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Qualitative Phytochemical Screening And *In Vitro* Antifungal Activity Of *Sterculia Urens* Roxb. Leaf Extracts From Narayanpur, Bastar, Chhattisgarh

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ABSTRACT

In the present investigation qualitative phytochemical screening and *in vitro* antifungal activity of petroleum ether, chloroform, acetone and aqueous leaf extracts of *Sterculia urens* Roxb. against six pathogenic fungal strains were compared against commonly used antifungal agent's Amphotericin-B antibiotics. Antifungal activity tests were performed by using poison food technique and agar well diffusion method on potato dextrose agar medium (PDA). Results of the present research work showed that poison food technique showed strong antifungal activity then agar well diffusion method. This might be due to better contact with the media. Antifungal activity of the different solvent plant extracts is directly proportional to the increase in concentration (100µl to 1000µl and 25% to 100%). A consistent increased and better growth inhibition in chloroform in *C. oxysporum* compared to antibiotics though statistically non-significant was examined. A significantly higher mean of GI % was reported for *C. oxysporum* (87.96 ± 1.85) in chloroform extract at 1000µl concentration, which did not statistically significantly differ from GI % of Amphotericin- B antibiotics (82.18 ± 1.51) for all six fungal strains. However, lowest growth inhibition % was observed for *A. niger* (20.89 ± 6.94) in aqueous extracts. In agar well diffusion method maximum zone of inhibition was exhibited against *C. albicans* (17.67 ± 1.45 mm) in acetone extract at 100% concentration. Qualitative phytochemical screening confirmed the presence of alkaloid, phenols, reducing sugar, saponin, tannins, terpenoids, flavonoids, anthraquinone, fixed oils and fats in different solvents. *S. urens* Roxb. have great medicinal importance and as it contains a number of bioactive compounds like phenols, flavonoids, saponins, tannins, terpenoids which possess antifungal activity. It can be used as an eco-friendly mode of disease treatment and can serve as a source of new antifungal agent with no or less side effects.

Keywords: Phytochemical, pathogenic fungal strain, antifungal activity, growth inhibition percentage (%), zone of inhibition, SPSS analysis, ANOVA.

INTRODUCTION

Bioactive chemicals obtained from natural sources like plants could be a potential source of antifungal, particularly in the present scenario in which human, animals, and plant parasitic pathogens have embraced resistance against antifungal agents (Krishna et al. 2008). The screening of several medicinal plants for their potential antimicrobial activity is rising because of the failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents (Rojas et al. 2003).

Sterculia urens Roxb. belongs to the family Sterculiaceae and subfamily Sterculioideae is commonly known as 'gum karaya tree' and often referred to as the chestnut family is a tree, shrubs or herbs found throughout the world. Sterculiaceae is a large family consisting of about 72 genera and 1500 species distributed mainly in tropical and subtropical regions. It is native to India and was introduced to Burma. In India, Sterculia species are found in tropical Himalayas, all the way through Eastern and Western Ghats, West and Central India, Rajasthan, Andhra Pradesh, Karnataka, Maharashtra, Gujarat, Odisha, Assam, Bihar, Uttar Pradesh, Madhya Pradesh, and Southwards to Western Peninsula (Khare 2007; Kumar 2016; Sukhadiya et al. 2019). In India its status of threat is critically endangered (Jadhav et al. 2001). *S. urens* Roxb. are also found in Chhattisgarh states (Khanna et al. 2005) and is facing the status of threat as vulnerable (http://www.envis.frlht.org). Its flowering season is during the month of December to January while the fruiting season is from April to June. *S. urens* Roxb. useful parts are stems, barks, leaves, and roots (Deshpande and Bhalsing 2015; Shukla and Modi 2020). Medicinally, leaves are used for activating parturition and source of vitamin A, pleuro-pneumonia in cattle, bark gum is used to treat lozenges, emulsions, lotions, sprays and pastes, throat infections and juice of bark is used to treat piles (Tandon and Sharma 2009). Gum is used to improve stamina, suppress the phenol content in the body, dysentery, cooling effect, constipation, diarrhoea. Gum is used as a substitute for tragacanth in throat affections. Stem bark is used to lessen pains during delivery, rheumatism, peptic ulcers, spermatorrhoea, leucorrhoea (Khanum and Khan 2005).

Pharmacologically, it has been reported that *S. urens* Roxb. is widely used in traditional medicine to fight and treat numerous illnesses due to its antimicrobial, antibacterial, antioxidant, anti-cancer, anti-inflammatory, anti-diabetic, antispasmodic, analgesic, and diuretic properties (Gritto et al. 2015; Shukla and Modi 2020).

Chemical Constituents

S. urens Roxb. leaves contains flavonol glycosides, phytol, sucrose, beta-amyrin, beta-sitosterol and an ester of terephthalic acid, ascorbic acid, tannin stercurens, quercetin, Galactose, rhamnose galacturonic, glucuronic acids, flavonol glycosides, xylose, acetyl derivatives, kaemperol derivatives, stercurensin, C-

methylchalcone,3,7,11,15-Tetramethyl-2-hexadecen-1-ol. Gum and mucilage contain aldobiuronic and aldotriouronic acids. Roots contains coumarin and scopoletin (Khatoon et al. 1989; Kumbhare and Bhargava 1999; Brito et al. 2004; Khan and Khanum 2005).

