IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

In Vitro Antifungal Activity And Preliminary Phytochemical Screening Of *Dillenia Indica* Linn. Leaf Extracts From Narayanpur, Bastar, Chhattisgarh

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ABSTRACT

Antifungal agents from plant origin have therapeutic potential for treatment of disease resistant fungal strains. *Dillenia indica* Linn. is a medicinal plant with a valuable source of natural compounds and traditionally used in therapeutic aid for various ailments. The present study aimed to evaluate preliminary phytochemical screening and *in vitro* antifungal activity of *Dillenia indica* Linn. leaves extract against six dermatophytic fungal strains, and it was compared against commonly used antifungal agent's Amphotericin- B antibiotics. Antifungal activity test was performed by using poison food technique on potato dextrose agar medium (PDA). Results of the present research work showed that antifungal activity of the different solvent plant extract is directly proportional to the increase in concentration (100µl, 250µl, 500µl and 1000µl). A consistent increased and better growth inhibition in chloroform in *A. niger* compared to antibiotics though statistically non-significant was examined. A significantly higher mean of GI % was reported for *A. niger* (85.77 ± 4.88) in chloroform extract at 1000µl concentration, which did not statistically significantly differ from GI % of Amphotericin- B antibiotics (87.55 ± 1.77) for all six fungal strains. However, lowest growth inhibition % was observed for *C. lunata* in aqueous extracts. Phytochemical screening confirmed the presence of alkaloid, phenols, reducing sugar, saponin, terpenoids, flavonoids, fixed oils and fats and anthraquinone in different solvents. *D. indica* Linn. have great medicinal importance and as it contains a number of bioactive compounds like phenols,

flavonoids, saponins, terpenoids which possess antifungal activity. It can be used as a source of new antifungal agent and as a source of new drug with no or less side effect, an ecofriendly mode of treatment.

Keywords: Phytochemicals, antifungal activity, growth inhibition percentage (%), SPSS analysis, ANOVA.

1. INTRODUCTION

In the present scenario with the emergence of new diseases and resistance to already available drugs, many medicinal plants will continue to be the best source of new and active drugs. Recent interests in traditional pharmacopoeias have meant that scientists around the globe are concerned not only with determining the scientific rationale for the usage of plants, but also with the discovery of novel compounds with pharmaceutical values (Fennell et al. 2004). Scientists and researchers across the world are watching plants as a future source of drugs because herbal medicines have a strong traditional or conceptual base and the potential leads for treating different diseases with fewer side effects.

Dillenia indica Linn. belongs to the family Dilleniaceae also commonly known as 'Chulta' or 'Elephant apple' is an evergreen flowering plant (angiosperms). There are about 60 species of evergreen trees that inhabit the Indo-Malayan region (Pandey et al. 2018; Kumar et al. 2018). Seven species are native to India which is used as traditional medicine. It is native to Southeastern Asia, India, Nepal to Bhutan, Bangladesh, Sri Lanka, East to Southwestern China (Yunnan) and Vietnam, and South through Thailand to Malaysia,Peninsula, Philippines and Indonesia. In India it is mainly found in Himalayan tract of Assam, North Bengal, Bihar, Orissa, Madhya Pradesh, Gujarat, and Chhattisgarh (Khanna et al. 2005; Khare 2007; Short et al. 2011; Gandhi and Mehta 2013; Rai and Sajwan 2020). In India its status of threat is vulnerable species (http://www.iucn.org).

Useful Parts: Fruits, leaves, aerial parts.

Pharmacologically, it has been reported that *D. indica* Linn. shows biological activities like antimicrobial, antibacterial, antifungal, antioxidant, anti-inflammatory, anti-cancer, anti-leukemic, anti-proliferative, anti-diabetic, anti-diarrheal, anti-malarial, anti-HIV, analgesic, cytotoxic, hepatoprotective, and chemoprevention and many more (Gandhi and Mehta 2013; Biswas and Pandita 2015; Kumar et al. 2018; Barua et al. 2018).

