Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2021; 9(6): 1798-1805
Received: 18-09-2020
Accepted: 19-10-2020

V Chandrasekaran
Assistant Professor, Department of chemistry, Government Arts College (Autonomous), Salem, Tamil Nadu, India

P Akilan
Assistant Professor, Department of chemistry, Government Arts College (Autonomous), Salem, Tamil Nadu, India

A Kannan
Assistant Professor, Department of chemistry, Government Arts College (Autonomous), Salem, Tamil Nadu, India

P Prasath
Research Scholar, Department of chemistry, Government Arts College (Autonomous), Salem, Tamil Nadu, India

Corresponding Author:
V Chandrasekaran
Assistant Professor, Department of chemistry, Government Arts College (Autonomous), Salem, Tamil Nadu, India

Studies on phytochemical and anti-microbial activities of natural plants extract

V Chandrasekaran, P Akilan, A Kannan and P Prasath

DOI: https://doi.org/10.22271/phyto.2020.v9.i6z.13212

Abstract
A green chemistry approach is employed for the biological studies of the *Aegle Marmelos (AM), Alypha Indica (AI), Acacia Arabica (AA), Ocimum Tenuiflorum (OT), Solanum Melongena (SM), Catharanthus Roseus (CR), Mongifera Indica (MI), Phylanthus Emblica (PE), Ficus Glomerata (FG), Acorus Calamus (AC), and AM, AI, AA,OT, SM, CR, MI, PE, FG, AC* with lemon using solvent like water. The plant extracts were characterized by UV-VISIBLE, FTIR, XRD, anti-bacterial and anti fungal studies. These extract were tested for anti-microbial activity against human pathogens *viz*., the result revealed that the plants extract contains phytochemical constitutions. The active compounds were identified by UV-VISIBLE, FT-IR and XRD studies. These biologically active compounds are found to be effective in controlling growth of human pathogens. This study concludes that the extract of *AM, AI, AA,OT, SM, CR, MI, PE, FG, AC* with lemon contains good anti-bacterial activity and anti fungal activities against tested microorganisms. It also concluded that *AM, AI, AA, OT, SM, CR, MI, PE, FG, AC* with lemon extract can be explored to discover the bioactive natural products that may serve as leads in the development of new pharmaceuticals.

Keywords: Natural plant extract, UV-Visible, FT-IR, XRD and anti-microbial activity.

1. Introduction
Phytochemistry is the study of the chemistry of plants. Phytochemistry or plant chemistry is a distinct field of study in between natural product plant biochemistry and organic chemistry and is related to each other. Man has sought out plants with medicinal properties since time immemorial. Evidence of this is the thousand years old traditions and records of proper healing. Even in this age of great development and progress in the field of chemistry, pharmaceutical and medicines; drugs of plant origin has never lost its their importance. Over the years scientific research has expanded and made more simple the knowledge of the chemical composition and effects of the active constituents of plants. It is thus possible to know more about their action and be more exact inprescribing their use in the treatment of various diseases [1].

Medicinal plants or rather the parts are collected and dried for example the roots, leaves, bark, flowers and seedsare the raw material used for the industrial preparation of spure active substances [2]. The synthetic preparations of many of these substances areeither unknown at present time or uneconomical for industrial purposes. The substances are employed either in their pure form or are used for the preparation of new substances, often with more significant therapeutic action. Example of active constituents from the plants which are now isolated and used by the medicinal profession in pre tableted form include the antimalarial drug Quinine from *Cinchona*, for treatment of heart failure digoxin from *Digitalis lanata*, antimicrotubule drug Vinblistine from *Vinca species*, analgesic drug Aspirin from bark of the willow tree [3]. The plants chiefly used for this purpose are those which contain aromatic substances like vitamins, aminoacids and enzymes to aid digestion and promote certain body functions [4, 5].

Medicinal plants are considered to be a chemical factory as it contain multiple of chemical components like alkaloids, terpenoids, steroids, glycosides, phenols, tannins, steroiods, flavonoids, oils and fats [6, 7]. There are approximately 270,000 species of higher plants [8], out of 5-10% have been systematically investigated for the presence of bioactive compounds [9]. The vast majority of plants have not been assessed pharmacologically for potential medicinal value, even those that are currently beingused for medicinal purposes by indigenous people [10]. Cancer is the disease area where investigators are anticipating for new molecules from natural products that can provide us with tools for fighting this dreadful disease [11]. Diabetes mellitus is another area in which researchers are focusing [12]. Arthritis and Inflammation is the other potent disease, where no satisfactory results are present in modern medicine. Thus, drug discovery from plants remains as required factor in the exploration for new medicines.

As natural products gain increasing importance and attention from chemists and pharmacologists, their discovery from new sources will continue to be essential in order to provide novel lead compounds which the synthetic chemist can never dream up. This is the major reason for embarking on research projects in the field of natural products [13].

In this present study the extraction, screening, characterization and biological activity of the *Aegle Marmelos (AM), Alypha Indica (AI), Acacia Arabica (AA), Ocimum Tenuiflorum (OT), Solanum Melongena (SM), Catharanthus Roseus (CR), Mongifera Indica (MI), Phylanthus Emblica (PE), Ficus Glomerata (FG), Acorus Calamus (AC), and AM, AI, AA,OT, SM, CR, MI, PE, FG, AC* with lemon has been carried out.