2. MATERIALS AND METHODS

2.1. Collection of plant sample

Mature green and disease free leaves of *S. urens* Roxb. were collected from Herbal Garden, Bakhrupara Kasthagar and tropical dry deciduous forest area of Narayanpur District during their flowering seasons (April - June). Geographically Narayanpur is situated in 19°13'41"- 19°56'46" N Longitude and 80°39'51"- 81°30'57" E latitude. Plants were identified by Vaidraj Ratan Dhar Department of Naturopathy and Yogic Science (Govt. Regd. No - MAH/394/06/THA).

2.2. Sequential Soxhlet extraction of plant material

Fresh and disease free leaves of *S. urens* Roxb. were washed 2-3 times with running tap water and rinsed once with distilled water. Air dried under shade at room temperature for 5-7 days till leaves become brittle enough to break easily. After complete drying, the leaves were crushed to fine powder using an electrical grinder and transferred into air tight poly bags and stored at room temperature. Extraction was carried out by Soxhlet extraction (hot continuous process) procedure as described by Hozowitz, 1984. About 25gm of powdered plant material was packed in manually prepared Whatman filter paper Grade 1 and placed in the thimble chamber of Soxhlet. In the round bottom flask of Soxhlet was taken 250 ml of different solvent *viz.,* aqueous, acetone, chloroform and petroleum ether respectively. During the cycling, the process continues until the liquid droplet from the Siphon arm does not leave any residues and becomes colorless. The extract was obtained and then filtered through Whatman filter paper Grade 1 (Yadav and Agrawala 2011). After filtration, the solvent was removed by evaporations in a water bath, which gave rise to a solid mass of the extract referred to as the crude extract. Further the crude extract was stored in labeled sterile brown bottles and eppendorf tubes and kept in the refrigerator at 4°C for further analysis (Venkatachalam and Muthukrishnan 2012).

2.3. Phytochemical screening analysis of plant extract

Qualitative phytochemical screening was based on the standard procedures described by Harborne (1973), Trease and Evans (1989), and Sofowora (1993). Preliminary Phytochemical screening was performed for characterization of different bioactive compounds present in *S. urens* Roxb.. **Alkaloids (Mayer's test):** 6 ml of extract was mixed with 6 ml 1% HCl in stream both and then it was filtered. 1 ml of Mayer's reagent was added. Presence of turbidity shows presence of alkaloids. Addition of a few drops of olive oil to form an

emulsion confirmed the presence of alkaloids. **Phenols** (**Ferric chloride test):** 2 ml of the crude extract was added to 4 ml distilled water then a few drops of 10% ferric chloride were added. Appearance of blue or green color will indicate the presence of phenols. **Reducing sugar** (**Fehling's test):** 1 gm of the extract was dissolved in 10 ml distilled water. Extracts were boiled with Fehling solution A and B in test tube color changes were observed. Presence of brick red color indicated the presence of reducing sugar. **Saponins (Froth test):** 0.5 gm of the extract was dissolved in 5 ml distilled water. The mixture was shaken vigorously. Formation of stable persistent forth shows the presence of saponins. Further addition of 6 drops of olive oil while shaking forms an emulsion, confirming the presence of saponins. **Tannins** (**Ferric chloride test):** 0.5 gm of the extract was dissolved in 10 ml of distilled water, a few drops of 1% ferric chloride solution was added to obtain a brownish green or blue black precipitate confirming the presence of tannins. **Terpenoids** (**Salkowski's test):** 0.5 gm extract was dissolved in 2 ml of chloroform then 3 ml concentrated sulfuric acid was added. A reddish brown color in the inter phase shows the presence of terpenoids. **Flavonoids** (**Ammonia reduction test):** 5 ml dilute ammonia was added into 5 ml extract then 5 ml concentrated sulfuric acid was added. Formation of yellow color shows the presence of flavonoids. **Fixed oil and fats** (**Filter paper test or stain test):** Small quantity of the extracts was pressed between two filter papers. Appearance of oil stain on first filter paper indicates the presence of fixed oils and fats. **Cardiac glycosides (Keller-killiani test):** 2.5 gm of extract was added to 2.5 ml distilled water 1 ml glacial acetic acid containing a few drops of ferric chloride was added then 0.5 ml of concentrated sulfuric acid was added. Presence of brown ring at the inter phase indicates the presence of deoxy sugar. A violet ring below the brown ring was observed, while a greenish ring also appears above the brown ring confirms the presence of cardiac glycosides. **Anthraquinone** (**Borntrager's test):** 2.5 gm extract was dissolved in 5 ml of concentrated sulfuric acid and filtered. The filtrate was dissolved in 2.5 ml of chloroform. Chloroform layer was pipette into a tube and 0.5 ml of 10% diluted ammonia was added. Formation of pink red or violet color shows the presence of anthraquinone.

2.4. Fungal strains

Fungal strains *Microsporum gypseum* (MTCC NO 4524), *Trichoderma viride* (MTCC NO 793), *Aspergillus niger* (MTCC NO 281) were purchased from Microbial type cell culture collection and Gene Bank (MTCC), Institute of microbial technology (IMTECH) Chandigarh. Also, cultures *Curvularia lunata* (NCFT), *Cladosporium oxysporum* (NCFT) were obtained from National Centre of Fungal Taxonomy (NCFT) New Delhi and *Candida albicans* from Govt. Bhim Rao Ambedkar Hospital Raipur (C.G.).

