ANTIOXIDANT PROFILING AND ANTIFUNGAL ASSESSMENT OF SOME MEDICINAL PLANTS AGAINST HUMAN PATHOGENIC FUNGI

Abstract

In the current investigation, seven different solvents were used to assess the antifungal abilities of four plants, *Datura stramonium*, *Lantana camara*, *Jatropha curcas*, and *Saraca asoca.* These plants were investigated for the qualitative and quantitative detection of secondary metabolites, antioxidant profile, and antifungal evaluation against two pathogenic fungi, *C. albicans* and *A. niger.* Alkaloids, flavonoids, and saponins are examples of secondary metabolites found in these plants. Alkaloids were discovered to be most prevalent in *D. stramonium*, followed by tannin in *L. camara*, and phenol in *S. asoca*. *L. Camara* demonstrated the strongest antifungal activity against *A. Niger*. *J. Curcas* demonstrated the highest antifungal activity against *C. albicans.*

Keywords: Antioxidant, antifungal, metabolites, infections

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I. INTRODUCTION

More and more experts agree that fungi can cause invasive and widespread illnesses in addition to superficial infections, which commonly affect a large number of individuals but are relatively simple to cure. The high death rates associated with fungi infections are a result of challenging diagnosis and a dearth of effective antifungal medications. Immunosuppressed persons, such as those undergoing solid organ or stem cell transplantations, are more likely to get serious fungal infections. This population has been growing as a result of advances in modern medicine (Jacobsen, 2019). The most common hospital infection, candidemia, which accounts for up to 15% of bloodstream infections, is caused by the Candida genus and ranges in severity from the mildest to the most serious diseases. About 50 to 70 percent of systemic fungal infections are caused by species of Candida (Barchiesi *et al*., 2016). The skin shields the internal organs from outside threats, protects against sunburn, and controls body temperature; nonetheless, microorganisms can enter the body and interfere with the skin's defences, resulting in skin illnesses or infections. Skin illnesses can be brought on by bacteria, viruses, parasites, and fungi. As they affect the third layer of skin, fungus infections are more severe (Shields *et al*., 2019). According to Rai *et al*., 2017, fungi affect keratin tissue, including skin, hair, and nails. According to Abd *et al*., 2020, indications of a fungal infection commonly include crusted spots, itchy red patches, and hair loss. Plants create and emit a wide range of secondary metabolites to defend themselves against pathogens. These metabolites are crucial components of many different illnesses' defence mechanisms. Traditional medicinal plant therapy is preferred because it has fewer adverse effects and is more effective (Giordani *et al*., 2020). Due to their lower toxicity and minimal environmental impact, the use of phytochemicals either alone or in conjunction with conventional drugs—could be a better treatment for fungal infections (De Andrade *et al*., 2019). In this regard, alcoholic plant extracts may provide a better extraction as some of these extracts have been shown to be more effective antifungal medications than traditional antibiotics (Keikha *et al*., 2018). As old as the usage of plants as food, medicine, and nutraceuticals is the utility and discovery of their bioactive components. Native people all around the world employ a wide variety of plants for the treatment of numerous diseases. It is said that 8% of the population in underdeveloped nations depends on medicinal plants for food and health maintenance for both humans and animals. Food preservation, quality maintenance, and safety are currently seen as key expanding concerns in the food sector. As a result of food contamination and spoiling, which are significantly accelerated by the presence of bacteria and other fungi, poor food quality can result in a number of infectious diseases. Due to the revolution in traditional culture and the decline of aboriginal knowledge of medicinal plants, the main benefits of obtaining medications from a natural source are their accessibility and relative lack of adverse effects (Aldughaylibi *et al* 2022). Antimicrobial resistance (AMR) poses a serious and continuous threat to public health on a worldwide scale. By 2050, it is predicted that AMR would have cost the world $100 trillion and caused ten million deaths. To address this issue, extensive research has been done on alternative therapeutic approaches based on natural resources, particularly medicinal plants, to create new antibiotics or therapeutic modalities, find substitutes for currently available antibiotics, and create alternative molecules that are effective against infectious diseases (Zouirech *et al*., 2022). Essential oils (EOs), a key source of bioactive molecules from aromatic and medicinal plants that could be used to treat infectious disorders, are also known as terpenes (Et-Touys *et al*., 2017). Almost all cultures have used medicinal herbs in traditional medicine for thousands of years. Approximately 200 years ago, herbal remedies dominated our pharmacopeia, and about 25%

