C.	<i>A.niger</i>	Petro. ether	Pure	50	Absent	None
7.	N.C.	<i>A.niger</i>	Distilled water	Pure	50	Absent	None
8.	<i>S.xanthocarpum</i> (mother extract)	<i>A.niger</i>	Distilled water	100	50	Absent	None
9.	<i>C.procera</i> (mother extract)	<i>A.niger</i>	Distilled water	100	50	Absent	None
10.	<i>A.marmalous</i> (mother extract)	<i>A.niger</i>	Distilled water	100	50	Absent	None
11.	<i>S.xanthocarpum</i>	<i>A.niger</i>	Hexane	100	50	Absent	None
12.	<i>S.xanthocarpum</i>	<i>A.niger</i>	Methanol	100	50	Absent	None
13.	<i>S.xanthocarpum</i>	<i>A.niger</i>	Ethanol	100	50	Absent	None
14.	<i>S.xanthocarpum</i>	<i>A.niger</i>	Benzene	100	50	Absent	None
15.	<i>S.xanthocarpum</i>	<i>A.niger</i>	Petro. ether	100	50	Absent	None
16.	<i>A.marmalous</i>	<i>A.niger</i>	Hexane	100	50	Absent	None
17.	<i>A.marmalous</i>	<i>A.niger</i>	Methanol	100	50	Absent	None
18.	<i>A.marmalous</i>	<i>A.niger</i>	Ethanol	100	50	Absent	None
19.	<i>A.marmalous</i>	<i>A.niger</i>	Benzene	100	50	Absent	None
20.	<i>A.marmalous</i>	<i>A.niger</i>	Petro. ether	100	50	Absent	None
21.	<i>C.procera</i>	<i>A.niger</i>	Hexane	100	50	Absent	None
22.	<i>C.procera</i>	<i>A.niger</i>	Methanol	100	50	Absent	None
23.	<i>C.procera</i>	<i>A.niger</i>	Ethanol	100	50	Absent	None
24.	<i>C.procera</i>	<i>A.niger</i>	Benzene	100	50	Absent	None
25.	<i>C.procera</i>	<i>A.niger</i>	Petro. ether	100	50	Absent	None

P.C.-Positive control, N.C.-Negative control, Conc.-Concentration, Vol.-Volume

**Agar Well-Diffusion Method in PDA Media:** Distil water (Mother) extract of selected plants was not effective against the selected fungal species. The inhibitions zones were not observed in experimental plates like the positive control, which contain fluconazole prepared in distil water. Diameter of zone present in positive control of *Aspergillus niger* is 4 mm in *Candida albicans* is 4.5mm and for *Phenerochaete chrysosporium* 20 mm. The Hexane extract of *Solanum xanthocarpum* and *Aegele marmalous* was effective against *Candida albicans* but not on *Aspergillus niger* and *Phenerochaete chrysosporium*. On comparison, it was observed that the antifungal activity of *Solanum xanthocarpum* and *Aegele marmalous* on *Candida albicans* was present in organic extract of leaf but not in distil water extract of leaf. The inhibition zone of 2 mm was seen in organic (Hexane) extract in *Solanum xanthocarpum* and 3mm for *Aegele marmalous* (Table 1-3)..

**Selection of Plant Extracts Showing Highest Antifungal Activity (Qualitative Analysis):** In Qualitative analysis of antifungal activity of *Solanum xanthocarpum*, on increasing concentration of plant extract, diameter of inhibition zone were also found to be increased and it shows maximum diameter (6 mm) in 500 mg/ml concentration (Table-4).

Table 2: Screening of plant extracts showing antifungal activity (Agar well diffusion method) For *Candida albicans*