2.5. ANTIFUNGAL ACTIVITY SUSCEPTIBILITY TEST

2.5.1. Poison food technique

Antifungal activity tests against pathogenic fungal strains were determined by Poisoned food technique using aqueous, acetone, chloroform, and petroleum ether solvents (Grover and Moore 1962; Nene and Thapliyal

2000). Potato dextrose agar (PDA) media was used as a culture medium. In 15 ml of cooled (45°C) molten PDA solid medium, different concentrations (100µl, 250µl, 500µl and 1000µl) were mixed and poured into the sterile petri plates and allowed to solidify at room temperature for thirty minutes. 5-7 days old fungal culture is punched aseptically with a sterile cork borer of generally 5 mm diameter. The fungal discs are then transferred aseptically at the center of each plate. PDA without extracts serves as negative control. Antibiotics Amphotericin- B was used as positive control (McCutcheon et al. 1994). Then, all inoculated petri plates were incubated at temperature 26 ± 1°C for fungi. After 72 hours, the radial mycelia growth was measured (in millimeters). Triplicates were maintained and the antifungal activity of each extract was calculated in terms of growth percentage inhibition of mycelia growth by using the following formula:

Growth % inhibition = (dc - dt) / dc x 100

Where, dc is the average increase in mycelia growth in control and dt is the average increase in mycelia growth in treated (Satish et al. 2007).

2.5.2. Agar Well Diffusion Method

Antifungal susceptibility activity was screened by agar well diffusion method (Perez et al. 1990). Potato Dextrose Agar (PDA) plates were swabbed (sterile cotton swabs) with 4 hours old-broth culture of respective fungi. Using sterile cork borer four wells of 5 mm diameter were prepared in the agar plates. Stock solution of the plant extract was prepared at a concentration of 4mg/ml in different solvent *viz.*, acetone, petroleum ether, chloroform and aqueous. About 20µl of different concentrations (25%, 50%, 75% and 100%) of solvent crude extract was introduced into the bored agar well using a micropipette and allowed to diffuse at room temperature for 2 hrs. Sterilized discs 5 mm prepared from Whatman filter paper Grade 1 were impregnated with 20µl pure solvents (negative control) and standard antibiotics Amphotericin- B were used as a positive control. Then plates were sealed and incubated at 26 ± 1°C for 72 hours. Triplicates were maintained and the diameter of the inhibition zone (mm) was measured which appears as a clear area around the wells (Cheesbrough 2006).

STATISTICAL ANALYSIS

All data values were expressed as mean ± standard error (SE) of triplicate measurements. Statistical analyses of the data were performed using the SPSS version 16.0 software, SPSS Inc (statistical package for Windows). The effect of individual factors (fungus (F), solvent (S) and the concentration (C)), and their interactions (F X S, F X C, S X C, F X S X C) on the studied variables (Growth inhibition percent (Poison food technique) and inhibition zone (mm) (Well diffusion method)) were observed through three-way Analysis of variance 3-way ANOVA. For evaluating the individual difference among treatments the Duncan's

multiple range Post hoc test was employed. All the tests were done at 5% level of significance ($p<0.05$) and carried out in arcsine log transformed data of growth inhibition percent in case of Poison food technique.

3. RESULT AND DISCUSSION

Results summarized in (**Table 1**). Qualitative phytochemical screening of leaves of ***S. urens* Roxb.** revealed the presence of various phytocompounds i.e., alkaloids, phenols, reducing sugar, saponins, tannins, terpenoids, flavonoids, fixed oils and fats, anthraquinones in one or the other solvent extracts. Whereas, cardiac glycosides were found to be absent in all four solvent extracts.

Table 1. Preliminary phytochemical screening of crude extract of *S. urens* Roxb.

Chemical constituents	Test Performed	Observation	Inference			
			AQ	AC	CF	PE
Alkaloids	Mayer's test	Presence of turbidity	+	+	+	+
Phenols	Ferric chloride test	Bluish green color	+	+	+	+
Reducing sugar	Fehling's test	Brick red color	++	++	++	-
Saponins	Froth test	Frothing persist 15 mins	+	+	+	+
Tannins	Ferric chloride test	Dark blue or greenish grey Cream ppt	-	++	-	-
Terpenoids	Salkowski's test	Bluish-green color at interphase reddish color	+	++	-	-
Flavonoids	Ammonia reduction test	Yellow color	+	+	+	++
Fixed oils	Filter paper test	Oil stain develops	+	++	++	+
Cardiac glycosides	Keller- Killani test	Violet ring below brown ring interphase	-	-	-	-
Anthraquinones	Borntrager's test	Deep red or Pink color of aqueous layer	++	-	-	-

AQ; aqueous, AC; acetone, CF; chloroform, PE; petroleum ether extracts; '**-**' indicates absence.

'+++' relatively a strong presence; '++' relatively moderate presence; '+' relatively low presence.