of the natural substances recommended globally were derived from plants. In the past, all medicines were made from plant materials, whether they were actual plant parts (such as leaves, rhizomes, roots, stems, bark, or fruits) or just simple extracts or mixes. Alkaloids, terpenoids, and polyphenols are among the bioactive substances found in plant extracts. These substances have a variety of functions, including antibacterial, antiviral, antifungal, antioxidant, anti-inflammatory, and anti-obesity properties (Allegra, 2019; Zielinska-Blizniewska *et al*., 2019). They are used to treat a variety of ailments, including rheumatoid arthritis, osteoporosis, cardiovascular problems, and skin conditions (Sitarek *et al*., 2020a). Traditional medicine has become increasingly popular as a primary form of healthcare worldwide over the past few decades, particularly in underdeveloped nations and nations where conventional medicine is the norm (Yuan *et al*., 2016). Additionally, plant medicines offer logical ways to treat numerous illnesses that are untreatable by conventional medical practices. The World Health Organisation (WHO) estimates that around 80% of the world's population lives in developing nations and relies primarily on plants for treatment because they do not have access to modern medicine. Additionally, of the 252 medications that the WHO considers to be basic and essential, 11% are sole of plant origin, and a sizable portion are synthetic medications made from natural precursors (Sitarek *et al*., 2020b). For present investigation fresh leaves of *Datura stramonium, Jatropha curcas, Lantana camara, and the* bark of *Saraca asoca* were used for antioxidant profiling and antifungal assessment of against human pathogenic fungal strains of *Aspergillus niger* and *Candida albicans*.

II. MATERIALS AND METHODS

1. **Collection of Medicinal Plants and their identification:** Fresh leaves of *D. stramonium, J. curcas, L.camara, and the* bark of *S. asoca* were collected from local areas of Chhattisgarh. For identification, plant parts were verified by a botanist at the Government Nagarjuna Post Graduate College of Science Raipur, Chhattisgarh.

2. **Preparation of plant extracts:** Various parts of the experimental plants were shed-dried at room temperature and crushed in small pieces followed by constant weight analysis prior to extraction by using the Soxhlet apparatus. Details of plant parts and various solvents used for extraction are mentioned below in Table 1.

Table 1: Details of Plant Parts and Various Solvents Used for Extraction Purpose

S. No.	Name of Plant	Plant Part to Used	Solvent Preferred
1	Datura Stramonium	Leaves	Chloroform and Acetone
2	Jatropha Curcas	Leaves	Ethanol and Methanol
3	Lantana Camara	Leaves	Acidified Methanol Methanol: Chloroform: Water
4	Saraca Asoca	Bark	Water

3. **Qualitative Estimation**

- ***Alkaloid:*** The 500µL of extract was added in 500µL of Hanger's reagent (saturated picric acid solution) and mixed thoroughly. The presence of alkaloids was confirmed by the formation of a yellow colour precipitate (Sharmila *et al*., 2021).

- ***Flavonoids:*** The 500µL of the extract was treated with 500µL of 10% lead acetate solution, the appearance of yellow colour indicated the presence of flavonoid (Gupta *et al*., 2015).

- ***Phenol:*** The 500µL of the extract was mixed with 1mL of 2% of freshly prepared chloride. The appearance of blue-green or black colour indicated the presence of phenol in the extract (Purushothaman *et al*., 2021).

- ***Saponin:*** The 500µL of the extract was dissolved in 2mL of distilled water and shaken vigorously. The foam's persistence for minutes demonstrate the presence of saponin's in the extract (Purushothaman *et al*., 2021).