S. No.	Name of plant species	Name of fungus species	Solvent used	Conc. Used (in mg/ml)	Volume Used (in µl)	Zone of inhibition (P/A)	Diameter (in mm)
1.	P.C.(fuconazole)	<i>C.albicans</i>	Distil water	25	50	Present	4.5 mm
2.	N.C.	<i>C.albicans</i>	Hexane	pure	50	Absent	None
3.	N.C.	<i>C.albicans</i>	Methanol	Pure	50	Absent	None
4.	N.C.	<i>C.albicans</i>	Ethanol	Pure	50	Absent	None
5.	N.C.	<i>C.albicans</i>	Benzene	Pure	50	Absent	None
6.	N.C.	<i>C.albicans</i>	Petro. ether	Pure	50	Absent	None
7.	N.C.	<i>C.albicans</i>	Distil water	Pure	50	Absent	None
8.	<i>S.xanthocarpum</i> (mother extract)	<i>C.albicans</i>	Distil water	100	50	Absent	None
9.	<i>C.procera</i> (mother extract)	<i>C.albicans</i>	Distil water	100	50	Absent	None
10.	<i>A.marmalous</i> (mother extract)	<i>C.albicans</i>	Distil water	100	50	Absent	None
11.	<i>S.xanthocarpum</i>	<i>C.albicans</i>	Hexane	100	50	Present	2 mm
12.	<i>S.xanthocarpum</i>	<i>C.albicans</i>	Methanol	100	50	Absent	None
13.	<i>S.xanthocarpum</i>	<i>C.albicans</i>	Ethanol	100	50	Absent	None
14.	<i>S.xanthocarpum</i>	<i>C.albicans</i>	Benzene	100	50	Absent	None
15.	<i>S.xanthocarpum</i>	<i>C.albicans</i>	Petro. ether	100	50	Absent	None
16.	<i>A.marmalous</i>	<i>C.albicans</i>	Hexane	100	50	Present	3 mm
17.	<i>A.marmalous</i>	<i>C.albicans</i>	Methanol	100	50	Absent	None
18.	<i>A.marmalous</i>	<i>C.albicans</i>	Ethanol	100	50	Absent	None
19.	<i>A.marmalous</i>	<i>C.albicans</i>	Benzene	100	50	Absent	None
20.	<i>A.marmalous</i>	<i>C.albicans</i>	Petro. ether	100	50	Absent	None
21.	<i>C.procera</i>	<i>C.albicans</i>	Hexane	100	50	Absent	None
22.	<i>C.procera</i>	<i>C.albicans</i>	Methanol	100	50	Absent	None
23.	<i>C.procera</i>	<i>C.albicans</i>	Ethanol	100	50	Absent	None
24.	<i>C.procera</i>	<i>C.albicans</i>	Benzene	100	50	Absent	None
25.	<i>C.procera</i>	<i>C.albicans</i>	Petro. ether	100	50	Absent	None

P.C.-Positive control, N.C.-Negative control, Conc.-Concentration, Vol.-Volume

Table 3: Screening of plant extracts showing antifungal activity (Agar well diffusion method) For *Phanerochaete Chrysosporium*

S. No.	Name of plant species	Name of fungus species	Solvent used	Conc. Used (in mg/ml)	Volume Used (in µl)	Zone of inhibition (P/A)	Diameter (in mm)
1.	P.C.(fuconazole)	<i>P.chrysosporium</i>	Distil water	25	50	Present	20 mm
2.	N.C.	<i>P.chrysosporium</i>	Hexane	pure	50	Absent	None
3.	N.C.	<i>P.chrysosporium</i>	Methanol	Pure	50	Absent	None
4.	N.C.	<i>P.chrysosporium</i>	Ethanol	Pure	50	Absent	None
5.	N.C.	<i>P.chrysosporium</i>	Benzene	Pure	50	Absent	None
6.	N.C.	<i>P.chrysosporium</i>	Petro. ether	Pure	50	Absent	None
7.	N.C.	<i>P.chrysosporium</i>	Distil water	Pure	50	Absent	None
8.	<i>S.xanthocarpum</i> (mother extract)	<i>P.chrysosporium</i>	Distil water	100	50	Absent	None
9.	<i>C.procera</i> (mother extract)	<i>P.chrysosporium</i>	Distil water	100	50	Absent	None
10.	<i>A.marmalous</i> (mother extract)	<i>P.chrysosporium</i>	Distil water	100	50	Absent	None
11.	<i>S.xanthocarpum</i>	<i>P.chrysosporium</i>	Hexane	100	50	Absent	None
12.	<i>S.xanthocarpum</i>	<i>P.chrysosporium</i>	Methanol	100	50	Absent	None
13.	<i>S.xanthocarpum</i>	<i>P.chrysosporium</i>	Ethanol	100	50	Absent	None
14.	<i>S.xanthocarpum</i>	<i>P.chrysosporium</i>	Benzene	100	50	Absent	None
15.	<i>S.xanthocarpum</i>	<i>P.chrysosporium</i>	Petro. ether	100	50	Absent	None
16.	<i>A.marmalous</i>	<i>P.chrysosporium</i>	Hexane	100	50	Absent	None
17.	<i>A.marmalous</i>	<i>P.chrysosporium</i>	Methanol	100	50	Absent	None
18.	<i>A.marmalous</i>	<i>P.chrysosporium</i>	Ethanol	100	50	Absent	None
19.	<i>A.marmalous</i>	<i>P.chrysosporium</i>	Benzene	100	50	Absent	None
20.	<i>A.marmalous</i>	<i>P.chrysosporium</i>	Petro. ether	100	50	Absent	None
21.	<i>C.procera</i>	<i>P.chrysosporium</i>	Hexane	100	50	Absent	None
22.	<i>C.procera</i>	<i>P.chrysosporium</i>	Methanol	100	50	Absent	None
23.	<i>C.procera</i>	<i>P.chrysosporium</i>	Ethanol	100	50	Absent	None
24.	<i>C.procera</i>	<i>P.chrysosporium</i>	Benzene	100	50	Absent	None
25.	<i>C.procera</i>	<i>P.chrysosporium</i>	Petro. ether	100	50	Absent	None