The present investigation revealed that in poison food technique the highest antifungal activity was exhibited by the chloroform extract followed by acetone, petroleum ether and least in the aqueous extract for *S. urens* Roxb.. Antifungal activity of plant extract in different solvents is directly proportional to the increase in concentration of low concentration (100µl) was weaker compared to high concentration (1000µl) of crude extracts.

The three-way ANOVA showed a significance independent effect of factors fungal strain, solvent and concentration on growth inhibition percentage (GI %) in *S. urens* Roxb..

The interaction effect of **fungal strain** and **solvent** was not found to be significance (p=1; **Table 2**). The DMRT post hoc test showed a significantly higher mean of GI % in was reported for *C. oxysporum* (87.96 ± 1.85) in chloroform extract at 1000µl concentration, which did not statistically significantly differ from GI % of Amphotericin- B antibiotics (82.18 ± 1.51) in all six fungal strains, *C. lunata* and *M. gypseum* in aqueous, *C. albicans*, *C. lunata*, *M. gypseum*, *C. oxysporum*, *T. viride*, and *A. niger* in acetone, *C. lunata*, *M. gypseum*, and *T. viride* in chloroform, *C. lunata*, *M. gypseum*, and *T. viride* in petroleum ether. However, lowest growth inhibition % was observed for *A. niger* in aqueous extracts. Rest of the treatments did not vary significantly with each other. Results summarized in (**Table 3)** and **Photoplate no. 1**. Similar studies using Poison food techniques work were performed by Adegbite Afeez et al. (2020) reported that the leaf extract of *P. zeylanica* showed highest antifungal activity against *C. oxysporum*, *L. theobromae*, and *A. flavus*. Marchi et al. (2019) aqueous extract *C. longa* L. against *Cladosporium oxysporum*, *C. subliforme*, *P. panemum*, and *P. citrinum*, Haddouchi et al. (2013) *Ruta angustifolia*, *R. chalepensis*, *R. graveolens*, and *R. tuberculata* against *Cladosporium herbarum* and *A. fumigates*. Gherib et al. (2014) *W. saharae* Benth. & Hook against *Cladosporium herbarum*, *F. oxysporum*, and *A. flavus*. Prashith Kekuda and Vinayaka (2017) *H. arborea* and *H. pentandra* against *Cladosporium* sp., *A. niger*, *A. flavus*, *A. fumigates*, *Penicillium* sp., *Helminthosporium* sp. and *Rhizopus* sp., Yehia et al. (2020) *Z. mandavillei* exhibited antifungal activity against *C. cladosporioides*.

In agar well diffusion method acetone extract exhibited highest antifungal activity followed by chloroform, petroleum ether and least in the aqueous extracts as compared to Amphotericin- B antibiotics. Antifungal activity of plant extract in different solvents is directly proportional to the increase in concentration of low concentration (25%) was weaker compared to high concentration (100%) of crude extracts.

The three-way ANOVA showed a significance independent effect of factors fungal strain, solvent and concentration on zone of inhibition (ZOI) in *S. urens* Roxb. (**Table 4**).

The interaction effect of **fungal strain** and **solvent** was also significance (p<0.001; **Table 5**). The DMRT post hoc test showed a significantly higher mean of ZOI for ***C. albicans*** showed a significantly higher mean

of ZOI in **acetone** extract (17.67 ± 1.45 mm) at **100%** concentration, which did not statistically significantly differ from ZOI of *C. oxysporum*, *T. viride*, and *A. niger* in acetone extract. No ZOI was observed for *A. niger* in aqueous, *C. lunata*, *M. gypseum*, *C. oxysporum*, and *T. viride* in chloroform, *C. albicans* and *C. oxysporum* in petroleum ether extract. Rest of the treatments did not vary significantly with each other.

The interaction effect of **fungal strain** and **concentration** was also significance (p=0.029). Results tabulated in (**Table 6**). ***C. albicans*** showed a significantly higher mean of ZOI in **100%** concentration (17.67 ± 1.45 mm) with **acetone** extract, which statistically significantly differ from ZOI of Amphotericin- B antibiotics (12.67 ± 1.20 mm) in four fungal strains and did not differ from other two fungal strains, *C. oxysporum*, *T. viride*, and *A. niger* in 100% concentration, *C. albicans* in 75% concentration. Rest of the treatments did not vary significantly with each other.

The interaction effect of **solvent** and **concentration** was also significance (p<0.001). Results summarized in (**Table 7).** The DMRT post hoc test showed a significantly higher mean of ZOI in **acetone extract** at **100%** concentration (17.67 ± 1.45 mm) for ***C. oxysporum***, which statistically significantly differ from ZOI of Amphotericin- B antibiotics (12.67 ± 1.20) in acetone and differ from other three solvents. No ZOI was observed in aqueous in 25% - 75% concentration, and chloroform and petroleum ether in 25% - 100% concentration. Rest of the treatments did not vary significantly with each other. Represented in **Photoplate no. 2**.

