International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 23-07-2020; Accepted: 08-08-2020: Published: 24-08-2020
www.botanyjournals.com
Volume 5; Issue 4; 2020; Page No. 281-285

Evaluation of antifungal activity of some selected plants of family fabaceae & the HPTLC analysis of most effective plant *Peltophorum pterocarpum* (DC.) K. Heyne

Asbaha K[1], Usman Arerath[2*]
[1-2] Post Graduate, Department of Botany, KAHM Unity Women's College, Manjeri, Malappuram, Kerala, India

Abstract
In recent years, reports exist on antifungal activity of peptides and proteins isolated from medicinal plants that have mainly concerned species of Fabaceae family. Here worked on extracts from leaves of selected plants of family Fabaceae and determined the antifungal activity against the fungal strain *Pochonia chlamydosporia*. In this study we evaluate the anti-fungal properties of aqueous extracts of leaves of 6 plants in fabaceae family *viz. Adenanthera pavonia* L., *Albizia saman* (Jacq.)Merr, *Bauhinia acuminata* L., *Peltophorum pterocarpum* (DC) K. Heyne, *Senna auriculata* (L.) Roxb, *Millettia pinnata* (L.) Pierre. against *Pochonia chlamydosporia* (Goddard) Zare& W. Gams by disc diffusion method. *Peltophorum pterocarpum* shows high anti-fungal activity against selected fungi *Pochonia chlamydosporia* as compared to others. Phytochemical effect of methanolic leaf extract of most effective plant *Peltophorum pterocarpum* (DC) K. Heyne also determined.

Keywords: antifungal property, *peltophorum pterocarpum*, *pochonia chlamydosporia*

1. Introduction
Antimicrobial agents are essentially important in reducing the global burden of infectious diseases (Bhatia and Narain, 2010) [3]. The use of antimicrobial medicines to treat infection is known as antimicrobial chemotherapy, while the use of antimicrobial medicines to prevent infection is known as antimicrobial prophylaxis. However, emergence and dissemination of multidrug resistant (MDR) strain in pathogenic bacteria have become a significant public health threat as there are fewer, or even sometimes no, effective antimicrobial agents available for the infection caused by pathogenic bacteria (Boucher *et al*., 2009) [4]. Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides, etc., which have been found *in vitro* to have antimicrobial properties (Dahanukar *et al*., 2000) [5]. In recent years, reports exist on antifungal activity of peptides and proteins isolated from medicinal plants that have mainly concerned species of Fabaceae family (Abad *et al*., 2007) [1].
For the soil borne pathogens, use of fungicides is not practical due to exorbitant cost and environmental hazards involved (Harman 1991) [9]. For the identification of alternative methods for plant protection, a serious search is needed which are less reliant on chemicals and are more environmentally friendly (Nair *et al*., 2002) [10]. Numerous reports are based on the potential use of biocontrol agents as alternatives for agrochemicals (Shimizu *et al*., (2000) [11], Yang *et al*., (2007) [12]. Plant extracts (Davidson and Parish, 1989) [13]. and essential oils (Singh *et al*., 1980) [14]. show antifungal activity against a wide range of fungi.
The present study shows the anti-fungal properties of aqueous extracts of leaves of 6 plants in fabaceae family *viz. Adenanthera pavonia* L., *Albizia saman* (Jacq.) Merr, *Bauhinia acuminata* L., *Peltophorum pterocarpum* (DC) K. Heyne, *Senna auriculata*(L.) Roxb., *Millettia pinnata* (L.) Pierre. against *Pochonia chlamydosporia* (Goddard) Zare& W. Gams. Also determine phytochemical effects of methonolic leaf extract of most effective plant *Peltophorum pterocarpum* (DC) K. Heyne and its alkaloid content.