P.C.-Positive control, N.C.-Negative control, Conc.-Concentration, Vol.-Volume

Table 4: Selection of plant extracts showing highest antifungal activity

S. No.	Name of plant species	Name of fungus species	Solvent used	Conc. Used (in mg/ml)	Volume Used (in µl)	Zone of inhibition (P/A)	Diameter (in mm)
1.	P.C.(fuconazole)	<i>C.albicans</i>	Distil water	25	50 µl	Present	5 mm
2.	N.C.	<i>C.albicans</i>	Hexane	Pure	50 µl	Absent	None
3.	<i>S.xanthocarpum</i>	<i>C.albicans</i>	Hexane	100	50 µl	Present	2.5 mm
4.	<i>S.xanthocarpum</i>	<i>C.albicans</i>	Hexane	200	50 µl	Present	3 mm
5.	<i>S.xanthocarpum</i>	<i>C.albicans</i>	Hexane	300	50 µl	Present	4 mm
6.	<i>S.xanthocarpum</i>	<i>C.albicans</i>	Hexane	400	50 µl	Present	4.5 mm
7.	<i>S.xanthocarpum</i>	<i>C.albicans</i>	Hexane	500	50 µl	Present	6 mm
8.	<i>A.marmalous</i>	<i>C.albicans</i>	Hexane	100	50 µl	Present	3 mm
9.	<i>A.marmalous</i>	<i>C.albicans</i>	Hexane	200	50 µl	Present	4 mm
10.	<i>A.marmalous</i>	<i>C.albicans</i>	Hexane	300	50 µl	Present	4.5 mm
11.	<i>A.marmalous</i>	<i>C.albicans</i>	Hexane	400	50 µl	Present	5 mm
12.	<i>A.marmalous</i>	<i>C.albicans</i>	Hexane	500	50 µl	Present	6 mm

P.C.-Positive control, N.C.-Negative control, Conc.-Concentration, Vol.-Volume

Table 5: Growth inhibition in different concentration of extract in Potato Dextrose broth

S.No.	Sample	Concentration of plant extract (mg/ml)	O.D. (in 348 nm)		
			24 hours	48 hours	72 hours
1.	Blank (control)	Media only	00	00	00
2.	P.C.	Media & strain ( <i>C.albicans</i> )	0.876	0.811	0.600
3.	N.C.	Media,A.A.(Fuconazole)& <i>C.albicans</i>	0.136	0.266	0.375
4.	<i>S.xanthocarpum</i> (Hexane)	100	0.701	0.808	0.595
5.	<i>S.xanthocarpum</i> (Hexane)	200	0.401	0.506	0.468
6.	<i>S.xanthocarpum</i> (Hexane)	300	0.334	0.504	0.468
7.	<i>S.xanthocarpum</i> (Hexane)	400	0.276	0.327	0.287
8.	<i>S.xanthocarpum</i> (Hexane)	500	0.179	0.202	0.101
9.	<i>A.marmalous</i> (Hexane)	100	0.702	0.511	0.382
10.	<i>A.marmalous</i> (Hexane)	200	0.481	0.414	0.234
11.	<i>A.marmalous</i> (Hexane)	300	0.334	0.334	0.234
12.	<i>A.marmalous</i> (Hexane)	400	0.276	0.413	0.301
13.	<i>A.marmalous</i> (Hexane)	500	0.179	0.333	0.176

P.C.-Positive control, N.C.-Negative control, Conc.-Concentration, Vol.-Volume, A.A. - Antifungal agent