- ***Tannin:*** One mL of water and a few drops of 5% freshly prepared ferric chloride solution, were mixed thoroughly. The appearance of a green or black precipitate indicated the presence of tannin in the extract (Dahanayake *et al*., 2019).

- ***Ascorbic Acid:*** The 500µL of the extract was treated with 500µL of dinitrophenyl hydrazine dissolved in concentrated sulphuric acid. The formation of a yellow precipitate demonstrated the presence of ascorbic acid in the extract (Bhandary *et al*., 2012).

4. Quantitative Estimation

- ***Alkaloid:*** The estimation of alkaloids was determined by preparing phosphate of pH 4.7 and bromocresol green (BCG) solution. Mixed 500µL of extract and add 500µL of the above reagent (phosphate buffer and BCG solution) to it. Mixed 3mL chloroform in the tubes. The yellow color layer obtained at the bottom is used to read the absorbance at 470nm against blank by discarding the upper blue layer. Atropine was used as a standard different concentration (Madhu *et al*., 2016).

- ***Flavonoids:*** The estimation of total flavonoid was carried out by the aluminum chloride colorimetric method with some modifications. 500µL of sample extract was mixed with 1.5mL of methanol, 0.1mL of 10% aluminum chloride, 0.1mL of 1M potassium acetate, and 2mL of distilled water and kept at room temperature for 30 min. The absorbance was measured at 420 nm. Quercetin was used as a standard (1 mg/mL). Flavonoid content was determined from the standard curve and was expressed as quercetin (mg/mL of extract).

- ***Phenol:*** The estimation of phenol was determined by the Folin-ciocalteu reagent method with some modification 1.5mL of 10% FCR and 1mL of 2% Na_2CO_3 were added to 0.5 mL of plant extract and shaken. The resulting mixture was incubated for 15 min at room temperature. The absorbance of the sample was recorded at 765nm. Gallic acid was used as standard (1mg/mL). The result was determined from the standard curve as gallic acid equivalence (mg/g of extracted compound) (Chen *et al*., 2015).

- ***Tannin:*** The estimation of total tannin was determined by the Vanillin-Hydrochloride method. Take an equal volume of 8% hydrochloric acid in methanol and 4% vanillin in methanol. The solution was mixed just before use, and avoid using it even if is slightly colored. 500µL of extract was treated with 500µL vanillin hydrochloride reagent. The absorbance was measured at 500nm. Tannic acid 1g/mL was taken as standard (Chen *et al*., 2015).

- ***Saponin:*** The estimationof total saponin was determined by taking 500µL of extract which was dissolved in 500µL of 80% methanol, 1 mL of vanillin in ethanol was added, mixed well, and 1 mL of 50% sulphuric acid solution was added, mixed well, and incubated on water bath at 60°C for 10 min, absorbance was measured at 544nm against blank. Diosgenin dissolved in hot ethanol was used as standard (1mg/mL) (Firthouse *et al*., 2020).

5. Antioxidant Profiling of Medicinal Plant

- **Scavenging Activity of DPPH Radical:** The DPPH free radical scavenging assay was carried out for the determination of antioxidant activity, 1.5 mL of each diluted extract, and 0.5 mL of ethanol solution of 0.1 mL DPPH were mixed. The mixture was incubated in the dark at room temperature for 30 min and absorbance was measured at 517nm against blank. 100, 200, 300, 400, and 500µLof different concentrations were taken to determine the scavenging activity by DPPH. Ascorbic acid is used as an antioxidant. The following equation was used to determine each extract's radical scavenging activity percentage.

  % of radical scavenging activity = OD Control -OD Sample/ OD control x 100

  The IC_{50} value (µg/mL) was the effective concentration at which DPPH radicals were scavenged by 50% and the value was obtained by interpolation from linear regression analysis (Shekhar *et al*., 2014).