2. Materials and Methods
Collection of plants
The preliminary effort of the study was collection of 6 members of family Fabacae includes *Adenanthera pavonina* L., *Albizia saman* (Jacq.) Merr., *Bauhinia acuminata* L., *Millettia pinnata* (L.) Pierre, *Peltophorum pterocarpum* (DC.) K. Heyne, *Senna auriculata* (L.) Roxb, collected from the campus of K.A.H.M. Unity Women's college Manjeri, and nearby area in the month of April 2019 (N 11^1 12°N, $76^1$12°E). The fresh plant leaves were washed, cleaned and dried under shade for 15 days. Air dried leaves were grinded into fine powder using electric blender. Then stored in refrigerator (4°C) until further study.
Preparation of aqueous extract
10g of dried and powdered leaves sample measured and make a pack with cotton cloth,put the sample in it,tied tightly. It soaked in 100ml of distilled water and placed in water bath at 75°C for 3 hours and leaved for overnight. The extract were collected and stored in sealed beaker. Then prepared different percentage solution of 25%, 50% and 75% from stock solution for testing the anti-fungal activity of plant.
Test microorganism.
The fungal strain selected for this study were *Pochonia chlamydosporia*, which is collected from K.A.H.M. Unity Women's College. The lactophenol cotton blue (LPCB) wet mount preparation is used for staining and observing fungi.
Antifungal activity.
The selected fungi *Pochonia chlamydosporia* were grown on PDA (Potato Dextrose sugar, Agar) medium in pH 5-6 ±0.2. Disc diffusion method is used to study antifungal activity of plant extracts of Fabaceae members. Solidified PDA medium in the sterilized petridishes were inoculated

by fungal cultures by streaking the L-shaped swab over the entire sterile agar surface.Using forceps 4 sterile disc P1, P2 (plant extract with different concentration),C as control (sterile water) and +ve(as positive control by Streptomycin.) were placed in each petriplates in equal distance. Plant extracts with different concentrations 25%, 50%, 75% were pipetted out 1ml each into sterile, blank disc. Finally the petriplates were covered with sterilized polythene cover to protect from contamination. Then kept it in the BOD incubator at 29º C for 4-5 days.

Inhibition zone is measured by using standard scale and anti-fungal activities of plants are measured by evaluating the inhibitory zone. Antifungal activity was then determined by measuring the diameter of the growth –inhibition zone in millimeters. Absorbance values of leaf extracts of different concentration were measured by spectrophotometer at 700nm. HPTLC (High Performance Thin Layer Liquid Chromatography) analysis High performance thin layer liquid chromatography is the most advanced form of thin layer chromatography. It is used for both qualitative and quantitative analysis.HPTLC has become a routine separation and analytical technique for standardization of a specific sample (reference).

Preparation of methanolic extract: Methanolic extract of most effective plant *Peltophorum pterocarpum* were prepared by the powdered leaf sample. 10 g of dried leaves sample in Soxhlet extraction apparatus filled with 200 ml of methanol gives the methanol extract. 10 ml of methanol extract were collected and its phytochemical analysis was evaluated by HPTLC method at Center for Medicinal Plant Research (CMPR), Arya Vaidya Sala, Kottakkal, Malappuram.

Test for Alkaloids

Total alkaloid content determination using Harborne, (1973) method.

3. Results and Discussion

The results of preliminary antifungal activity investigation of aqueous extract of 6 Fabaceae members using disc diffusion method revealed that all plants leaves extracts taken under the study show anti-fungal activity against *Pochonia chlamydosporia.* Antifungal activity of *Peltophoprum pterocarpum* shows good results as compared with other leaf extracts. In these, *Peltophoprum pterocarpum* had the best antifungal activity even at 25%. The scale of potency of the 6 extracts inhibiting the *Pochonia chlamydosporia* mycelial growth is as follows:*Peltophoprum pterocarpum>Millettia pinnata> Senna auriculata> Bauhinia acuminata>Albizia saman>Adenanthera pavonina.* The last plant extract shown to be the least effective one in reducing *in vitro* mycelial growth of fungi. The most effective *Peltophorum pterocarpum* shows maximum zone diameter of inhibition at higher concentration 75% (diameter of 25.5 ± 0.70 mm), Minimum diameter of zone of inhibition at 25% (13.75 ± 0.35 mm) and at 50% it shows 19.5 ± 3.53 mm diameter of zone of inhibition. In drug designing OD values were considered. OD values of the 6 selected plants of Fabaceae members were calculated. Given in table