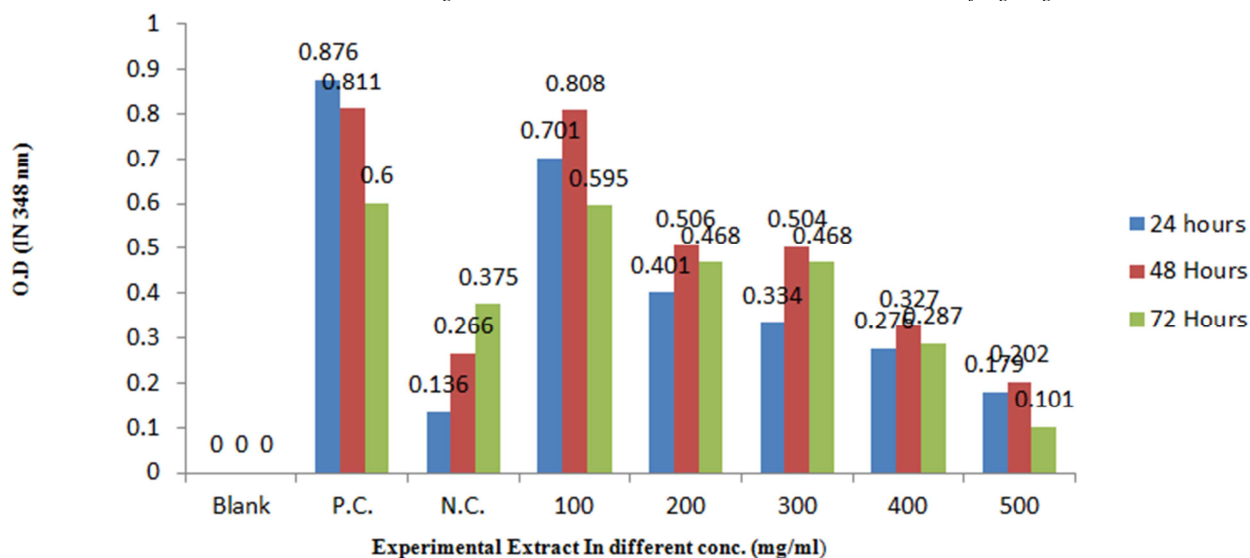
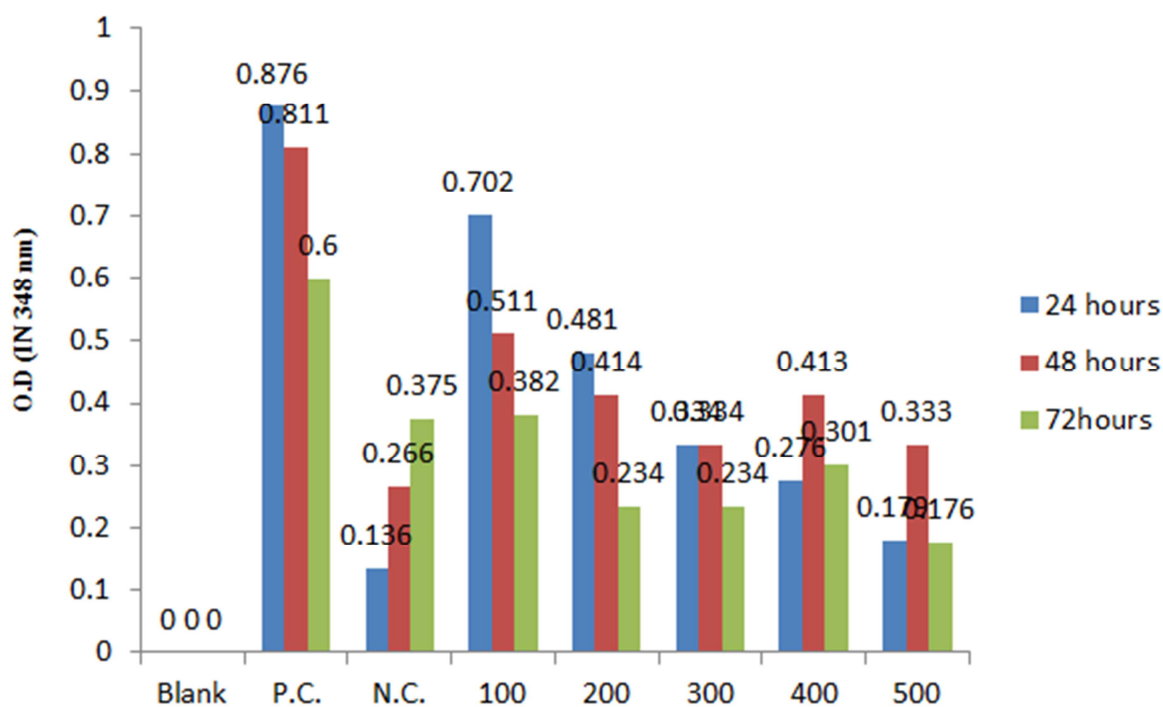