2. Material and Methods

2.1 Collection of plant materials

The fresh plant sample often different medicinal plants were collected randomly from the Uthangarai and Singarapettai forest area in Krishnagiri district,Tamil Nadu. Sample materials were washed under running tap water, it was dried in shade and then homogenized to fine powder and stored in air tight polythene bags and refrigerated at 4ºC. Lemon peel was derived from the fresh lemon and it also dried under shade, this powder was made from by using mortar and pestle.

2.2 Preparation of plant extract

2.2.1 Decoction method

Crude drug samples were prepared by Decoction method by using water as solvents. About 50 gm of powdered plant material was weighed and transferred into the beaker, and extracted with 500 ml of water at 80ºC. The process of extraction has to be continued for 4 hours or till the extraction completed. After that the extract was taken in a beaker and solvent got evaporated until the full volume reduced to quarter. Dried extract was kept in refrigerator at 4ºC for future use.

2.2.2 Preparation of plant extract with lemon peel:

About 25g of plant powder and 25g of lemon peel powder was taken in 1:1 ratio, and then the extraction has been done using the 500ml of distilled water. Above the mixture was kept on induction stove, the temperature was maintained at 80ºC as well as the above standard procedure.

2.2.3 Extraction of plant materials

The extraction of plant material was carried out were extracted by using soxhlet apparatus at 50-60^{0}C. In the extraction procedure a total amount of 50 gm powered was used. The extraction was carried out by using water. The extraction was carried out with 500 ml of each solvent for the period of 8 hours. At the end of the extraction the respective solvents were concentrated by using rotary evaporator at 80^{0}C under reduced pressure.

2.3 Phytochemical Qualitative Analysis

2.3.1 Test for Alkaloids

**Wager`s Test:** About 2ml of extract and 1ml of dilute hydrochloric acid and 1ml of wagner`s (Iodine solution) was added. The formation of reddish brown precipitate indicate the presence of Alkaloids.

**Hager`s Test:** To the 2ml of extract and 1ml of dilute hydrochloric acid and 1ml of Hager`s reagents (saturated solution of Picric acid) was added. Formation of yellow color shows the presence of alkaloids.

2.3.2 Test for Flavonoids

Lead acetate test: Add few drops of lead acetate to the 2ml of extract. Formation of yellow precipitate confirms the presence of flavonoids compounds.

Sodium Hydroxide test: To the 2ml of crude extracts, add two drops of 10% sodium hydroxide. Disappearance of color obtained by addition of dilute hydrochloric acid, intense yellow color indicates the flavonoids.

2.3.3 Test for Phenol

Ferric chloride Test: To the 2ml of extract, add 2ml of 5% ferric chloride solution. Appearance of blue or green color confirms the Phenolic compounds.

2.3.4 Test for Tannin

Ferric chloride Test: To the 2ml of extract, add 2ml of 5% ferric chloride solution. Appearance of greenish black precipitate may be presence of Pyragalcol or Catechol kind of tannin.

Lead acetate test: Add few drops of lead acetate to the 2ml of the extract. Formation of yellow precipitate or white precipitate confirms the presence of tannin.

2.3.5 Test for Amino acid and Protein

Xanthoproteic Test: To the 2ml extract add few drops of concentrated nitric acid was added, formation of yellow color.

Biureate Test: To the 2ml of crude sample, add 2ml of 10% sodium hydroxide was added and heating, then add one drop of copper sulphate solution. Violet or pink color confirms the presence of protein.

2.3.6 Test for Terpenoids

Salkowski Test: To 0.5g of each of extracts was added 2ml of chloroform and add then 3ml of concentrated sulphuric acid, deep red color were formed at the junction of two layers.

2.4 Anti-Microbial Assay

Agar- Well Diffusion Method

The antibacterial activities of all test compounds were carried out by well diffusion method. The concentrations of the test compounds were taken in DMSO and used in the concentration of 25, 50, 75, 100µg. The target microorganisms (in nutrient broth) were cultured in Mueller–Hinton Broth (MHB). After 24h the suspensions were adjusted to standard sub culture dilution. The Petri dishes containing Muller Hinton Agar (MHA) medium. The agar plates were seeded with freshly prepared different pathogens. Agar wells with diameter of 6 mm were made with the help of a sterile stainless steel cork borer. The standard drug streptomycin (10µg) was used as a positive reference standard to determine the sensitivity of each microbial species tested. Then the plates were incubated at 37 ºC for 24 h. The diameter of the clear zone around the disc was measured and expressed in millimeters as its anti-microbial activity.

Procedure

Petriplates containing 20ml Muller Hinton medium were seeded with 24hr culture of appropriate bacterial strains. Wells were cut and 1ml of respective compound solutions were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring

the diameter of the inhibition zone formed around the well (NCCLS, 1993).
Streptomycin powder was used as a positive control. The agar plates were then incubated at 37ºC. After 16 to 18 hours of incubation, each plate was examined. The resulting zones of inhibition were uniformly circular with a confluent lawn of growth. The diameters of the zones of complete inhibition were measured, which includes the diameter of the control well of streptomycin.