In Northeast India the juices of leaves, bark and fruits are mixed and given orally for the treatment of cancer and diarrhea. When mixed with sugar and water, it is used as a cooling beverage in fever and cough remedies along with leukemic and cardio tonic effects (Sharma 2001). The leaves and bark are used as laxative, tonic and astringents. Bruised bark is applied as a cataplasm for patients with arthritis (Singh 2016). *D. indica* Linn. leaves used as an astringent, anti-amphetamine and the bark of *D. indica* Linn. used as an oral thrush and to remove offensive odor from the mouth. Fruits for the treatment of jaundice, treatment for fever and fatigue, and heart related diseases. The fruit possesses tonic and laxative properties and is used to cure abdominal pain. Fresh juice of the fruit is mixed with honey or sugar and given for treatment against high fevers, cough and

dyspnea. The fresh juice of bhavya fruit can be used to improve digestive capacity and lack of appetite. Fresh juice and cold infusion of *D. indica* Linn. applied over the scalp to regenerate in hair fall and used as a good hair tonic. Scabies and skin pigmentation of the skin can be cured by applying the paste of the bark of *D. indica* Linn. in the affected areas. It is generally consumed as chutney or made into jam. The fruit is used in Indian cuisine to prepare various dishes and desserts. Flower used for treatment of dysentery (Tandon and Sharma 2009; Rai and Lalramnghinglove 2010; Choudhary et al. 2018).

Chemical Constituents

Dillenia indica Linn. is rich in various chemical compounds like betulinic acid (Dillenetin), betulin, betulinaldehyde, cycloartenone, n-hentricontanol, β-sitosterol, stigmasterol, glycoside, steroids, flavonoids, saponins, tannins, triterpene, malic acid, arabinogalactan, quercetin, reducing sugar, Kaempferol 4-methyl ether, Dihydrokaempferide, β-Amyrin, n-hentriacontanol.Leaves contain an acyl ester n-nonyl oleate, two aliphatic ketones n-heptacosan-9-one, n-nonatriacontan-20-one, and stigmasteryl palmitate (Shah 1978; Kumar et al. 2013; Kumar et al. 2018; Ali et al. 2018).

2. MATERIALS AND METHODS

2.1. Collection of plant sample

Mature green and disease free leaves of *D. indica* Linn. was collected from Herbal Garden, Bakhrupara Kasthagar and tropical dry deciduous forest area of Narayanpur District during their flowering seasons (July-August). 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 *D. indica* Linn. 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 with proper labeling 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. The upper part was fitted with a condenser where cool water inflow and outflow were provided for liquid condensation throughout the process of successive extraction. 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^{o}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), Sofowora (1993). Preliminary Phytochemical screening was performed for characterization of different bioactive compounds present in *D. indica* Linn..

1) Test for the presence of 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.

2) Test for the presence of 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.

3) Test for the presence of 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.

4) Test for the presence of 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.

5) Test for the presence of 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.

6) Test for the presence of 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.

7) Test for the presence of 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.

8) Test for the presence of 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.

9) Test for the presence of 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.

10) Test for the presence of 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

Antifungal activity tests against pathogenic fungal strains were determined by Poisoned food technique using aqueous, acetone, chloroform, and petroleum ether extracts (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 concentration (100µl, 250µl, 500µl and 1000µl extracts of each plant) was 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 in an inverted position. Proper control (PDA without extract) serves as negative control. Antibiotics Amphotericin- B was used as positive control (McCutcheon et al. 1994). Then, all inoculated dishes were incubated at temperature 26 ± 1°C for fungi. After that, the radial mycelia growth was measured after 72 hours of inoculation (in millimeters) by measuring the two opposite circumferences of the colony growth. For each treatment, three replicates were maintained. Finally, the antifungal activity of each extract was calculated in terms of growth inhibition percentage 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).

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) 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 of preliminary phytochemical analysis are summarized in Table 1. Leaves of ***D. indica*** **Linn.** in **aqueous** extract revealed the presence of various phytocompounds i.e., alkaloids, phenols, reducing sugar, saponins, terpenoids, flavonoids, fixed oils and fats, anthraquinones while tannins, cardiac glycosides were absent. **Acetone** extract revealed the presence of alkaloids, phenols, reducing sugar, saponins, flavonoids, fixed oils and fats, rest of the compounds tannins, terpenoids, cardiac glycosides, anthraquinones were absent. **Chloroform** extract revealed the presence of phenols, reducing sugar, saponins, flavonoids, fixed oils and fats, the rest of the compounds alkaloids, tannins, terpenoids, cardiac glycoside, anthraquinones were absent. However, in **petroleum ether** extract only alkaloids, phenols, saponins, flavonoids, fixed oils and fats were found to be present while rest reducing sugar, tannins, terpenoids, cardiac glycoside, anthraquinones these compounds were absent.