Fig1. Graphical representation of antifungal activity of *Solanum xanthocarpum* extract on *Candida albicans* in PDB Media  
P.C.-Positive control, N.C.-Negative control, Conc. – Concentration



Experimental Extract In different conc. (mg/ml)

Fig2. Graphical representation of antifungal activity of *Agle marmelos* extract on *Candida albicans* in PDB Media  
P.C.-Positive control, N.C.-Negative control, Conc. – Concentration

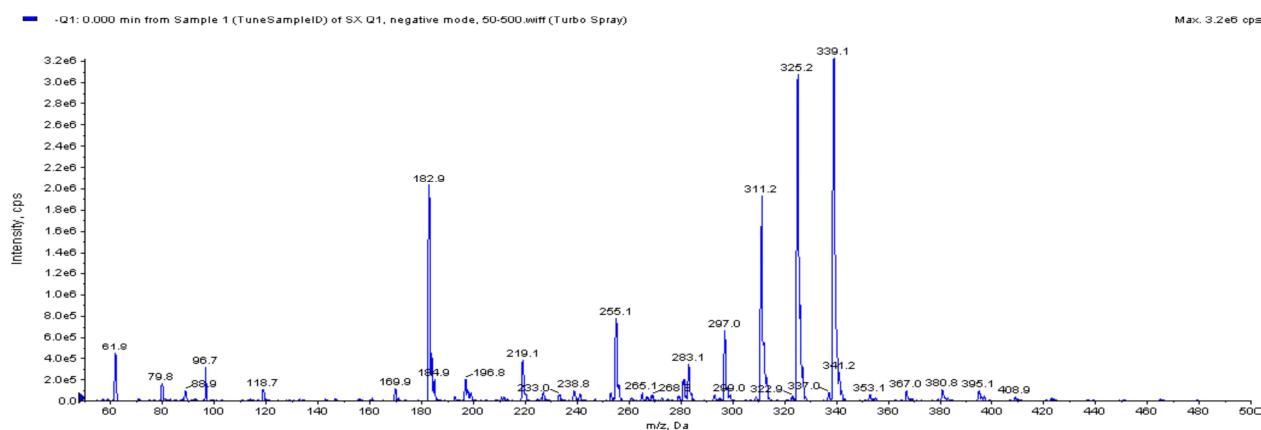
Table 6: Analysis of Antifungal compounds present in plant extracts by LC-MS/MS

S. No.	Name of compounds	Molecular formula	Molecular weight	(m/z) observed	Component comments
1.	Solasodine(S.X.)	C <sub>27</sub> H <sub>43</sub> NO <sub>2</sub>	413.6	413.3	Major pick
2.	Carpesterol(S.X.)	C <sub>37</sub> H <sub>54</sub> O <sub>4</sub>	562.82	564.4	Major pick
3.	B-sitosterol(S.X.)	C <sub>29</sub> H <sub>50</sub> O	414.7	413.4	Minor pick
4.	Diosgenin(S.X.)	C <sub>27</sub> H <sub>42</sub> O <sub>3</sub>	414.61	414.4	Minor pick
5.	Campesterol (S.X.)	C <sub>28</sub> H <sub>48</sub> O	400.68	401.8	Minor pick
6.	γ- sitosterol(A.M.)	C <sub>29</sub> H <sub>50</sub> O	414.70	413.3	Major pick
7.	Phenylethyl cinnamamide(A.M.)	C <sub>15</sub> H <sub>12</sub> BrNO	302.17	301.2	Major pick
8.	Aegelin(A.M.)	C <sub>18</sub> H <sub>19</sub> NO <sub>3</sub>	297.35	297.2	Major pick
9.	Marmesinin(A.M.)	C <sub>20</sub> H <sub>24</sub> O <sub>9</sub>	408.41	409	Minor pick