Similar studies using well diffusion method works were performed by Baloch et al. (2013) reported that the chloroform leaves extracts of *M. lupulina* exhibited good antifungal activity against *C. albicans* and *C. glaberata*. Gauniyal and Teotia (2014) reported that *A. nilotica* showed the highest activity while *M. indica* and *L. coromandelica* showed lowest activity against *C. albicans.* Studied twelve plants also showed activity against *C. tropicalis*, *L. acidophilus*, *E. faecalis* and *Strep. mutans.* Gaherwal et al. (2014) reported that *S. xanthocarpum* Kantkari hexane extract showed better activity than aqueous extract against *C. albicans* and showed no activity against *A. niger*. Abd-Ulgadir et al. (2015) reported that the methanolic leaves extracts of *H. sabdariffa* showed strong activity against *C. albicans* and *A. niger* as compared to *R. communis*. Uppala et al. (2016) chloroform extract of *C. guianensis* showed better activity against the fungus *C. albicans* and *A. niger* compared to aqueous extract. Prastiyanto et al. (2021) ethanolic extract of five members of family Zingiberaceae (*C. longa*, *A. galanga*, *Z. officinale* var. *rubrum*, *Z. officinale* var. *officinarum* and *Z. officinale* var. *amarum*) against *C. albicans* and *T. rubrum*.

CONCLUSION

In the concluding remarks, the present investigation established a successful attempt in identification of *Sterculia urens* Roxb. as potential antifungal plant species. These plants have similar effects like antibiotics Amphotericin- B used as a positive control. Poison food technique exhibited good antifungal potential. It can

be used either in combination with traditional medicines or used alone as an antibiotic. Further research should be conducted for isolation and characterization of bioactive compounds responsible for antifungal activity and for more effective outcomes clinical trials should be performed. Thus selected plants could be seen as a good source for useful drugs that can provide a great help in the preparation of new drugs to treat many diseases and have a high therapeutic value. Thus, natural plant derived fungicides may be a source of new alternative active compounds, in particular with antifungal activity with low cost and less side effects in order to assess its tolerance in the human body when administered.

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Photoplate No. 1. *S. urens* Roxb. leaves extract showing growth inhibition percent (%) against six fungal strains at 1000µl concentration using Poison food technique.

C. albicans (Acetone)	*C. lunata* (Acetone)	*M. gypseum* (Acetone)	*C. oxysporum* (Chloroform)	*T. viride* (Acetone)	*A. niger* (Acetone)

Photoplates No. 2. *S. urens* Roxb. leaves extract showing zone of inhibition (mm) using Agar well diffusion method.

C. albicans (Acetone)	*C. lunata* (Acetone)	*M. gypseum* (Acetone)	*C. oxysporum* (Acetone)	*T. viride* (Acetone)	*A. niger* (Acetone)

Table 2. Effect of *S. urens* Roxb. leaves extract on fungal strain, solvents and concentration on growth inhibition percentage (%) (Mean inhibition zone, mm) using poison food technique.

Factors	ANOVA Summary		
	F (Factors)	df (Degree of freedom)	*p* (Significance level)
Fungus type (F)	16.985	5, 240	<0.001
Solvent (S)	27.308	4, 240	<0.001
Concentration (C)	17.053	3, 240	<0.001
F X S	9.959	20, 240	1.00
F X C	0.165	15, 240	0.977
S X C	0.355	12, 240	1.00
F X S X C	0.231	60, 240	1.00

Analysis based on transformed data.

Table 3. DMRT to rank the mean values of growth inhibition (%) (Mean inhibition zone, mm) based on fungal strain, solvents, and concentration in *S. urens* Roxb. (leaves extract).

Fungal strain	Growth inhibition % (Mean inhibition zone in mm ± Std. Error)				
	Solvent				
	Aqueous	Acetone	Chloroform	Petroleum ether	Amphotericin- B
At concentration 100 µl					
C. albicans	42.59 ± 4.9^{bcde}	53.7 ± 1.85^{abcde}	44.44 ± 0.00^{bcde}	44.44 ± 0.00^{bcde}	57.4 ± 1.85^{abcd}
C. lunata	56.56 ± 6.62^{abcde}	62.62 ± 8.08^{abc}	57.57 ± 1.74^{abcd}	54.54 ± 0.00^{abcde}	77.95 ± 0.54^{ab}
M. gypseum	54.38 ± 3.51^{abcde}	57.89 ± 10.52^{abcd}	57.89 ± 5.26^{abcd}	61.4 ± 1.75^{abc}	54.38 ± 4.64^{abcde}
C. oxysporum	50.92 ± 14.01^{abcde}	29.16 ± 25.00^{def}	76.85 ± 3.78^{ab}	48.61 ± 23.91^{bcde}	68.32 ± 1.50^{abc}
T. viride	44.44 ± 18.09^{bcde}	53.77 ± 11.93^{abcde}	63.55 ± 1.60^{abc}	76.44 ± 0.44^{ab}	81.77 ± 0.88^{a}
A. niger	4.89 ± 0.88^{f}	76.89 ± 5.40^{ab}	24.44 ± 14.57^{ef}	38.22 ± 16.44^{cde}	64.88 ± 1.17^{abc}
At concentration 250 µl					
C. albicans	46.29 ± 4.9^{cde}	53.7 ± 1.85^{abcde}	50.00 ± 5.55^{bcde}	48.15 ± 1.85^{bcde}	62.96 ± 1.85^{abcd}
C. lunata	61.61 ± 5.62^{abcd}	70.7 ± 4.04^{abcd}	64.64 ± 1.01^{abcd}	58.58 ± 2.02^{abcde}	80.64 ± 0.00^{ab}
M. gypseum	63.15 ± 0.00^{abcd}	61.4 ± 7.02^{abcd}	57.89 ± 5.26^{abcde}	66.66 ± 1.75^{abcd}	61.4 ± 1.75^{abcd}