2.5 UV-Visible Spectroscopy
The plant extracts of *AM, AI, AA,OT, SM, CR, MI, PE, FG, AC* with and without lemon extracts was monitored by measuring UV-VIS spectrum of the reaction mixture after diluting a small aliquot of the sample with DMSO. The periodic scans of the optical absorbance between 300 nm and 900nm with a UV-Visible spectrophotometer (Elico BL 180 Bio) were recorded.

2.6 FT-IR: (Fourier Transform Infra-Red) Spectroscopy
A single-beam FT-IR spectrometer (FT-IR-7600, Lambda Scientific). The FT- IR spectra has been recorded using KBr disc for the successive extracts.

2.7. XRD Analysis
X-ray powder diffraction (XRD) is a rapid analytical technique primarily used for identification of metal ion present in the plant extract.

3. Result and Discussion
The results of the crude extracted compound obtained by using UV-Visible, FT-IR, XRD, antimicrobial activities are discussed.

3.1 Phytochemical Qualitative Analysis
The preliminary phytochemical investigation of ten different medicinal plants were reported. This ten medicinal plants are named as following *AM, AI, AA,OT, SM, CR, MI, PE, FG, AC* with and without lemon.
Our present investigation carried out into two different way of analysis, the first one was reported as plants extracts only and other thing has been reported as plant extract combined with lemon peel powder. These results were presented in the Table 1.
From the table, alkaloids, flavonoids, tannin, carbohydrate, amino acids and terpenoids were obtained in various plants. These six phyto constituents were generally investigated. From the report from table 3.1 we concluded the presence of alkaloids in AM, AM+L,OT, OT+L, PE, PE+L, AC, AC+L plants. Flavonol were presents in AM, AM+L, AA, AA+L, OT, OT+L, PE, PE+L, AC, AC+L, FG, FG+L.
From the ten plants tannin compounds presents only in CR, CR with lemon peel and FG, FG with lemon. Carbohydrate presented in the all plants. There was no amino acids. Terpenoids compounds were reported in only OT, CR, FG, AC plants were with and without lemon.

Table 3.1: Qualitative Analysis of medicinal plants

S. No	Phytoconstituents	Tests	Name of The Plants																			
			AM	AM+L	AI	AI+L	AA	AA+L	OT	OT+L	SM	SM+L	CR	CR+L	MI	MI+L	PE	PE+L	FG	FG+L	AC	AC+L
1	Alkaloids	1.Wagner`s	+	+	-	-	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
		2.Hager`s	+	+	-	-	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
2	Flavonoid	1.Lead acetate	+	+	-	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	+
		2.NaOH	+	+	-	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	+
3	Tannin	1.Ferric Chloride	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	+	-	-
		2.Lead aceate	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	+	-	-
4	Carbohyd-rate	1.Fehling test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	Amino acids	1.Biuret test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	Terpenoid	1.Salkowski Test	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	+	+	+	+	+

3.2 UV-VIS Spectrum
UV- VIS spectroscopy could be used to examine the size and shape of the sample. The absorption spectrum of the plant extract most formed in the absorption peak at 245nm (water). The UV-VIS spectrum of PL, PS, PSL shown in Figure 3.1,3.2, and 3.3 and respectively as shown in below.

Absorption value of PL = 1.9
Absorption value of PS = 2.2
Absorption value of PSL = 1.9
It could be concluded that the range is almost nearest to presence of alkaloids.