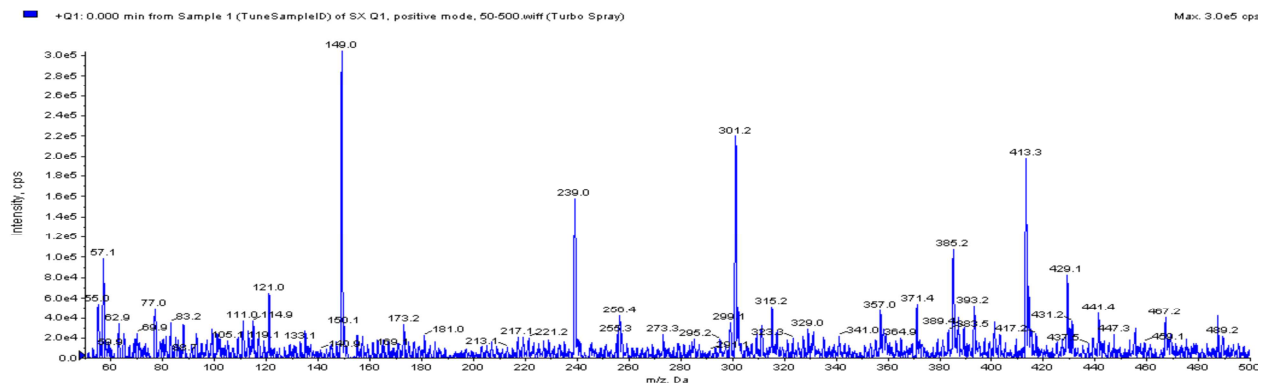
S.X.-*Solanum xanthocarpum*, A.M.- *Aegle marmalouus*

Mass data for bioactive compounds present in *Solanum xanthocarpum* leaves in Hexane extract are as follows:-

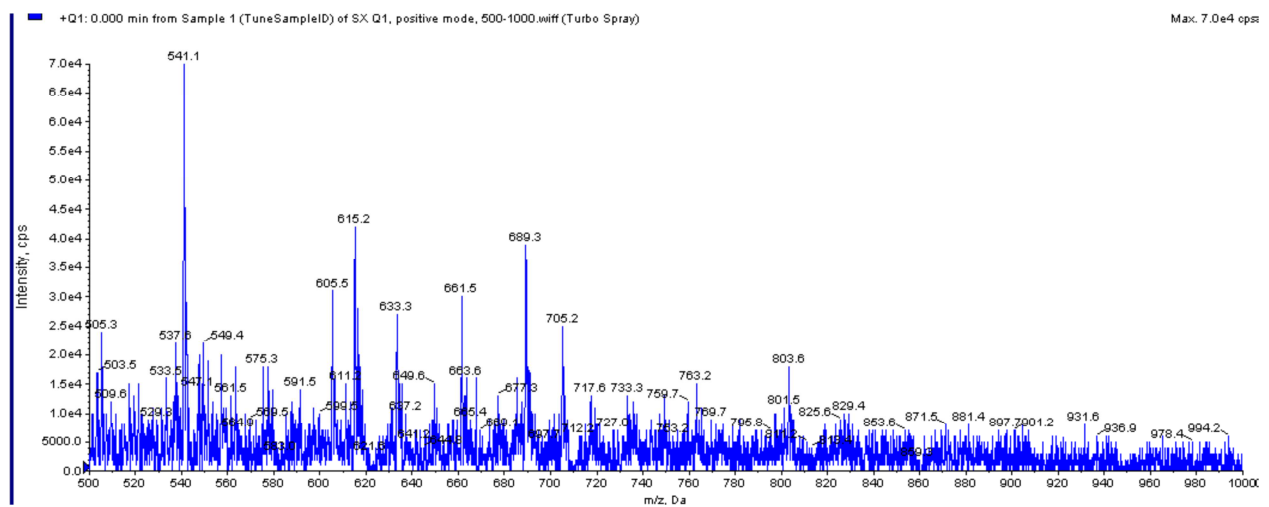
Graph 1: LC/MS of Leaf extract of *S.xanthocarpum* in Hexane (negative mode, 50-500 m/z Da)