- **Hydroxyl Radical Scavenging Activity:** The 1.0mL of extract solution, 0.5mL of ortho-phenanthroline (7.5 m mol^{-1}), 2.0mL of phosphate buffer (0.2M, pH 6.6), 0.5mL of ferrous sulfate and 0.5mL H_2O_2 (0.1%) were mixed and diluted with 1mL of distilled water. After incubation at room temperature for 30 min, absorbance was measured at 510nm. Different concentrations of 200, 400, 600, and 800µL were taken to determine hydroxyl radical scavenging activity. The scavenging percentage (P%) was calculated as P% = (A-A1) (A2-A1)*100 (Shekhar *et al*., 2014).

- **Reducing Power Assay:** The 1mL of diluted extract was mixed with 1.5mL of phosphate buffer (0.2m, pH 6.6) and 1.5mL of potassium ferrocyanide (1%). The mixture was incubated at 50°C for 20 min. 1.5 mL of aliquots of trichloroacetic acid (10%) was added to the mixture and centrifuged at 3000 rpm for 10 min. The upper layer of the solution was transferred to another tube and diluted with distilled water and 0.5mL of $FeCl_3$ (0.1%). 200, 400, 600, and 800µL of different concentrations were taken to determine the reducing activity of the extract. The increased absorbance

was measured at 700nm against the blank indicating the increasing of reducing power (Keshari *et al*., 2018).

- **Total Antioxidant Activity by FRAP Assay:** The FRAP method was used to estimate the total antioxidant activity which measures the reduction of ferric ion to the ferrous form in the presence of antioxidant compounds. The fresh FRAP reagent consists of 500µL of acetate buffer (300Nm pH 3.6), 50mL of 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) (10mM), and 50mL of $Fecl_{3.}$ $6H_2O$ (50Mm). For the assay, 75µL of each extract was mixed with 2 mL of FRAP reagent, and the absorbance was recorded after 2 minutes at 593nm against blank (Munteanu *et al*., 2021).

6. **Antifungal Assay:** Two pathogenic strains of fungi were used in the study, *Candida albicans* (ITCC 5479) and *Aspergillus niger* (ITCC 4718) were procured from ITCC, Division of Plant Pathology, IARI, New Delhi.

   - **Anti-Fungal Assessment by Disc Diffusion Method:** The antifungal activity of the extracts was evaluated by the disc diffusion method. The fungal strains were aseptically spread with the help of a spreader on the sterile and solidified PDA plates uniformly. The four layers of 5mm sterilized paper disc (Whatman no.1) were impregnated on the surface of the PDA plates. The plates were kept for a few minutes at room temperature, for the diffusion of the extract, thereafter the plates were incubated at 30°C for 48 h for the *A. niger* colony and 24 h for the *C. albicans* colony. The disc devoid of extract and the presence of the same amount of DMSO will serve as a negative control. The antifungal disc impregnated with extract was used as a positive control. After incubation, the zone of inhibition if any around the disc will be measured in mm. Ketoconazol was taken as standard for both strains of fungi. Each assay in this experiment was replicated three times (Alhajj *et al*., 2019).

7. **Statistical analysis:** All tests were performed in triplicate and the result was expressed as mean ± standard. DPPH scavenging activity was calculated using linear regression analysis.

III. RESULTS

1. **Qualitative Estimation:** The outcomes of preliminary phytochemical screening for six secondary metabolites viz. alkaloid, flavonoid tannin, saponin, phenol, and acid were carried out in seven different extracts prepared from leaves of *D. stramonium, J. curcas, L. camara,* and bark of *S. asoca.* This screening revealed the presence of flavonoid, saponin, and phenol in all four plants, alkaloid, and tannin in the rest of the three except *S. asoca and* ascorbic acid did not show its presence in any sample (Table 2).