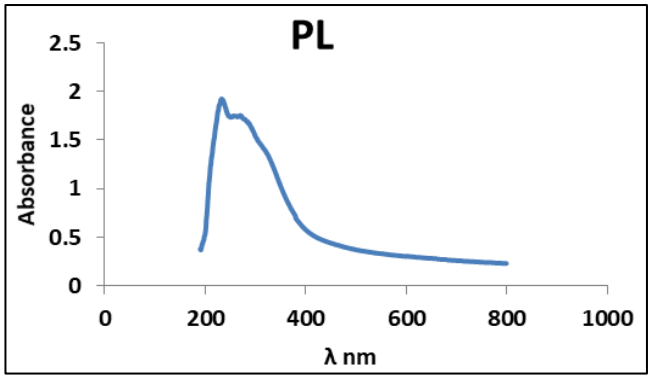

Fig 3.1: UV Vis Spectrum of PL

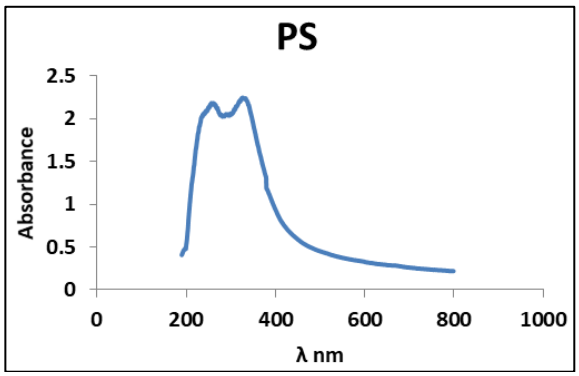

Fig 3.2: UV Vis Spectrum of PS

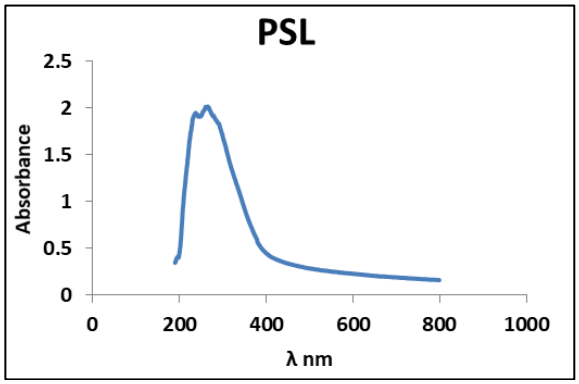

Fig 3.3: UV Vis Spectrum of PSL

3.3 FT-IR Spectrum

FT-IR Spectrum of PL, PS, PSL taken for plant material. The region in the FT-IR spectrum shows spectral bands or peaks due to the vibrational of individual bonds or functional groups. FT- IR spectroscopy is most frequently used in phytochemical studies as finger print device by comparing natural products.

FT-IR spectra were taken for water extracts of PL, PS, PSL. The FT- IR spectrum profile are illustrated in the fig 3.4-3.6. The FT-IR spectrum confirmed the presence of alkaloids, phenols, alkenes, alkanes, ketenes, ethers, amino acids and aromatic of alkaloids in the extracts. Some common functional groups stretching frequency are given below.

Table 3.2: FT-IR peak values PL, PS and PSL

S.NO	TYPES OF BOND	FUNCTIONAL GROUP	WAVE NUMBER(cm^{-1})
1	C-H	Alkenes	>3000
	C-H(Stretch)	Alkanes	2853
	C-H(Bend)	Alkanes	1450
	C-H(Bend)	Alkenes	900-1000
	C-H(Bend)	Aromatic	1750-1950
2	C-C(Stretch)	Alkanes	800-1200
3	C=C(Stretch)	Alkene	1655
		Aromatic	1600-1500
4	C=O	Amide	1655
		Aldehyde	1720-1740
		Ketone	1705-1725
		Ether	1736
5	C-O(Stretch)	Alcohol	1000-1200
		Ether	1000-1300
		Acid	1342
6	O-H(Stretch)	Alcohol	3200-3600
	O-H(Stretch)	Alcohol	3600-3700
	O-H(Stretch)	Alcohol	1000-1200
	O-H	Acid	2500-3300
7	N-H	Amine	3300-3500
8	N-O(Stretch)	Nitro compounds	1400-1550