Table 1: Average diameter zone of inhibition, Absorbance and % of Transmittance

Si No	Plant Name	Conc;	PC (mm)	P1 (mm)	P2 (mm)	Average D.Z.I (mm)	Absorbance	%T
1.	Adenantherapavonia	25%	30	0	12	6	-0.0008	100.13
		50%	29	12	10	11	-0.0015	100.13
		75%	30	11	12	11.5	-0.0016	100.32
2.	Albiziasaman	25%	33	0	11	5.5	0.0016	99.68
		50%	32	9	10	9.5	0.002	100
		75%	33	15	16	15.5	0.008	100.13
3.	Bauhinia acuminata	25%	30	18	13	15.5	0.0013	99.77
		50%	27	16	15	15.5	0.0013	99.77
		75%	31	16	17	16.5	0.002	100
4.	Millettiapinnata	25%	26	15	10	12.5	-0.006	100.07
		50%	30	22	21	21.5	0.0016	99.69
		75%	30	23	21	22	0.0016	99.69
5.	Peltophorumpterocarpum	25%	21	13.5	14	13.75	-0.0013	100.23
		50%	26	22	17	19.5	0.0002	100
		75%	26	26	25	25.5	0.0009	99.83
6.	Senna auriculata	25%	34	18	12	15	-0.009	100.16
		50%	29	21	18	19.5	-0.006	100.07
		75%	31	21	21	21	-0.006	100.07