**Graph2: LC/MS Leaf extract of *S.xanthocarpum* in Hexane (positive mode, 50-500 m/z Da)**

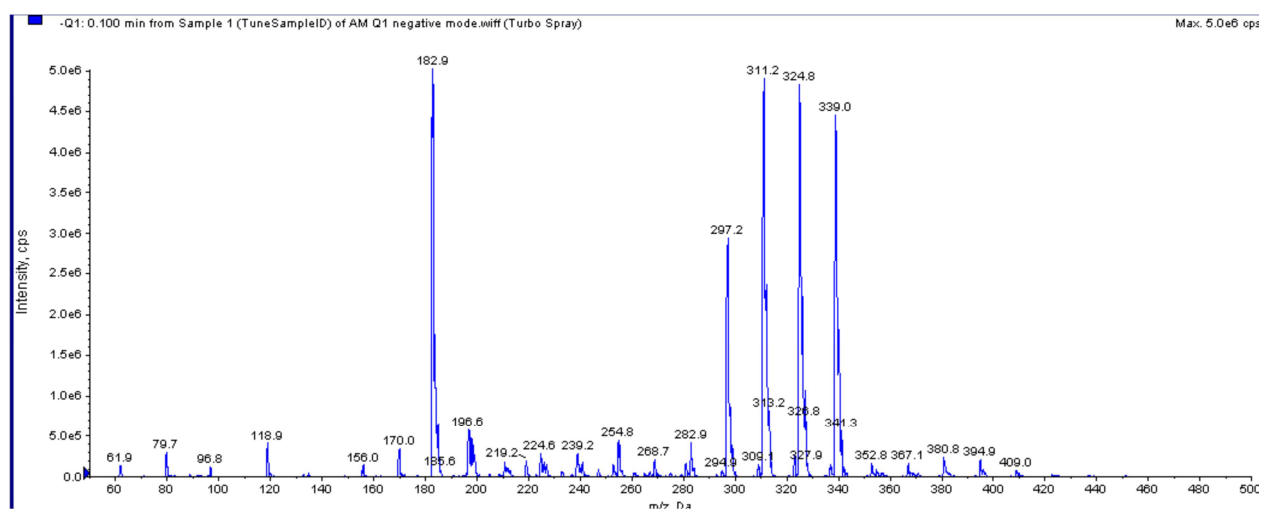


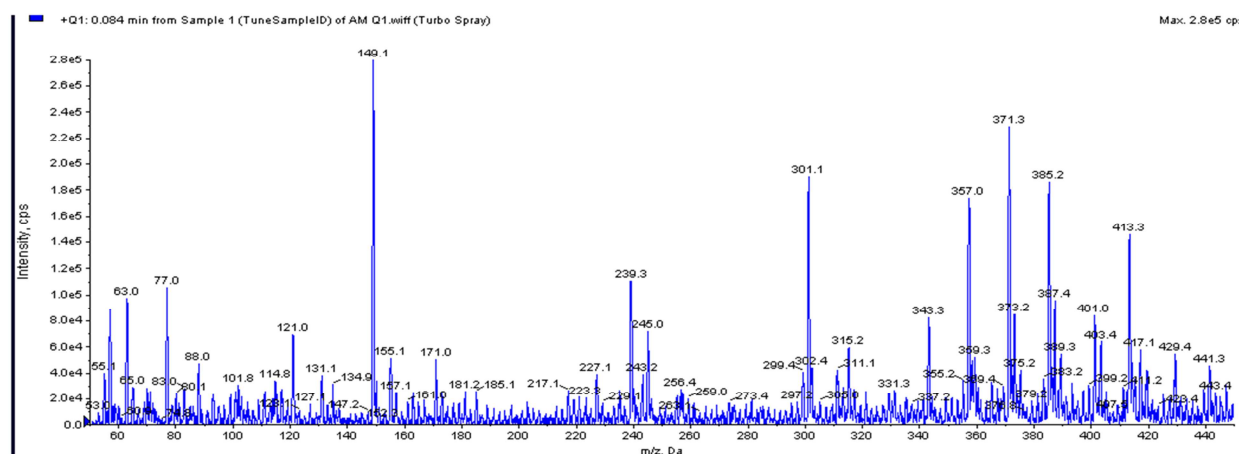
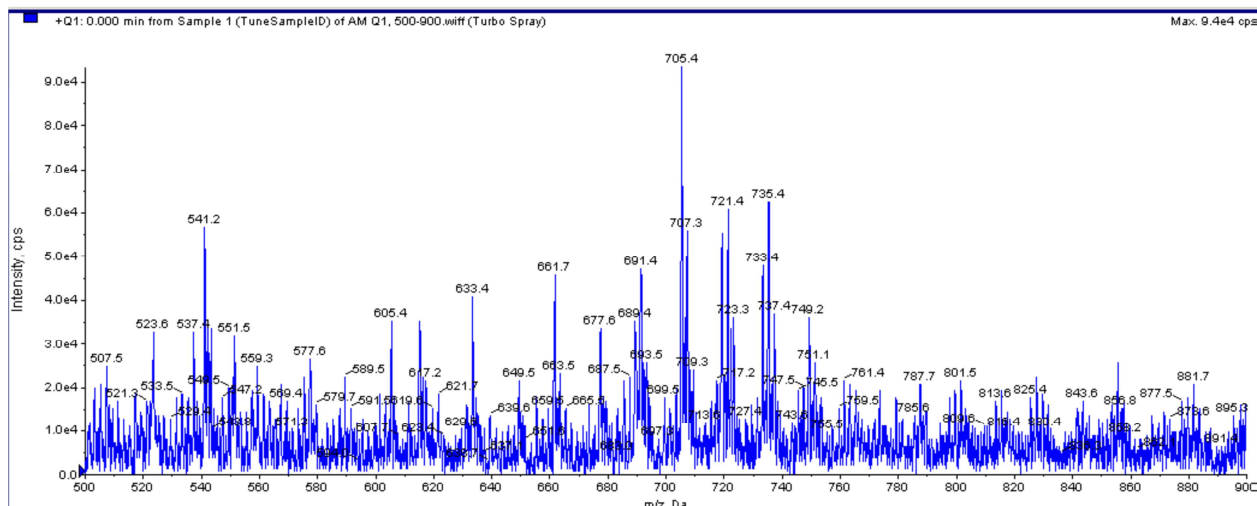
**Graph 3: LC/MS Leaf extract of *S.xanthocarpum* in Hexane (positive mode, 500-1000 m/z Da)**