2. **Quantitative Estimation:** The quantitative estimation of these medicinally active compounds present in different fraction of plants was done by standard.. Quantitative estimation of alkaloid revealed its presence in the highest amount in chloroform extract of *D. stramonium*and *J. curcas* (400µg) followed by *L. camara.* Total flavonoid content was showed maximum by *D. stramonium* (260µg) lead by *J. curcas, L. camara,*and *S. asoca.* Total tannin content was evaluated in *L. camara* (480µg) at the highest amount compared to *J. curcas and D. stramonium.* Quantitative estimation of saponin indicated the presence

in all extracts but in *J. curcas* it was available in the highest conc. The foremost amount of total phenol content was shown by *S. asoca (390*µg) than *J. curcas, L. camara,* and, *D. Stramonium.*

Table 2: Phytochemical screening of secondary metabolites measured in microgram (µg)

Datura stramonium							
Solvent > Metabolites[V]	**Chloroform**	**Acetone**	**Ethanol**	**Methanol**	**Acidified Methanol**	**M:C:W**	**Water**
Alkaloids	400	180	185	150	180	-	-
Flavonoids	-	-	180	260	90	-	-
Tannins	-	55	-	34	-	30	65
Saponins	-	-		-	65	40	60
Phenol	-	-		-	-	160	-
A. acid	-	-		-	-	-	-
Jatropha curcas							
Alkaloids	400	180	160	215	160	-	-
Flavonoids	-	90	90	165	165	165	145
Tannins	-	-		-	-	55	355
Saponins	-	-	-	195	-	135	145
Phenol	-	-	300	355	-	25	-
A. acid	-	-	-	-	-	-	-
Latana camara							
Alkaloids	195	-	200	160	-	-	-
Flavonoids	-	-	80	95	-	-	-
Tannins	-	55	480	260	345	255	250
Saponins	-	80	-	-	90	30	150
Phenol	-	155	160	360	220	165	55
A. acid	-	-	-	-	-	-	-
Saraca asoca							
Alkaloids	-	-	-	-	-	-	-
Flavonoids	-	70	-	-	80	-	-
Tannins	-	-	-	-	-	-	-
Saponins	-	-	50	85	-	145	60
Phenol	-	-	70	390	245	95	45
A. acid	-	-	-	-	-	-	-

Data given are the mean of three replicates; Symbol (–) indicates absence; M:C:W Methanol:Chloroform:Water

3. **Hydroxyl Radical Scavenging Activity:** The hydroxyl radical scavenging activity of the various extracts was investigated. All extracts exhibit scavenging abilities for the hydroxyl radical. M.C.W extract of *D. stramonium* was found to be the most powerful scavenger of hydroxyl radical, with inhibition of up to 97% in M.C.W and 77% in chloroform extract of *J. curcas*, 85% in aqueous extract of *D. stramonium*, 80% in M.C.W and 77% in chloroform extract of *S. asoca* and 75% in acetonic extract of *L. camara* at concentration of 800µL. 3% inhibition was shown by methanolic extract and 7% by acidified methanolic of *D. stramonium* at the concentration of 100 µL. The results showed that the extracts obtained had excellent scavenging activities for the hydroxyl radical (Table 3).

Table 3: Hydroxyl Radical Scavenging Activity in Percentage (%)

D. stramonium							
Solvent⟶ Dose (µL)↓	**Chloroform**	**Acetone**	**Ethanol**	**Methanol**	**Acidified Methanol**	**M:C:W**	**Water**
200	15	53	38	7	3	21	31
400	76	76	39	9	23	37	48
600	77	77	46	13	32	67	64
800	79	79	55	29	62	97	85
J. curcas							
200	41	8.0	36	48	15	48	15
400	42	54	45	56	31	56	31
600	45	66	63	62	47	62	47
800	48	66	65	71	90	71	62
L. camara							
200	56	44	54	64	26	17	11
400	73	55	60	66	66	54	26
600	73	71	66	67	67	62	39
800	74	75	70	72	71	68	60
S. asoca							
200	47	18	68	49	48	18	41
400	48	31	68	55	54	43	43
600	55	56	73	57	56	60	43
800	77	67	87	72	61	80	64