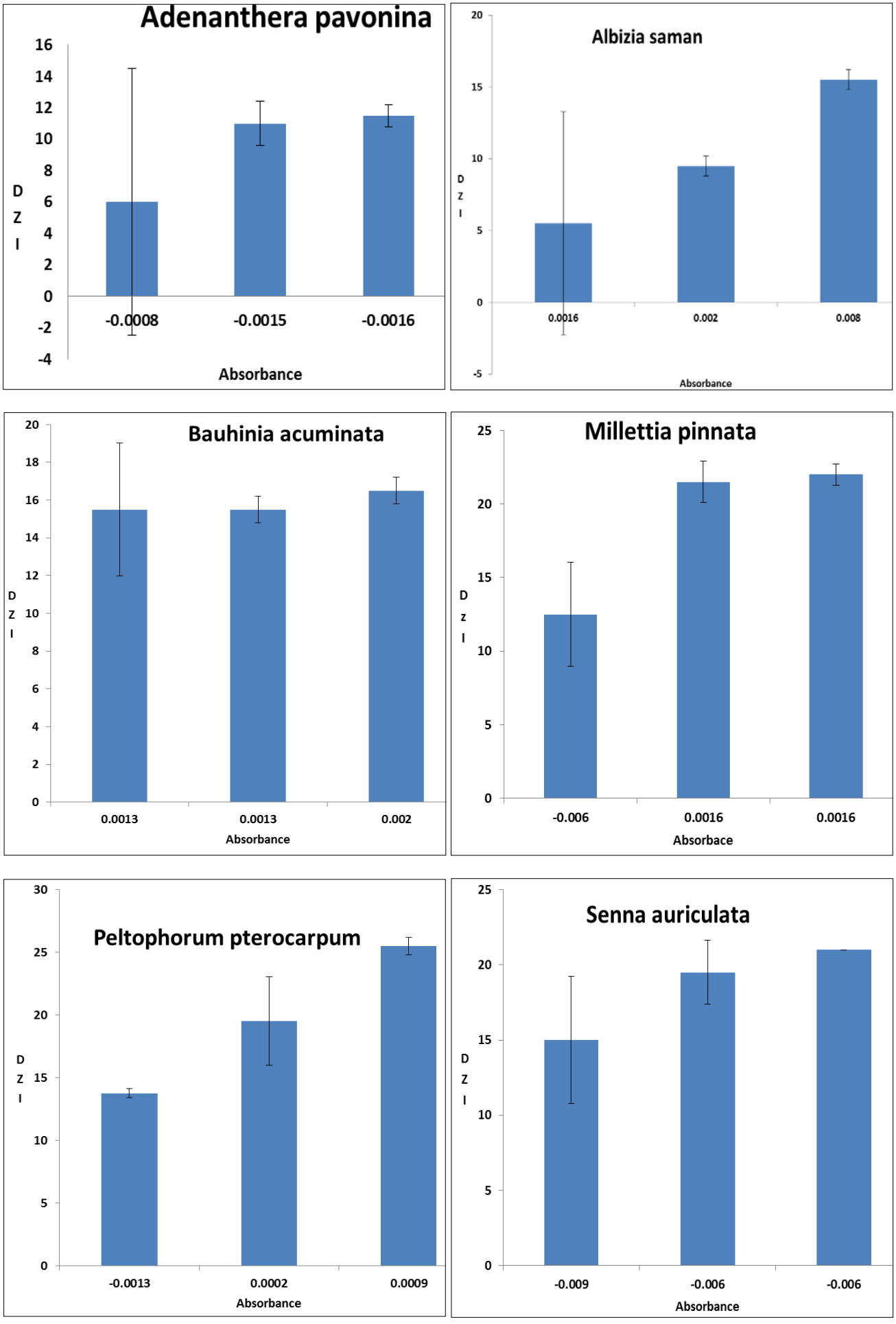

Fig 1: Graphs plotted with average zone of inhibition against its OD values.

Graphs showing Diametre zone of inhibition of each plants at different concentration against it's absorbance.

HPTLC ANALYSIS OF *Peltophorum pterocarpum* (DC.) K. Heyne

HPTLC shows clear separation of components present in the methanol extract of the plant powder of *Peltophorum pterocarpum* (DC.) K. Heyne. The method may be applied to identify the plant *Peltophorum pterocarpum* (DC.) K. Heyne of from other species. HPTLC fingerprint enables a particular plant to be identified and distinguished from closely related species (Houghton 1998). Methanolic extract of *Peltophorum pterocarpum* (DC.) K. Heyne was subjected to phytochemical analysis at 254 and 366 nm and showed 13 and 12 different phytoconstituents respectively. Rf values obtained during the analysis were presented in the Table 2 and 3 respectively.

Table 2: Rf values at 254 nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.00	2.4	0.02	263.1	21.77	0.05	82.9	4434.1	17.01	unknown *
2	0.05	83.2	0.06	95.9	7.94	0.08	46.5	1563.0	6.00	unknown *
3	0.09	46.6	0.10	51.5	4.26	0.12	31.7	937.0	3.59	unknown *
4	0.40	25.3	0.43	107.0	8.85	0.44	34.4	1422.7	5.46	unknown *
5	0.50	28.1	0.56	42.7	3.54	0.58	37.9	1684.2	6.46	unknown *
6	0.63	40.7	0.64	48.7	4.03	0.67	29.5	1160.7	4.45	unknown *
7	0.71	35.4	0.73	39.5	3.27	0.76	31.6	1364.5	5.23	unknown *
8	0.78	32.2	0.79	42.1	3.49	0.81	31.3	804.0	3.08	unknown *
9	0.82	31.6	0.85	43.3	3.58	0.88	30.2	1414.4	5.43	unknown *
10	0.97	34.0	1.00	51.5	4.27	1.02	47.7	1508.8	5.79	unknown *
11	1.05	43.9	1.06	59.5	4.92	1.07	52.2	682.0	2.62	unknown *
12	1.07	53.3	1.09	67.2	5.56	1.10	65.7	1180.6	4.53	unknown *
13	1.10	64.6	1.14	296.4	24.53	1.17	117.7	7913.3	30.35	unknown *