C. oxysporum	57.86 ± 10.15^{abcde}	30.55 ± 25.00^{ef}	79.63 ± 3.70^{abc}	50.46 ± 22.19^{bcde}	72.98 ± 0.57^{abcd}
T. viride	49.33 ± 17.75^{bcde}	63.11 ± 6.72^{abcd}	69.33 ± 3.35^{abcd}	77.77 ± 0.88^{abc}	84.44 ± 0.44^{a}
A. niger	6.22 ± 0.44^{f}	79.55 ± 3.87^{abc}	27.55 ± 16.26^{ef}	46.22 ± 20.44^{de}	79.55 ± 0.44^{abc}
At concentration 500 µl					
C. albicans	51.85 ± 4.90^{defg}	61.11 ± 0.00^{abcdefg}	55.55 ± 3.20^{cdefg}	59.26 ± 1.85^{bcdefg}	66.66 ± 0.00^{abcdef}
C. lunata	71.71 ± 1.01^{abcdef}	76.76 ± 3.64^{abcde}	70.7 ± 2.67^{abcdef}	63.63 ± 3.03^{abcdefg}	81.71 ± 0.53^{abc}
M. gypseum	66.66 ± 1.75^{abcdef}	68.42 ± 0.00^{abcdef}	61.4 ± 7.02^{abcdefg}	68.42 ± 0.00^{abcdef}	68.42 ± 0.00^{abcdef}
C. oxysporum	58.79 ± 11.06^{bcdefg}	56.01 ± 13.71^{cdefg}	81.94 ± 5.00^{abc}	54.16 ± 20.45^{cdefg}	77.58 ± 0.99^{abcde}
T. viride	49.33 ± 17.75^{efg}	80.44 ± 1.93^{abcd}	78.22 ± 0.44^{abcde}	78.66 ± 1.53^{abcde}	87.11 ± 0.44^{a}
A. niger	9.77 ± 1.93^{h}	84.89 ± 3.10^{ab}	38.22 ± 19.01^{g}	46.66 ± 20.00^{fg}	85.77 ± 0.88^{ab}
At concentration 1000 µl					
C. albicans	59.26 ± 1.85^{cde}	64.81 ± 1.85^{abcde}	61.11 ± 0.00^{bcde}	61.11 ± 3.20^{bcde}	72.22 ± 0.00^{abcde}
C. lunata	74.74 ± 2.02^{abcde}	80.80 ± 1.01^{abcd}	79.79 ± 1.01^{abcd}	65.65 ± 2.02^{abcde}	84.41 ± 0.53^{abc}
M. gypseum	66.66 ± 1.75^{abcde}	68.42 ± 0.00^{abcde}	64.91 ± 3.51^{abcde}	68.42 ± 0.00^{abcde}	71.93 ± 1.75^{abcde}
C. oxysporum	60.64 ± 12.89^{bcde}	68.98 ± 8.64^{abcde}	87.96 ± 1.85^{a}	58.79 ± 17.31^{bcde}	82.18 ± 1.51^{abc}
T. viride	53.77 ± 15.78^{de}	85.33 ± 2.03^{abc}	78.66 ± 0.77^{abcd}	79.55 ± 0.89^{abcd}	88.89 ± 0.44^{a}
A. niger	20.89 ± 6.94^{g}	86.22 ± 2.70^{ab}	48.44 ± 20.54^{f}	51.11 ± 22.22^{f}	87.55 ± 1.77^{a}

Each value represents the mean of three measurements ± std. error from three petri plates. Duncan's Multiple Range Test at the level of $p<0.05$ denotes the significance difference in mean. Highest to lowest mean indicated by 'a' to 'h' alphabets, where, same alphabet denotes non-significant difference.

Table 4. Effect of *S. urens* Roxb. leaves extract on fungal strain, solvents and concentration on growth diameter of zone of inhibition (mm) using well diffusion method.

Factors	ANOVA Summary		
	F (Factors)	df (Degree of freedom)	*p* (Significance level)
Fungus type (F)	16.619	5, 240	<0.001
Solvent (S)	523.035	3, 240	<0.001
Concentration (C)	75.944	4, 240	<0.001
F X S	6.456	15, 240	<0.001
F X C	1.731	20, 240	0.029
S X C	18.006	12, 240	<0.001
F X S X C	2.569	60, 240	<0.001

Table 5. Comparison of diameter of zone of inhibition (mm) as function of fungal strain and solvents at different concentration in *S. urens* Roxb. (leaves extract).