Data given are the mean of three replicates; M:C:W Methanol:chloroform:water

4. **Total Antioxidant Activity by FRAP:** The total antioxidant activity by FRAP was shown maximum in *S. asoca* acetonic extract (3.8µML^{-1}) followed by *D. stramonium* ethanolic extract (3.7µML^{-1}), *J. curcas* ethanol extract (3.0 µML^{-1}) and *L. camera's* M:C:W extract (3.1 µML^{-1}) respectively. The minimal total antioxidant activity was recorded in the acetonic extract of *D. stramonium* (1.2 µML^{-1}) (Table 4).

Table 4: Total antioxidant activity by ferric reducing antioxidant power measured in μML^{-1}

D. stramonium Dose (75 µL)↓	**Chloroform**	**Aceton**	**Ethanol**	**Methanol**	**Acidified methanol**	**M:C:W**	**Water**
	2.6	1.2	3.7	3.2	0.2	2.6	2.4
J. curcas	2.9	2.0	3.0	2.2	2.7	2.6	3.1
L. camara	2.6	2.8	2.2	2.8	2.4	3.1	2.1
S. asoca	2.7	3.8	3.5	2.4	2.9	3.1	2.4

Data given are the mean of three replicates; M:C:W Methanol:Chloroform:Water

5. **Antifungal Activity:** The study was conducted to investigate the antifungal action of plant extract prepared in seven different organic solvents with eight different concentrations. *In vitro*, antifungal assessment for *A. niger* was performed by measuring the zone of inhibition in mm and was calculated by conducting the test in triplicates (mean±SD). Among the seven considered solvents, the zone of inhibition was measured maximum in ethanolic extract of *L. camara* 12.0± 4.0 at a concentration of 2.5µL followed by methanolic extract 7.6µL±1.1 at a concentration of 2.5µL and 6.0±0.7 for chloroform extract at the concentration of 5.0µL. After *L. camara, D. stramonium* ethanolic extract exhibitsa higher zone of inhibition 10.0±0.7 at the concentration of 1.25µL and methanolic extract 6.6±0.7 at the concentration of 10µL. *S. asoca* in acetonic extract 9.3±0.5 at a concentration of 20µL exhibits a minimum zone of inhibition followed by methanolic extract 7.3±1.5 at a concentration of 0.312µL and ethanolic extract 7.0±1.1 at a concentration of 0.625µL. There was no antifungal activity in *J. curcas* with the respective solvents (Table 5).

Table 5: Antifungal Activity of Different Solvent Extracts of Selected Plants Against *A. Niger*

D. stramonium		**Zone of inhibition in mm**		
Solvent→ Dose (µL)↓	Chloroform	Acetone	Ethanol	Methanol
20	-	-	6.3±0.5	6.3±1.1
10	-	-	7.3±1.5	6.6±0.7
05	-	-	6.6±0.5	-
2.5	-	-	6.0±0.5	-
1.25	-	-	10.0±3.4	-
0.625	-	-	7.0±1.0	-
0.312	-	-	-	-
0.156	-	-	-	-
L. camara				
20	6.3±0.5	-	7.0±1.7	5.6±0.5
10	6.6±1.1	-	8.0±2.0	6.3±0.5

05	6.6±0.5	-	10.0±2.0	6.6±0.5
2.5	6.3±0.5	-	12.0±2.0	7.6±1.1
1.25	-	-	11.6±3.0	6.3±0.5
0.625	-	-	10.0±3.0	-
0.312	-	-	-	-
0.156	-	-	-	-
S. asoca				
20	-	9.3±0.5	7.3±1.1	6.0±1.5
10	-	7.6±0.5	6.3±0.5	7.0±1.0
05	-	6.3±1.1	6.3±0.5	6.6±1.1
2.5	-	7.0±1.0	6.6±0.5	7.0±1.0
1.25	-	6.3±0.5	6.3±1.0	6.6±0.5
0.625	-	6.6±1.1	7.0±1.0	7.3±0.5
0.312	-	7.3±0.5	-	7.3±1.5
0.156	-	-	-	-