Table 1. Preliminary phytochemical screening of crude extract of *Dillenia indica* Linn.

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, PE; petroleum ether, CF; chloroform extracts; '-' indicates absence

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

The three-way ANOVA showed a significant independent effect of factors fungal strain, solvent and concentration on growth inhibition percentage (GI %) in *D. indica* Linn. (**Table 2**). The interaction effect of fungal strain and solvent was found to be significant (p<0.001; **Table 3**).

1) Growth inhibition percentage in 100µl concentration

The DMRT post hoc test showed a significantly higher mean of GI % for *A. niger* (82.66 ± 4.00) in chloroform extract at 100µl concentration, which did not statistically significantly differ from GI % of Amphotericin- B antibiotics (64.88 ± 1.17) for four fungal strains and significantly differ from other two fungal strains, *T. viride* in aqueous, *M. gypseum* in acetone, *C. lunata*, *M. gypseum*, *C. oxysporum* and *T. viride* in chloroform.

However, lowest growth inhibition % was observed for *A. niger* in acetone, consequently *C. lunata* in aqueous extract (**Figure 3.1**). Rest of the treatments did not vary significantly with each other.

2) Growth inhibition percentage in 250µl concentration

The DMRT post hoc test showed a significantly higher mean of GI % was observed for *A. niger* (83.55 ± 3.79) in chloroform extract at 250µl concentration, which did not statistically significantly differ from GI % of Amphotericin- B antibiotics (79.55 ± 0.44) for five fungal strains and significantly differ from one fungal strain, *T. viride* in aqueous, *M. gypseum* in acetone, *C. lunata*, *M. gypseum*, *C. oxysporum* and *T. viride* in chloroform. However, lowest growth inhibition % was observed for *A. niger* acetone, consequently *C. lunata* in aqueous extract (**Figure 3.2**). Rest of the treatments did not vary significantly with each other.

3) Growth inhibition percentage in 500µl concentration

The DMRT post hoc test showed a significantly higher mean of GI % was observed for *A. niger* (85.77 ± 4.88) in chloroform extract at 500µl concentration, which did not statistically significantly differ from GI % of Amphotericin- B antibiotics (85.77 ± 0.88) in all six fungal strains, *T. viride* in aqueous, *M. gypseum* in acetone, *C. lunata*, *M. gypseum*, *C. oxysporum* and *T. viride* in chloroform. However, lowest growth inhibition % was observed for *A. niger* in acetone, *C. lunata* in aqueous extract (**Figure 3.3**). Rest of the treatments did not vary significantly with each other.

4) Growth inhibition percentage in 1000µl concentration

The DMRT post hoc test showed a significantly higher mean of GI % was observed for *A. niger* (85.77 ± 4.88) in chloroform extract at 1000µl concentration, which did not statistically significantly differ from GI % of Amphotericin- B antibiotics (87.55 ± 1.77) in all six fungal strains, *T. viride* in aqueous, *C. lunata* and *A. niger* in acetone, *C. lunata*, *C. oxysporum* and *T. viride* in chloroform. However, lowest growth inhibition % was observed for *C. lunata* in aqueous, consequently *C. lunata* and *A. niger* in petroleum ether extract (**Figure 3.4**). Rest of the treatments did not significantly vary with each other **(Photoplate 1)**.