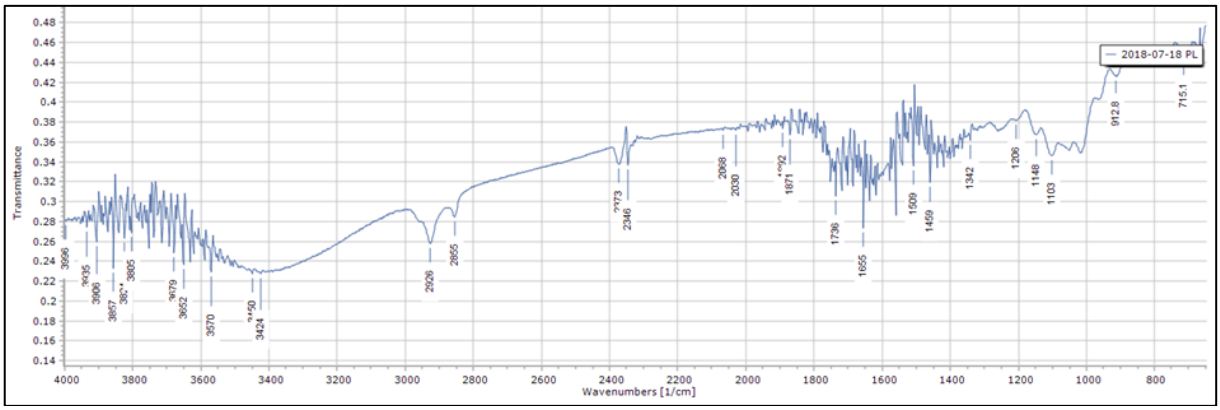

Fig 3.4: FT-IR Spectrum of PL

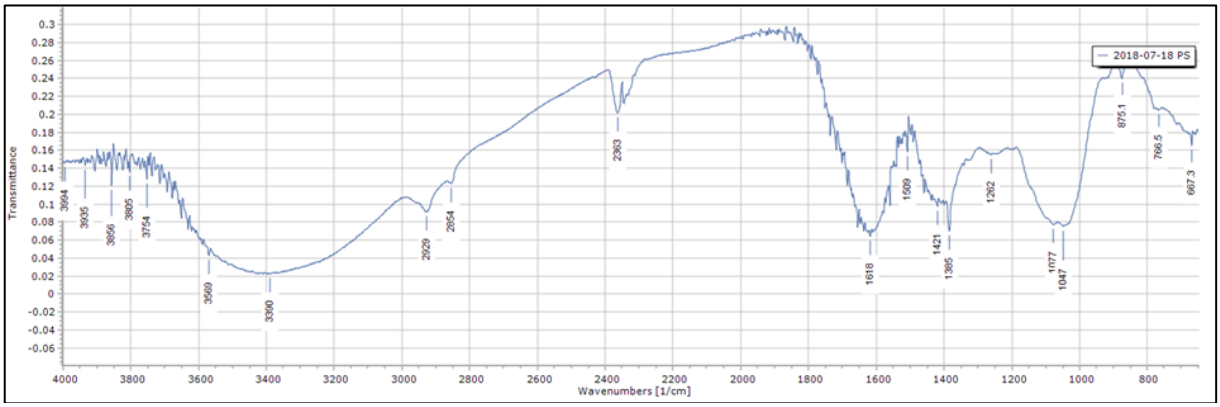

Fig 3.5: FT-IR Spectrum of PS

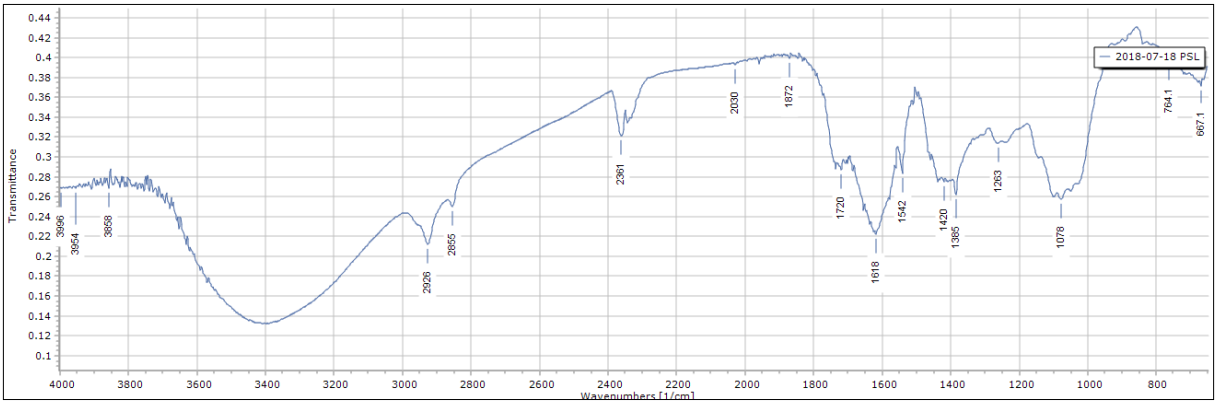

Fig 3.6: FT-IR Spectrum of PSL

3.4 Antimicrobial Activity

The ten different types of medicinal plants were investigated against the two human pathogens. Here we examine two microorganism *Escherichia coli* and *Candida albicans.* Antibacterial activity of the samples mentioned in Table 3.3. From the results comparatively PSL exits the higher zone of inhibition than others (PS, PL) decrease in concentration also showed increase in inhibition efficiency.

Antifungal activity also same as the bacterial activity. Table 3.4 showed the antifungal activity, there also PSL compound exhibit the higher efficiency.

3.4.1 Anti-bacterial activity

Compound PS

At 25µL concentration was found with negligible zone size around 10 mM. PS compound at 50µL, 75µL and 100µL concentrations were observed with prominent zone size namely 15, 16 and 17mm respectively. After comparison with positive control (23mm), this compound was confirmed with anti-bacterial activity against *Escherichia coli.*

Compound PL

At25 µL concentration was found with negligible zone size around 10 mm. PS compound at 50µL, 75µL and 100µL concentrations were observed with prominent zone size namely 11, 14 and 17mm respectively. After comparison with positive control (24 mm), this compound was confirmed with moderate anti bacterial activity against *Escherichia coli.*

Compound PS

At 25µL concentration was found with negligible zone size around 10mm. PS compound at 50 µL, 75µL and 100µL concentrations were observed with prominent zone size namely 12, 15 and 18mm respectively. After comparison with positive control (23mm), this compound was confirmed with moderate anti bacterial activity against *Escherichia coli.*

In the three different samples, PSL exhibits the higher inhibition efficiency than PS and PL.

Antibacterial activity (*Escherichia coli*):