Data given are the mean of three replicates ± SD; no activity was observed around the control (DMSO impregnated) disc; symbol (-) indicatesthe absence

J. curcas showed a maximum zone of inhibition for extract 12.6±2.0 at a concentration of 20µL followed by ethanolic extract 10.0±1.0 at a concentration of 0.625µL, M.C.W extracts aqueous extract 7.3±0.5 at a concentration of 2.5µL against *C. albicans, D. stramonium* possess zone of inhibition next to *J. curcas* using methanolic extract 11.0±1.0 at the concentration of 1.25µL followed by M.C.W extract of 10.3±3.0 at the concentration of 10µL, chloroform extract 9.6±1.1 at a concentration of 10µL, and ethanolic extract 9.3±1.1 at concentration of 2.5µL. The pattern followed by *S. asoca* and *L. camara* is depicted in Table 6.

Table 6: Antifungal Activity of Different Solvent Extracts of Selected Plant Against *C. albicans*

D. stramonium	**Zone of inhibition in mm**						
Solvent→ Dose (µL) ↓	Chloroform	Acetone	Ethanol	Methanol	Acidified methanol	M:C:W	Water
20		-	8.6±1.5	10.0±1.7	-	10.3±1.5	9.3±1.5
10	9.6±1.1	-	8.0±1.7	9.0±1.7	-	10.3±1.5	8.6±1.5
05	9.3±2.5	-	7.3±1.1	6.6±1.1	-	9.3±1.1	10.3±1.5
2.5	8.6±1.5	-	9.3±1.1	9.0±1.0	-	9.6±1.5	9.3±0.5
1.25	6.6±0.5	-	8.6±1.5	11.0±1.1	-	9.6±1.0	7.6±1.5
0.625	7.0±1.0	-	7.6±1.5	8.6±1.1	-	9.6±1.1	6.6±0.5
0.312	7.6±0.5	-	7.6±0.5	9.2±1.0	-	-	10.6±0.5
0.156	-	-	-	8.3±0.5	-	-	-
L.camara							
20	-	-	8.6±0.5	-	-	-	-

10	-	-	7.3±0.5	-	-	-	-
05	-	-	7.0±1.0	-	-	-	-
2.5	-	-	8.0±1.0	-	-	-	-
1.25	-	-	8.3±2.5	-	-	-	-
0.625	-	-	7.6.0±2.0	-	-	-	-
0.312	-	-	7.3±0.5	-	-	-	-
0.156	-	-	7.6±0.5	-	-	-	-
S. asoca							
20	-	7.3±0.5	9.0±1.0	9.3±1.5	7.3±1.5	8.3±1.5	7.3±0.5
10	-	7.0±1.0	9.3±1.5	9.6±2.5	6.6±1.1	7.3±2.3	7.6±0.5
05	-	6.6±0.4	8.6±1.1	9.6±1.5	7.0±1.0	8.3±2.0	6.6±0.5
2.5	-	8.3±0.5	9.3±1.1	9.3±0.5	9.3±2.5	10.0±3.0	7.6±0.5
1.25	-	7.3±0.5	8.0±1.0	8.3±0.5	9.0±1.0	9.3±1.5	6.6±0.5
0.625	-	6.3±1.5	9.6±2.0	10.0±2.0	8.6±0.5	6.6±0.5	8.6±0.5
0.312	-	6.3±0.5	7.3±1.5	10.3±3.0	7.6±0.5	9.0±2.6	8.3±1.5
0.156	-	6.6±1.1	-	10.3±1.5	-	9.0±2.6	7.6±2.0

Data given are the mean of three replications± SD; no activity was observed around control (DMSO impregnated) disc symbol (-) indicated absence.