The present investigation revealed that the highest antifungal activity was exhibited by the chloroform extract followed by aqueous, acetone and least in the petroleum ether extract of *D. indica* Linn.. A consistent increased and similar growth inhibition in chloroform against *A. niger* compared to Amphotericin- B antibiotics though statistically non-significant was examined. The minimum growth inhibition % was variable in each concentration. Similar studies using Poison food techniques were performed by Apu et al. (2010) reported leaves of *D. indica* Linn. showed antifungal activity against *A. niger*, *C. albicans*, and *S. cerevisiae*. Acetone and alcoholic seeds extracts of *D. indica* showed good antimicrobial activity while chloroform extract found to have mild activity (Uppalapati and Rao 1980), acetone and alcoholic seeds extract of *D. indica f. Elongate* (Miq.) Miq. showed strong antimicrobial activity while chloroform extract was found to have mild activity

(Boparai et al. 2016), chloroform extracts of *R. sceleratus* and *P. pinnata* showed highest activity against *M. gypseum*, *M. fulvum*, *T. rubrum*, *T. mentagophytes*, and *T. tonsourons* than methanol extracts. The water extract showed the least activity (Sharma et al. 2012), *H. arborea* and *H. pentandra* exhibited antifungal activity against *Cladosporium* sp., *A. niger*, *A. flavus*, *A. fumigates*, *Penicillium* sp., *Helminthosporium* sp. and *Rhizopus* sp. (Prashith Kekuda and Vinayaka 2017), methanolic extracts of *S. species* had antifungal activity against *C. lunata* while extracts of *S. rostratum* and *S. erianthum* showed stronger activity than the reference fungicide (Hernandez-Rodrigcuez et al. 2018), *Z. mandavillei* showed activity against *C. cladosporioides* (Yehia et al. 2020), methanolic extracts of *A. ilicifolius* L. leaves exhibited activity against *C. albicans* (Andriani et al. 2020).

Table 2. Effect of *D. indica* Linn. leaves extract on fungal strain, solvents and concentration on growth inhibition percentage (mm) (Mean inhibition zone, mm).

Factors	ANOVA Summary		
	F (Factors)	**df (Degree of freedom)**	***p* (Significance level)**
Fungus type (F)	16.911	5, 240	<0.001
Solvent (S)	108.534	4, 240	<0.001
Concentration (C)	30.086	3, 240	<0.001
F X S	17.839	20, 240	<0.001
F X C	1.285	15, 240	0.212
S X C	1.257	12, 240	0.245
F X S X C	0.78	60, 240	0.873