Mass data for bioactive compounds present in *Aegle marmalous* leaves in Hexane extract are as follows:-

**Graph 4: LC/MS Leaf extract of *A.marmalous* in Hexane (Negative mode, 50-500 m/z Da)**



Graph 5: LC/MS Leaf extract of *A.marmalous* in Hexane (positive mode, 50-500 m/z Da)Graph 6: LC/MS Leaf extract of *A.marmalous* in Hexane (positive mode, 500-900 m/z Da)

**Growth Inhibition in Potato Dextrose Broth Media Method:** *A. marmalous* and *S. xanthocarpum* extracted with Hexane is only effective against the *C.albicans*. So in broth media Hexane extract of plant is used to check the antifungal activity. More concentrated extract of *A. marmalous* and *S. xanthocarpum* is more effective against *C.albicans*. Extract is more effective in 500 mg/ml and less effective in 100 mg/ml conc. The O.D. for 24, 48 and 72 hrs respectively were estimated for different concentration of extract has been summarized in Table-5.

*Aegle marmalous* and *S. xanthocarpum* is an important medicinal herb in Ayurvedic medicine having different phytochemical and pharmacological activities leaf extract shows Antifungal activity on dermatophytes. *Aegle marmelos* leaf extracts significantly inhibited the growth of all dermatophytic fungi studied. A number of chemical constituents and various therapeutic effects of *A. marmelos* have been reported by different workers. Extensive investigations have been carried out on different parts of *A.marmelos* and as a consequence, varied classes of compounds Coumarins (Marmelosin, marmesin, imperatorin), alkaloids (Aeglin, aegelenine), Tannins (skimmianine), Carotenoids [14]. MS analysis of Bael (*Aegle marmelos* is an Indian medicinal plant; which has enormous traditional values against various diseases and many bioactive compounds have been isolated from this plant) contain Skimmianine, Aegeline, Lupeol, Cineol, Citral, Citronella, Cuminaldehyde, Eugenol, and Marmesinin. Various traditional claims like immunomodulation, anti-inflammatory, antiallergic, antianaphylactic and antitumor effects of the plant still remain to be validated scientifically [15].

Various studies indicated that *Solanum xanthocarpum* possesses antiasthmatic, hypoglycemic hepatoprotective, antibacterial and insect repellent properties [16, 17]. It has been reported that *Solanum nigrum* is a rich source of one of plants most dreaded toxins solanine and its potential has been demonstrated as a reservoir of antioxidants having hepatoprotective, anti-tumor, cytostatic, anti-convulsant anti-ulcerogenic, antifungal and anti-inflammatory effects [16]. LC-MS results of plant extract showed the presence of some chemical compounds which are responsible for



antifungal property. *Aegle marmelos* leaves contained  $\gamma$ -sitosterol, aegelin, lupeol, rutin, marmesinin,  $\beta$ -sitosterol, flavone, glycoside, Oisopentenyl halfordiol, marmeline and Phenylethyl cinnamamide. *Solanum xanthocarpum* Leaf contains different type of alkaloids (solasodine, solanine) and terpenoids, which are responsible for antifungal activity.

The finding of our study indicates that *Aegle marmelos* and *S. xanthocarpum* has got intense antifungal activity. Hexane extract of *Aegle marmelos* and *S. xanthocarpum* leaf showed good antifungal activity against the *Candida albicans* in solid and broth media. Thus, the results of present investigation support the view of above mentioned authors.

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