IV. DISCUSSION

Secondary metabolites contribute significantly to the biological activities of medicinal plants such as hypoglycemia, anti-diabetic, antioxidant, anti-inflammatory, anti-carcinogenic, anti-malarial, anti-cholinergic, anti-leprosy activities, etc. The four medicinal plants screened for six secondary metabolites alkaloid, flavonoid, tannin, saponin, phenol, and ascorbic acid. The present screening of phyto-constituents revealed the presence of flavonoids, saponin, and phenol in all four plants. Alkaloids and tannins are also present in three plants. Excluding *S. asoc* ascorbic acid was not detected in any of these medical plants (Mohan *et al*., 2016).

Quantitative estimation measured the amounts of secondary metabolites in the plant extracts. Alkaloid was found maximum in *D. stramonium* and *J. curcas* (400µg), the highest amount of tannin was present in *L. camara* (480µg), saponin in *J. curcas* (150µg), the flavonoid in *D. stramonium* (260µg) and phenol in *S. asoca* (390µg). These plants are also known to possess specific amounts of secondary metabolites.

The antioxidant activity in a relatively shorter time in the DPPH free radical scavenging model. The results were expressed as IC_{50}, ascorbic acid was used as standard, whose IC_{50} is 57.61 µg/mL whereas the crude ethanolic extract of leaves of *D. stramonium* showed an IC_{50} value of 249.58 µg/mL followed by acetonic,methanolic, acidified methanolic M.C.W and chloroform extract. *L. camara* showed IC_{50} of 164.74µg/mL for methanolic extracts. *J. curcas* showed IC_{50} of 162.44 µg/mL with ethanolic extract followed by methanolic, M.C.W, chloroform, acidified methanolic, aqueous, and acetone extracts. *S. asoca* showed IC_{50} 104.37 µg/mL with acetonic extract followed by aqueous, chloroform, M.C.W, methanolic ethanolic, and acidified methanolic extract. The hydroxyl radical

scavenging activity of the various extracts was analyzed. All extracts exhibit scavenging abilities for hydroxy radicals. *D. stramonium* possesses higher scavenging activity of 97% followed by *J. curcas at* 85%, *S. asoca at* 77%, and *L. camara* showed 75% of scavenging activity. Reducing power assay is used to measure the reductive ability of antioxidants by the transformation of Fe^{3+} to Fe^{2+} in the presence of leaf and bark extract. The observation suggests that, as the concentration of different extracts with their respective solvents increases their reducing power also increases proportionally. FRAP assay uses antioxidants as reductants in a redox link colorimetric method the principle of this method is based on the ferric complex of TPTZ to its ferrous colored form in the presence of antioxidants. The total antioxidant activity by FRAP was estimated maximum in *S. asoca* 3.8 μML^{-1}, followed by *D. stramonium, J. curcas,* and *L. camara.* Antioxidant profiling by DPPH assay, hydroxyl radical scavenging activity, reducing power assay, and FRAP were previously reported by many researchers in the following plants with satisfactory results (Ananth *et al*., 2015; Rajlakshmi *et al*., 2016).

Antimicrobial activities of four medicinal plant leaves and bark extract have been reported by many researchers. Successful prediction of bioactive compounds from plant material is largely dependent on the type of solvents used in the extraction procedure.

The result of the antifungal assay carried out with seven different organic extracts prepared from *D. stramonium, J. curcas, and L. camara* leaves and *S. asoca* bark showed antifungal activity against two pathogenic fungi *C. albicans* and *A. niger* which was done in a dose-dependent manner. The antifungal activity against *A. niger* was shown by *L. camara* with a zone of inhibition of 12.0±4.0 at a concentration of 2.5µL, followed by *D. stramonium* and *S. asoca* and there was no antifungal activity observed in *J. curca* against *A. niger.* Evaluation of antifungal activities against *C. albicans* for medicinal plant *J. curcas*was a maximum of 12.6±2.0 at a concentration of 20µL followed by *D. stramonium, S. asoca,*and *L. camara.* Previous studies conducted on*L. camara* and *J. curcas* weresatisfied with the present finding (Sailaja *et al*., 2014).

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