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 *D. indica* Linn. (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	53.7 ± 1.85^{cdefghi}	44.44 ± 0.00^{efghij}	31.48 ± 8.07^{hij}	44.44 ± 5.55^{efghij}	57.4 ± 1.85^{bcdefgh}
C. lunata	9.09 ± 0.00^{kl}	27.27 ± 9.09^{ijk}	68.68 ± 10.1^{abcde}	33.33 ± 3.49^{ghij}	77.95 ± 0.54^{abc}
M. gypseum	40.19 ± 1.90^{fghij}	59.64 ± 1.75^{abcdefg}	59.64 ± 1.75^{abcdefg}	47.36 ± 0.00^{defghij}	54.38 ± 4.64^{cdefgh}
C. oxysporum	46.76 ± 1.22^{defghij}	34.25 ± 3.70^{ghij}	70.36 ± 18.51^{abcd}	48.61 ± 8.37^{defghij}	68.32 ± 1.50^{abcdef}
T. viride	79.55 ± 0.44^{abc}	36.88 ± 15.42^{ghij}	75.55 ± 1.17^{abc}	43.55 ± 1.93^{efghij}	81.77 ± 0.88^{ab}
A. niger	32.88 ± 13.77^{hij}	4.89 ± 2.35^{l}	82.66 ± 4.00^{a}	26.22 ± 19.57^{jk}	64.88 ± 1.17^{abcdef}
At concentration 250 µl					
C. albicans	55.55 ± 0.00^{cdefg}	48.15 ± 1.85^{efg}	46.29 ± 6.67^{efgh}	48.14 ± 7.40^{efg}	62.96 ± 1.85^{abcdef}
C. lunata	12.12 ± 3.03^{ij}	37.37 ± 4.04^{fgh}	73.73 ± 5.05^{abcd}	37.37 ± 2.02^{fgh}	80.64 ± 0.00^{ab}
M. gypseum	43.85 ± 1.75^{efgh}	63.15 ± 0.00^{abcde}	63.15 ± 3.04^{abcde}	52.63 ± 0.00^{defg}	61.4 ± 1.75^{bcdefg}
C. oxysporum	47.68 ± 2.01^{efgh}	39.81 ± 4.63^{efgh}	75.92 ± 13.66^{abc}	48.61 ± 8.37^{efg}	72.98 ± 0.57^{abcd}
T. viride	81.33 ± 0.76^{ab}	44.00 ± 14.98^{efgh}	78.66 ± 1.33^{abc}	45.33 ± 2.77^{efgh}	84.44 ± 0.44^{a}
A. niger	39.11 ± 16.26^{gh}	5.33 ± 2.03^{j}	83.55 ± 3.79^{a}	27.55 ± 19.57^{hi}	79.55 ± 0.44^{ab}
At concentration 500 µl					
C. albicans	61.11 ± 0.00^{bcdef}	51.85 ± 1.85^{defg}	51.85 ± 4.9^{defg}	53.7 ± 7.41^{defg}	66.66 ± 0.00^{abcdef}
C. lunata	22.22 ± 1.01^{hi}	53.53 ± 4.04^{defg}	75.75 ± 6.06^{abcd}	41.41 ± 4.04^{fgh}	81.71 ± 0.53^{ab}
M. gypseum	49.12 ± 1.75^{efg}	66.66 ± 1.75^{abcdef}	66.66 ± 1.75^{abcdef}	54.38 ± 1.75^{cdefg}	68.42 ± 0.00^{abcde}
C. oxysporum	51.84 ± 0.46^{defg}	47.22 ± 0.00^{efg}	77.31 ± 13.66^{ab}	51.39 ± 6.94^{efg}	77.58 ± 0.99^{abc}
T. viride	84.89 ± 0.88^{a}	45.77 ± 15.85^{efg}	82.67 ± 1.33^{ab}	46.66 ± 3.84^{efg}	87.11 ± 0.44^{a}
A. niger	47.55 ± 19.49^{efg}	7.55 ± 1.17^{i}	85.77 ± 4.88^{a}	32.89 ± 18.48^{gh}	85.77 ± 0.88^{a}
At concentration 1000 µl					
C. albicans	62.96 ± 1.85^{cdefg}	53.7 ± 3.70^{defgh}	55.55 ± 6.41^{defgh}	57.4 ± 6.67^{defg}	72.22 ± 0.00^{abcd}
C. lunata	30.3 ± 1.74^{i}	69.69 ± 6.06^{abcdef}	79.79 ± 2.02^{abc}	47.47 ± 2.02^{ghi}	84.41 ± 0.53^{ab}
M. gypseum	57.89 ± 0.00^{defg}	68.42 ± 0.00^{bcdef}	68.42 ± 0.00^{bcdef}	57.89 ± 0.00^{defg}	71.93 ± 1.75^{abcde}
C. oxysporum	58.33 ± 0.00^{defg}	51.38 ± 2.12^{fgh}	79.61 ± 12.5^{abc}	56.48 ± 6.02^{defgh}	82.18 ± 1.51^{abc}
T. viride	87.55 ± 0.44^{ab}	50.67 ± 15.72^{fgh}	84.89 ± 0.88^{ab}	52.00 ± 2.77^{efgh}	88.89 ± 0.44^{a}
A. niger	64.00 ± 8.88^{cdefg}	70.66 ± 1.53^{abcdef}	85.77 ± 4.88^{ab}	37.78 ± 17.77^{hi}	87.55 ± 1.77^{ab}

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 'l' alphabets, where, same alphabet denotes non-significant difference.

CONCLUSION

In the concluding remarks, the present investigation established a successful attempt in identification of *D. indica* Linn. 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. This might be due to strong occurrence and combined effect of active compounds. It can be used either in combination with traditional medicines or used alone as an antibiotic. Hence it can be concluded that the degree of antifungal activity is solvent and concentration dependent. Leaves of *D. indica* Linn. indicate the more

positive result in aqueous and acetone plant extract, as compared to chloroform and petroleum ether. Alkaloids, phenols, reducing sugar, saponins, terpenoids, flavonoids, fixed oils and fats and anthraquinones were present. Each bioactive compound shows novel biological behavior which may increase the chances of the discovery of new compounds like antibiotics against pathogens. 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.

ACKNOWLEDGMENT

The author would like to thank Pt. Ravishankar Shukla University Raipur (C.G.) for providing funds for this research work.