Fungal strain	Zone of inhibition (diameter in mm): Mean ± Std. Error (n=3)			
	Solvent			
	Aqueous	Acetone	Chloroform	Petroleum ether
At concentration 25%				
C. albicans	NI	11.67 ± 0.66^{a}	NI	NI
C. lunata	NI	7.00 ± 3.51^{b}	NI	NI
M. gypseum	NI	9.33 ± 1.33^{ab}	NI	NI
C. oxysporum	NI	10.00 ± 0.00^{a}	NI	NI
T. viride	NI	10.67 ± 0.66^{a}	NI	NI
A. niger	NI	10.00 ± 0.00^{a}	NI	2.33 ± 0.00^{c}
At concentration 50%				
C. albicans	NI	13.33 ± 0.88^{a}	2.33 ± 0.00^{c}	NI
C. lunata	NI	9.33 ± 1.20^{b}	NI	NI
M. gypseum	NI	10.00 ± 1.00^{b}	NI	NI
C. oxysporum	NI	10.67 ± 0.66^{b}	NI	NI
T. viride	NI	11.67 ± 0.33^{ab}	NI	NI
A. niger	NI	10.67 ± 0.66^{b}	NI	2.67 ± 0.00^{c}
At concentration 75%				
C. albicans	2.33 ± 0.00^{ef}	15.33 ± 0.33^{a}	8.00 ± 1.00^{cd}	NI
C. lunata	NI	10.00 ± 1.00^{bc}	NI	NI
M. gypseum	NI	11.00 ± 0.57^{bc}	NI	NI
C. oxysporum	NI	12.33 ± 0.66^{ab}	NI	NI
T. viride	2.33 ± 0.00^{ef}	13.33 ± 0.88^{ab}	NI	5.00 ± 2.51^{de}
A. niger	NI	12.67 ± 0.66^{ab}	2.33 ± 0.00^{ef}	6.00 ± 3.05^{de}
At concentration 100%				
C. albicans	7.00 ± 0.00^{efg}	17.67 ± 1.45^{a}	8.33 ± 0.88^{def}	NI
C. lunata	7.00 ± 0.00^{efg}	11.00 ± 1.00^{cde}	NI	2.33 ± 0.00^{hi}
M. gypseum	7.00 ± 0.00^{efg}	12.00 ± 0.00^{bcd}	NI	2.33 ± 0.00^{hi}
C. oxysporum	7.00 ± 0.00^{efg}	14.67 ± 0.33^{abc}	NI	NI
T. viride	2.67 ± 0.00^{ghi}	15.67 ± 1.20^{ab}	NI	8.33 ± 0.88^{def}
A. niger	NI	16.33 ± 1.85^{a}	4.67 ± 2.33^{fgh}	6.67 ± 3.33^{efg}

Each value represents the mean of three measurements ± std. error from three petri plates. Duncan's Multiple Range Test at the level of $p<0.05$ denotes the significance difference in mean. Highest to lowest mean indicated by 'a' to 'i' alphabets, where, same alphabet denotes non-significant difference.

Table 6. Comparison of diameter of zone of inhibition (mm) as function of fungal strain and concentration in each solvent in *S. urens* Roxb. (leaves extract).

Fungal strain	Zone of inhibition (diameter in mm): Mean ± Std. Error (n=3)				
	Concentration				
	25%	50%	75%	100%	Amphotericin- B
Aqueous					
C. albicans	NI	NI	2.33 ± 0.00^{bc}	7.00 ± 0.00^{a}	5.1 ± 2.57^{ab}
C. lunata	NI	NI	NI	7.00 ± 0.00^{a}	NI
M. gypseum	NI	NI	NI	7.00 ± 0.00^{a}	NI
C. oxysporum	NI	NI	NI	4.67 ± 2.33^{ab}	NI
T. viride	NI	NI	2.33 ± 0.00^{bc}	2.67 ± 0.00^{bc}	NI
A. niger	NI	NI	NI	NI	NI
Acetone					
C. albicans	11.67 ± 0.66^{cdef}	13.33 ± 0.88^{bcdef}	15.33 ± 0.33^{abc}	17.67 ± 1.45^{a}	12.67 ± 1.20^{bcdef}
C. lunata	7.00 ± 3.51^{g}	9.33 ± 1.20^{fg}	10.00 ± 1.00^{efg}	11.00 ± 1.00^{defg}	10.33 ± 0.88^{efg}
M. gypseum	9.33 ± 1.33^{fg}	10.00 ± 1.00^{efg}	11.00 ± 0.57^{defg}	12.00 ± 0.00^{cdef}	10.67 ± 0.66^{defg}
C. oxysporum	10.00 ± 0.00^{efg}	10.67 ± 0.66^{defg}	12.33 ± 0.66^{bcdef}	14.67 ± 0.33^{abcd}	14.00 ± 1.52^{abcde}
T. viride	10.67 ± 0.66^{defg}	11.67 ± 0.33^{cdef}	13.33 ± 0.88^{bcdef}	15.67 ± 1.20^{abc}	9.33 ± 0.66^{fg}
A. niger	10.00 ± 0.00^{efg}	10.67 ± 0.66^{defg}	12.67 ± 0.66^{bcdef}	16.33 ± 1.85^{ab}	14.00 ± 3.05^{abcde}
Chloroform					
C. albicans	NI	2.33 ± 0.00^{cd}	8.00 ± 1.00^{b}	8.33 ± 0.88^{b}	12.2 ± 1.2^{a}
C. lunata	NI	NI	NI	NI	8.87 ± 0.59^{b}
M. gypseum	NI	NI	NI	NI	NI
C. oxysporum	NI	NI	NI	NI	8.33 ± 0.33^{b}
T. viride	NI	NI	NI	NI	9.00 ± 1.00^{b}
A. niger	NI	NI	2.33 ± 0.00^{cd}	4.67 ± 2.33^{c}	11.67 ± 1.66^{a}
Petroleum ether					
C. albicans	NI	NI	NI	NI	11.33 ± 1.85^{a}
C. lunata	NI	NI	NI	2.33 ± 0.00^{de}	8.67 ± 0.33^{ab}
M. gypseum	NI	NI	NI	2.33 ± 0.00^{de}	9.33 ± 0.66^{ab}
C. oxysporum	NI	NI	NI	NI	7.33 ± 0.33^{abc}
T. viride	NI	NI	5.00 ± 2.51^{bcde}	8.33 ± 0.88^{ab}	8.00 ± 0.57^{ab}
A. niger	2.33 ± 0.00^{de}	2.67 ± 0.00^{cde}	6.00 ± 3.05^{bcd}	6.67 ± 3.33^{abcd}	6.33 ± 3.18^{bcd}