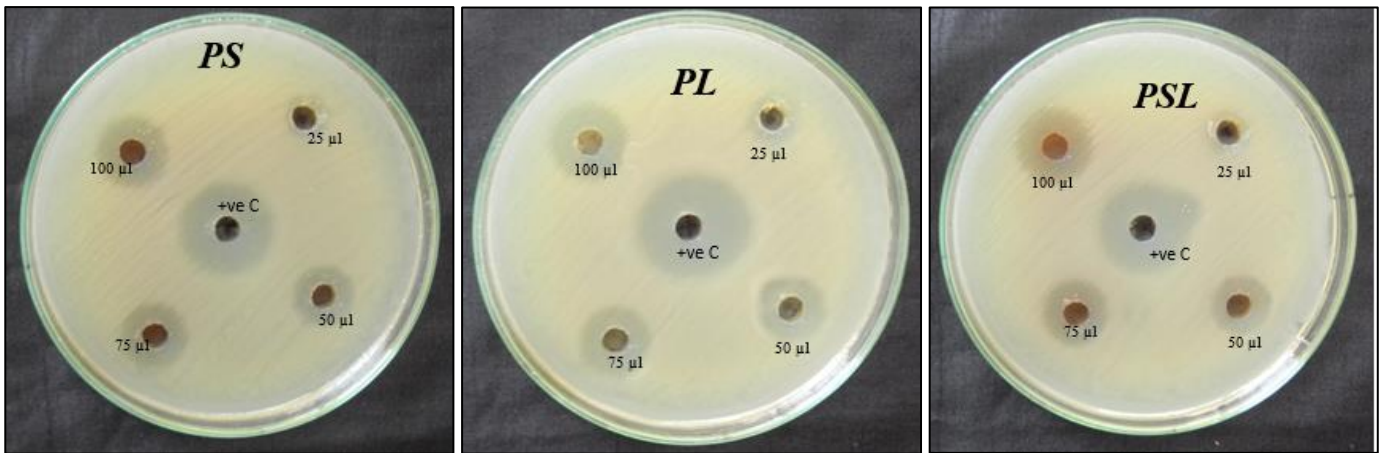

Fig 3.7: Antibacterial activity of PS,PL and PSL

Table 3.3: Anti-bacterial activity of PS, PL and PSL compounds (*Escherichia coli*):

S. No.	Sample	Positive Control	Zone of Inhibition (mm)			
			25µl	50µl	75µl	100µl
1.	PS	23	10	15	16	17
2.	PL	24	10	11	14	17
3.	PSL	23	10	12	15	18

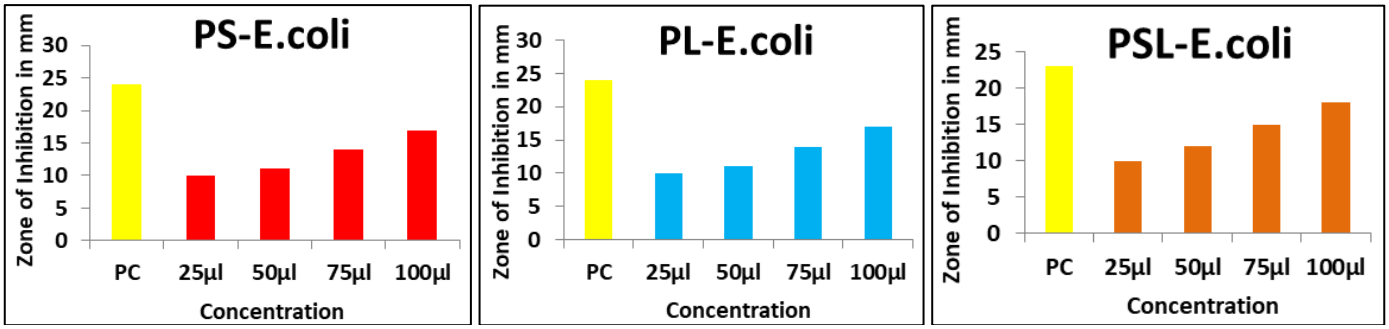

Fig 3.7: Antibacterial activity of PS, PL, PSL

3.4.2 Antifungal activity of PS,PL and PSL:

Candida albicans were treated with different samples (PS, PL, PSL) respect with various concentration, higher concentration shows the higher inhibition efficiency.

Compound PS

There was no measurable zone observed at 25µL concentration. Followed by 50µL, 75µL and 100µL concentrations are found with negligible zone of inhibition *viz.*, 6, 10 and 11mm respectively. When compared to control zone size 19mm compound PS does not show prominent activity against *Candida albicans* fungal strain.

Compound PL

There was 8mM zone observed at 25 µL concentration. Followed by 50 µL, 75 µL and 100 µL concentrations are found with negligible zone of inhibition *viz.*, 10, 14 and 17mm respectively.

When compared to control zone size 22 mm compound PL showed prominent activity against *Candida albicans* fungal strain.

Compound PSL

There was 8mm zone observed at 25µL concentration. Followed by 50µL, 75µL and 100µL concentrations are found with negligible zone of inhibition *viz.*, 10, 12 and 15mm respectively.

When compared to control zone size 20 mm compound PSL showed prominent activity against *Candida albicans*fungal strain.