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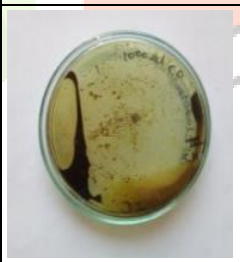
C. albicans **(Aqueous)**	***C. lunata*** **(Chloroform)**	***M. gypseum*** **(Chloroform)**	***C. oxysporum*** **(Chloroform)**	***T. viride*** **(Chloroform)**	***A. niger*** **(Chloroform)**
					
					

Photoplate 1. *D. indica* Linn. leaves extract showing growth inhibition percent (%) against six fungal strains at 1000µl concentration.

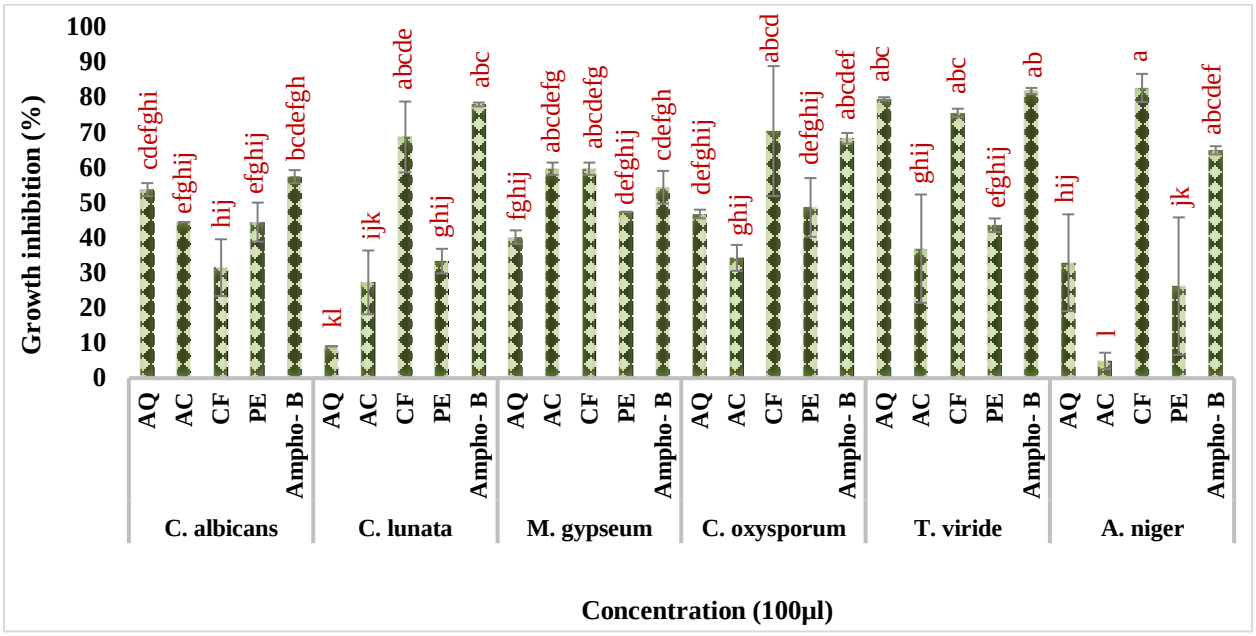


Figure 3.1. Effect of *D. indica* Linn. leaves extract at 100µl concentration on growth inhibition percentage against six fungal strains: ANOVA summary represents F= 7.755; df= 29,60; $p<0.05$. Similar alphabets, which are not statically significant from each other at $p<0.05$ (Based on Duncan's test, DMRT).