Each value represents the mean of three measurements ± std. error from three petri plates. Duncan's Multiple Range Test at the level of $p<0.05$ denotes the significance difference in mean. Highest to lowest mean indicated by 'a' to 'g' alphabets, where, same alphabet denotes non-significant difference.

Table 7. Comparison of diameter of zone of inhibition (mm) as function of concentration and solvent in each fungal strain at different concentration in *S. urens* Roxb. (leaves extract).

% Concentration	Zone of inhibition (diameter in mm): Mean ± Std. Error (n=3)			
	Solvent			
	Aqueous	Acetone	Chloroform	Petroleum ether
Candida albicans				
25%	NI	11.67 ± 0.66^{bcd}	NI	NI
50%	NI	13.33 ± 0.88^{bc}	2.33 ± 0.00^{fg}	NI
75%	2.33 ± 0.00^{fg}	15.33 ± 0.33^{ab}	8.00 ± 1.00^{de}	NI
100%	7.00 ± 0.00^{e}	17.67 ± 1.45^{a}	8.33 ± 0.88^{de}	NI
Amphotericin- B	5.1 ± 2.57^{ef}	12.67 ± 1.20^{bc}	12.2 ± 1.2^{bc}	11.33 ± 1.85^{cd}
C. lunata				
25%	NI	7.00 ± 3.51^{b}	NI	NI
50%	NI	9.33 ± 1.20^{ab}	NI	NI
75%	NI	10.00 ± 1.00^{ab}	NI	NI
100%	7.00 ± 0.00^{b}	11.00 ± 1.00^{a}	NI	2.33 ± 0.00^{c}
Amphotericin- B	NI	10.33 ± 0.88^{ab}	8.87 ± 0.59^{ab}	8.67 ± 0.33^{ab}
M. gypseum				
25%	NI	9.33 ± 1.33^{b}	NI	NI
50%	NI	10.00 ± 1.00^{ab}	NI	NI
75%	NI	11.00 ± 0.57^{ab}	NI	NI
100%	7.00 ± 0.00^{c}	12.00 ± 0.00^{a}	NI	2.33 ± 0.00^{d}
Amphotericin- B	NI	10.67 ± 0.66^{ab}	NI	9.33 ± 0.66^{b}
C. oxysporum				
25%	NI	10.00 ± 0.00^{de}	NI	NI
50%	NI	10.67 ± 0.66^{cd}	NI	NI
75%	NI	12.33 ± 0.66^{bc}	NI	NI
100%	4.67 ± 2.33^{g}	14.67 ± 0.33^{a}	NI	NI
Amphotericin- B	NI	14.00 ± 1.52^{ab}	8.33 ± 0.33^{ef}	7.33 ± 0.33^{f}
T. viride				
25%	NI	10.67 ± 0.66^{bcd}	NI	NI
50%	NI	11.67 ± 0.33^{bc}	NI	NI
75%	2.33 ± 0.00^{fg}	13.33 ± 0.88^{ab}	NI	5.00 ± 2.51^{de}
100%	2.67 ± 0.00^{fg}	15.67 ± 1.20^{a}	NI	8.33 ± 0.88^{cd}
Amphotericin- B	NI	9.33 ± 0.66^{cd}	9.00 ± 1.00^{cd}	8.00 ± 0.57^{de}
A. niger				
25%	NI	10.00 ± 0.00^{bcd}	NI	2.33 ± 0.00^{ef}
50%	NI	10.67 ± 0.66^{abcd}	NI	2.67 ± 0.00^{ef}
75%	NI	12.67 ± 0.66^{ab}	2.33 ± 0.00^{ef}	6.00 ± 3.05^{cdef}
100%	NI	16.33 ± 1.85^{a}	4.67 ± 2.33^{def}	6.67 ± 3.33^{cde}
Amphotericin- B	NI	14.00 ± 3.05^{ab}	11.67 ± 1.66^{abc}	6.33 ± 3.18^{cdef}

Each value represents the mean of three measurements ± std. error from three petri plates. Duncan's Multiple Range Test at the level of $p<0.05$ denotes the significance difference in mean. Highest to lowest mean indicated by 'a' to 'g' alphabets, where, same alphabet denotes non-significant difference.