Table 3: Rf values at 366 nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.04	1.8	0.05	30.4	3.41	0.07	1.5	297.8	1.49	unknown *
2	0.13	1.9	0.15	14.5	1.63	0.18	5.5	311.2	1.56	unknown *
3	0.40	12.8	0.42	83.7	9.39	0.44	8.1	1010.3	5.06	unknown *
4	0.45	8.8	0.46	24.7	2.77	0.47	14.4	153.9	0.77	unknown *
5	0.54	12.8	0.55	21.8	2.45	0.58	8.5	316.8	1.59	unknown *
6	0.58	8.9	0.61	23.1	2.60	0.62	17.6	518.0	2.59	unknown *
7	0.74	16.6	0.77	25.5	2.86	0.78	14.0	498.1	2.49	unknown *
8	0.81	13.5	0.85	23.8	2.67	0.86	19.0	586.6	2.94	unknown *
9	0.86	19.5	0.86	22.9	2.57	0.88	9.6	305.5	1.53	unknown *
10	0.97	14.2	1.01	33.8	3.79	1.04	26.8	1099.7	5.51	unknown *
11	1.06	28.6	1.14	346.4	38.84	1.17	153.9	9893.2	49.53	unknown *
12	1.17	155.8	1.19	241.1	27.03	1.22	5.4	4984.9	24.95	unknown *

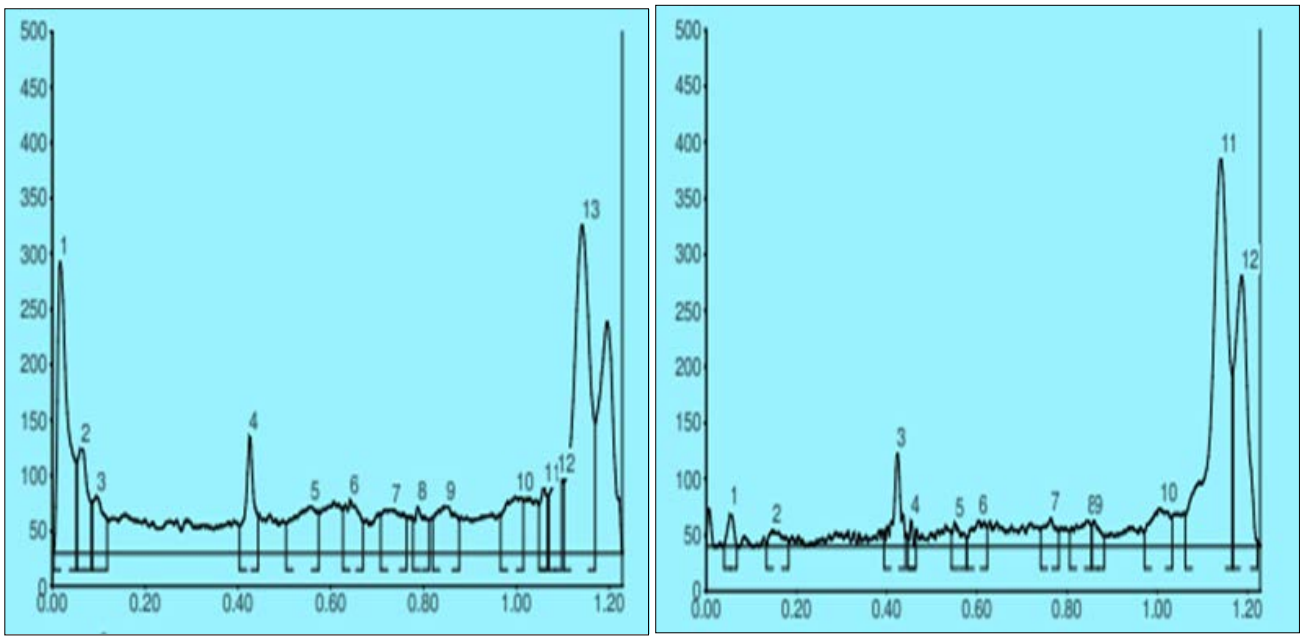

Fig 2: Densitogram display scanned at (A):254 nm and at (B): 366 nm.