Antifungal activity (*Candida albicans)*

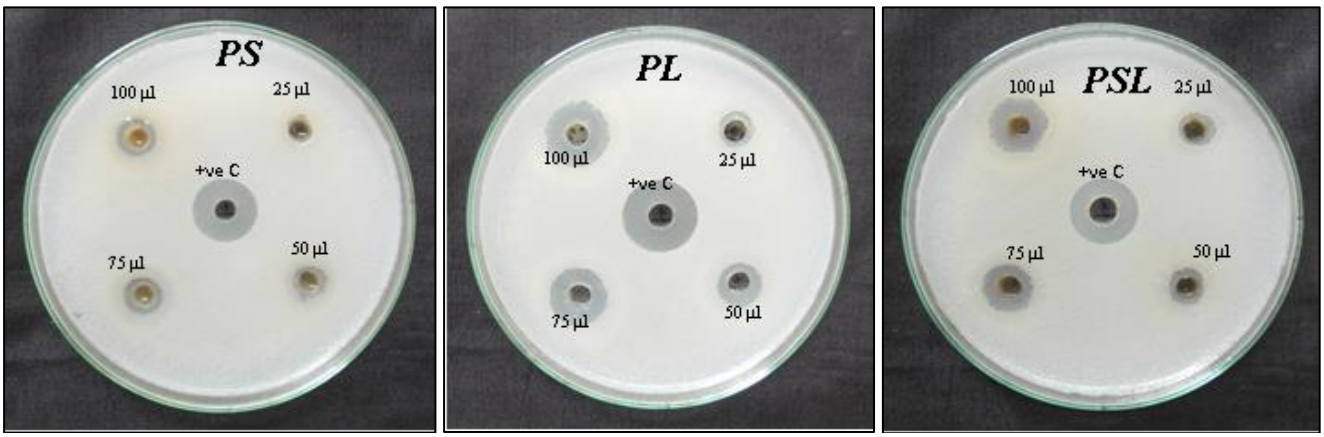

Fig.3.8 Antifungal activity of PS, PL, PSL

Table 3.4: Anti-fungal activity of PS, PL and PSL compounds (*Candida albicans):*

S.No.	Sample	Positive Control	Zone of Inhibition (mm)			
			25µl	50µl	75µl	100µl
1.	PS	19	00	06	10	11
2.	PL	22	08	10	14	17
3.	PSL	20	08	10	12	15

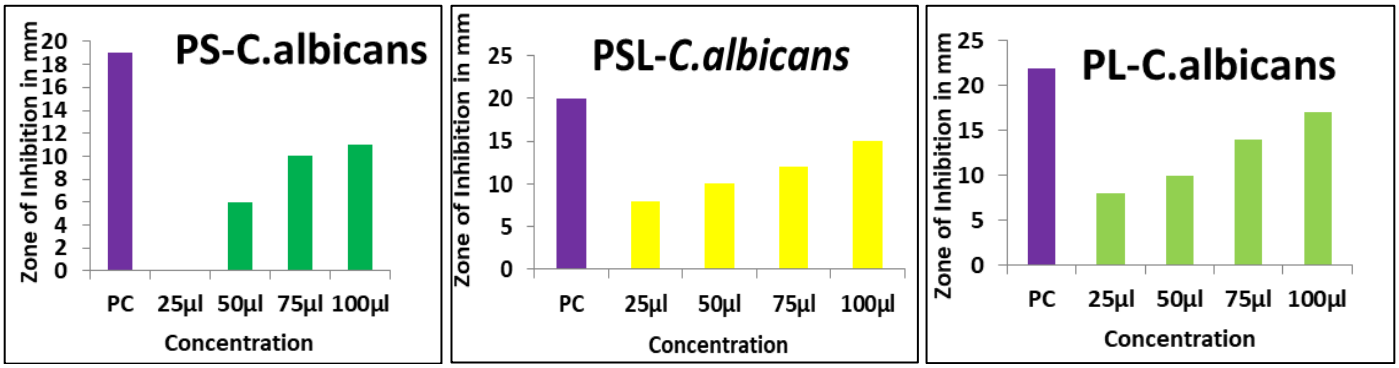

Fig 3.9: Antifungal avtivity of PS, PL and PSL

3.5 XRD analysis

X-ray diffraction (XRD) is a hasty analytical skill mostly used for phase detection of a powder material and can provide information on unit cell dimensions. The analyzed material has been finely ground, homogenized, and average bulk composition was determined. On examining XRD pattern of PS (Fig.3.10) and PSL (Fig3.11), the prominent peaks at 2ɵ =28.43 º, 40.60 º, 31.77 º (PS)and2ɵ=19.81º, 24.50 º, 9.4 º (PSL) represented. The size of the particles was also determined from X-ray line broadening using the Debye–Scherrer formula given as D = 0.9/ˇ cos, where D is the average crystalline size (A), ˚ the X-ray wavelength used (nm), ˇ the angular line width at half maximum intensity (radians) and the Braggs angle (degrees). In the sample PS and PSL were three strong peaks aroused, these peaks may be shows the appearance of sample.

Here we have done the interpretation from the 2θ values, the unknown minerals have mentioned by using (D_1 using 1.54056 – Cu Ka_1 for 2θ) listed out the some minerals for PS (Ca, Si, Na) and PSL (In, As, Al). The above metal ions (minerals) may present in the sample.