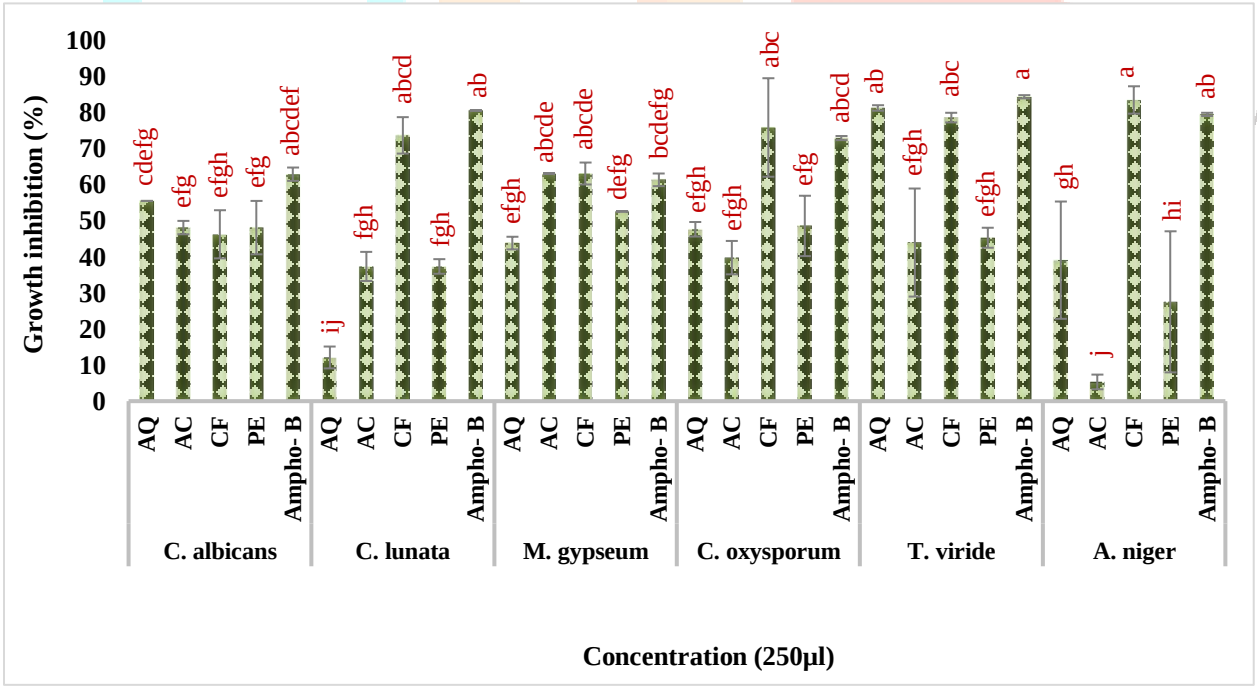


Figure 3.2. Effect of *D. indica* Linn. leaves extract at 250µl concentration on growth inhibition percentage against six fungal strains: ANOVA summary represents F= 9.356; df= 29,60; $p<0.05$. Similar alphabets, which are not statically significant from each other at $p<0.05$ (Based on Duncan's test, DMRT).