Alkaloid content in the most effective leaf extract *Peltophorum pterocarpum* (DC.) K. Heyne may be one of the reason for antifungal activity. *Peltophorum pterocarpum* (DC.) K. Heyne leaf extract contains 1.19 ± 0.020 of alkaloids. The study focused that *Peltophorum pterocarpum* shows high anti-fungal property against selected fungi *Pochonia chlamydosporia* as compared to others. There were somany studies are done by different scientists on the anti-fungal activities of selected plant species in this study. Abida *et al.*, (2016) [2]. evaluate the antifungal activity of leaves and seed oil of Pongame (*Pongamia pinnata*) with the help of Poisoned Food Technique against *Sclerotium rolfsii*. The results from the study of Dahikar and Bhutada, (2017) [6]. were approximately similar to this present study by using disc diffusion method. the present study also conducted in the aqueous extract which is an traditional approach also useful to traditional drug designers. As compared to other study on *Peltophorum pterocarpum* aqueous leaf extract this study also shows a significant antifungal activity against this fungus, *Pochonia chlamydosporia*. It shows more anti-fungal activity than the control which is used as the sterile water.

3. Conclusion

Aqueous extracts of the leaves show significant activity against fungus as compared with sterile water which is used as control. It shows that even at aqueous extraction the phytochemicals of the leaves show antifungal activity. It were more useful for the traditional farmers than using other costly extraction methods. Investigations performed on the aqueous extract proved that the leaves contain higher amount of alkaloid compounds which represents that the extract have phytochemical property.However further studies are needed to better evaluate the potential effectiveness of the crude extracts as the antimicrobial agents

4. References

1. Abad MJ, Ansuategui M, Bermejo P. Active antifungal substances from natural sources. Archive for organic chemistry. 2007; 7:116-145.
2. Abida A, Iram S, Shaista A, Brian GN, Wajiha S. *In vitro* antifungal activity of *Pongamia pinnata* against collar rot pathogen (*Sclerotium rolfsii*) of Chickpea. Pure and applied biology. 2016; 5(3):520-528.
3. Bhatia R, Narain JP. The growing challenge of antimicrobial resistance in the South-East Asia Region - are we losing the battle? Indian journal of medical research. 2010; 132(5):482-486.
4. Boucher HW, Talbot GH, Bradley JS. Bad bugs, no drugs: no eskape! An update from the infectious disease's society of America. Clinical infectious diseases. 2009; 48(1):1-12.
5. Dahanukar SA, Kulkarni RA, Rege NN. Pharmacology of medicinal plants and natural products. *Indian journal of pharmacology*. 2000; 32:81-118.
6. Dahikar SB, Bhutada SA. Evaluation of antifungal properties of leaves of *Pongamia pinnata* Linn. Department of microbiology, sanjivani arts. Pharmacogyonline. 2017; 2:40-44.
7. Harborne JB. Phytochemical methods, London. *Chapman and Hall, Ltd*, 1973, 49-188.
8. Houghton PJ. Establishing identification criteria for botanicals. Drug information journal. 1998; 32:461-469.
9. Harman G. Seed treatments for biological control of plant disease. Crop Protection. 1991; 10:166-171.
10. Nair JR, Singh G, Sekar V. Isolation and characterization of a novel Bacillus strain 6from coffee phyllosphere showing antifungal activity. Journal of applied microbiology. 2002; 93:772-780.
11. Shimizu M, Nakagawa Y, Sato Y, Furumai T, Igaroshi Y, Onaka H, *et al*. Studies on endophyticactinomycetes I Streptomyces sp. isolated from *Rododendron* and its antifungal activity. Journal of general plant pathology. 2000; 66:360-366.
12. Yang L, Xie J, Jiang D, Fu Y, Li G, Lin F, *et al*. Antifungal substances produced by *Penicillium oxalicum* strain PY-1- potential antibiotics against plant pathogenic fungi. World journal of microbiology and biotechnology. 2007; 24:909-915.
13. Davidson PM, Parish ME. Methods for testing the efficacy of food antimicrobials. Food Technology. 1989; 43:148-155.
14. Singh AK, Dickshit A, Sharma ML, Dixit SN. Fungitoxic activity of some essential oils. Economic botany. 1980; 34:186-190.