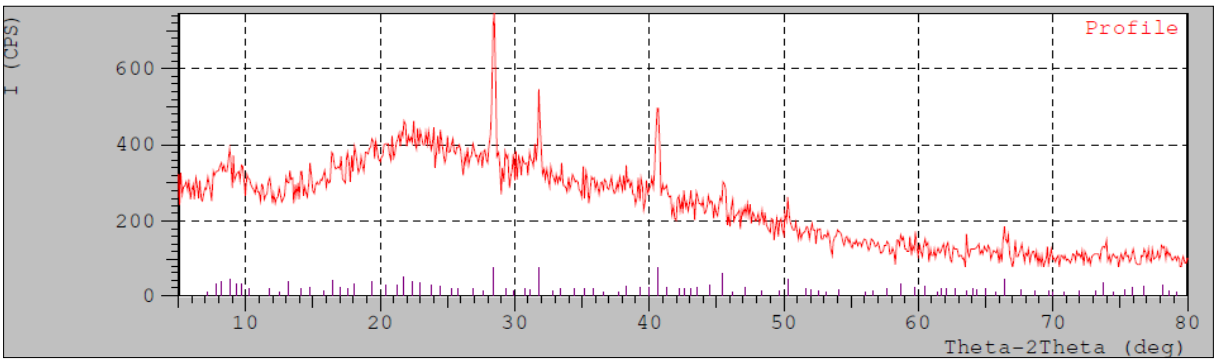

Fig 3.9: XRD pattern of PS sample

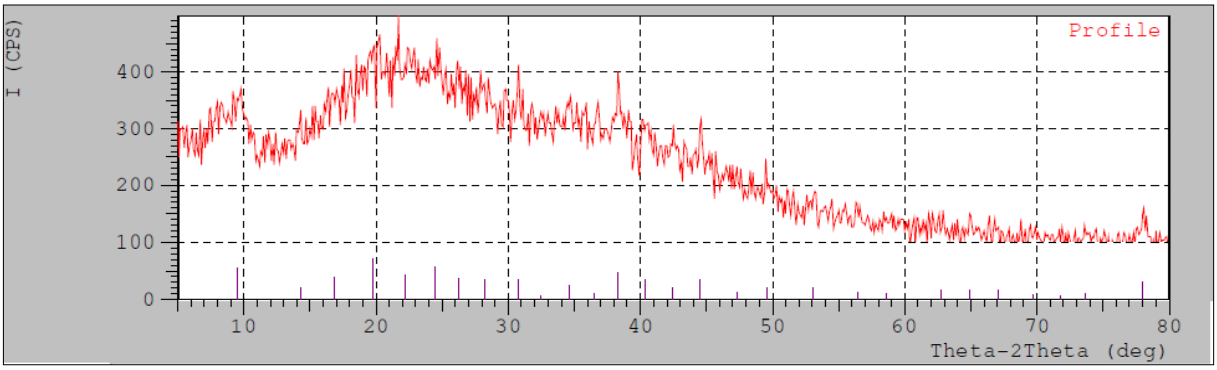

Fig 3.10: XRD pattern of PSL sample

4. Conclusion

The following conclusions were made from the investigation

- It could be concluded that *AM, AI, AA,OT, SM, CR, MI, PE, FG, AC* with and without lemon contain a number of pharmaceutically important photochemical like alkaloids, saponin. Phytosterols, terpenes, carbohydrates, tannins, flavonoids and amino acid, Glycosides, Quinine, Lacton.
- UV–Vis spectra of the extracts shows that the sharp bands of extract were observed at 245nm absorption spectra It could be concluded that the range is almost nearest to presence of alkaloids.
- From the XRD studies, it could be concluded that some minerals are present in PS (Ca, Si, Na) and PSL (Ca, Si, Na, In, As and Al).
- The anti-bacterial and antifungal potential of water extracts of the plants with Lemon showed the inhibition on *Escherichia coli* and *Candida albicans*

5. References

1. Butlet MS. The role of natural product chemistry in drug discovery, J Nat Prod 2004;67:2141-2153.

2. Stepp, John R. The role of weeds as sources of pharmaceuticals, J. Ethnopharmacol 2004;92(.2-3):163.
3. Lichterman BL. Aspirin: The Story of a Wonder Drug, British Medical Journal 2004;329:1408. Doi: http://dx.doi.org/10.1136/bmj.329.7479.1408.
4. Kumar BR, Suryanarayana B. Promising antidote plant species from the tribals of Sriharikotaisland, Andhra Pradesh, Life sciences leaflets 2011;19:769-779.
5. Unschuld PU. Medicine in China: A History of Pharmaceutics, University of California Press, Berkely 1986.
6. Aggarwal BB, Sundaram C, Malani N, Ichikawa H. Curcumin: the Indian solid gold. Adv. Exp. Med. Biol 2007;595:1-75.
7. Rasool Hassan BA. Medicinal Plants (Importance and Uses), Pharmaceut Anal Acta 2012;3:e139.
8. Fabricant DS, Farnsworth NR. Environmental Health Perspectives 2001;109:69-75.
9. Ghosal S, Mazumder UK, Mehta R. Indole bases of Desmondiumgyrans. Phytochemistry 1972;11(5):1863-1864.
10. Akerele OMD. Importance of Medicinal Plants. Nat. Resour. Hum.HealthPlants Med. Nutr. Value, Proc. WHO Symp. Plants Health All: Sci. Adv, 1st 1992, pp.66-91.
11. Cragg GM, David NJ. Plants as a source of anti-cancer agents, J Ethnopharmacol 2005;100(1-2):72-79.
12. Bailey CJ, Day C. Traditional plant medicines as treatment for diabetis, Diabetis Care, Americandiabetis association 1989, pp. 553-564.
13. Leelavathi M, Anitha L, Maheswarlu M, Prasad NN. Standardization of the technology for the development of a Solanumtorvum- Herbal based therapeutic lehyam. Asian Journal of Experimental Biological Sciences 2010;1:758- 764.