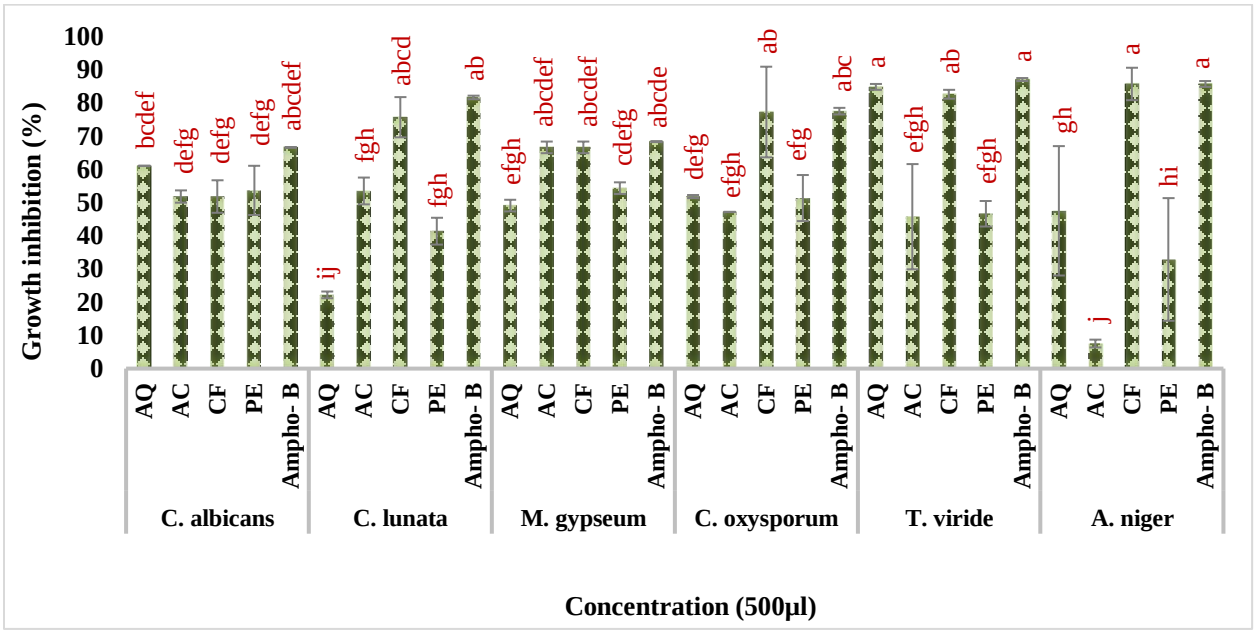


Figure 3.3. Effect of *D. indica* Linn. leaves extract at 500µl concentration on growth inhibition percentage against six fungal strains: ANOVA summary represents F= 8.334; df= 29,60; $p<0.05$. Similar alphabets, which are not statically significant from each other at $p<0.05$ (Based on Duncan's test, DMRT).

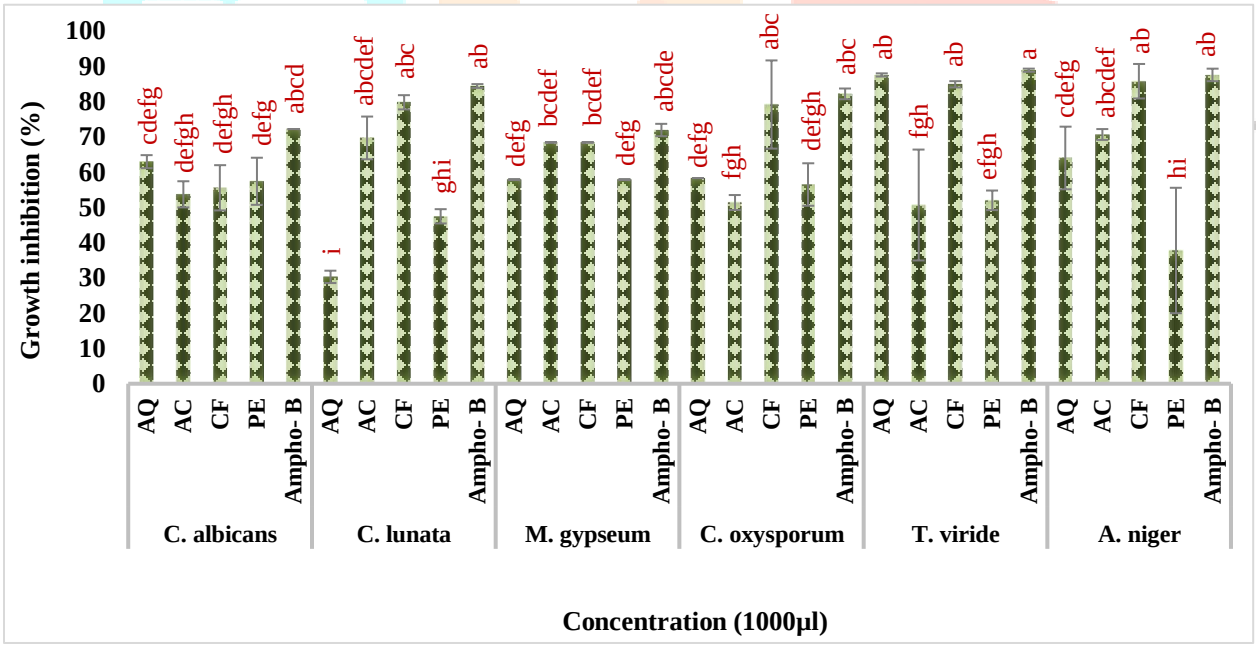


Figure 3.4. Effect of *D. indica* Linn. leaves extract at 1000µl concentration on growth inhibition percentage against six fungal strains: ANOVA summary represents F= 6.978; df= 29,60; $p<0.05$. Similar alphabets, which are not statically significant from each other at $p<0.05$ (Based on Duncan's test, DMRT).