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Studies on phytochemical and anti-microbial activities of selected medicinal plants

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Abstract
A green chemistry approach is investigated for the biological studies of the Adhatoda Vasica (AV), Azadirachta Indica (AI), Cassia Auriculata (CA), Ficus Benghalensis (FG), Ficus Religiosa (FR), Leucas Aspera (LA), Murraya Koenigii(MK), Punica Granatum (PG), Tinospora Cordifolia (TC), and AV, AI, CA, FB, FG, FR, LA, MK, PG, TC, 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. The present study concluded that the extract of AV, AI, CA, FB, FG, FR, LA, MK, PG, TC with lemon contains good anti-bacterial activity and anti-fungal activities against tested microorganisms. It also concluded thatv AV, AI, CA, FB, FG,FR, LA, MK, PG, TC, 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
Natural products, also known as secondary metabolites, are compounds produced by plants, terrestrial, and marine organisms. While it is not known exactly why these compounds are produced, it is thought that they may provide the organism with a possible chemical defense mechanism [1-2] or to enhance the organisms overall fitness (reproduction, chemical communication, cell function) [3-4]. Since historic times natural products have proved to be an important resource for the identification of potential new pharmaceuticals. Both terrestrial and marine sources have yielded a variety of compounds, displaying unique carbon skeletons and important therapeutic properties. Newman and Cragg reported that over the past 30 years (1981-2010), approximately 50% of the new drug entities have been discovered or are derived from a natural product origin [5].

In the area of anti-biotics, there is a pressing need for the discovery of new drugs. With the existence of drug resistant strains of bacteria, the discovery of new anti-biotics has never been more important. For instance the occurrence of resistant strains such as *Pseudomonas aeruginosa* and other multidrug resistant pathogens is well documented [6-7], and there is a need to discover new drugs to target these strains. In addition, the emergence of new diseases also necessitates the need for new drugs. Natural product sources, particularly marine invertebrates, provide an ideal avenue for potential new drug discovery.

Plants are composed of different components, such as the bulbs, stems, leaves, flowers and, at times, fruit. When undertaking an investigation of a plant, it is important to evaluate all parts of the plant individually, as each component/part may contain different chemistry, and therefore, different biological activity. Terrestrial organisms such as plants and fungi have been studied extensively and have large precedence historically in drug discovery. Plants and fungi are generally easily accessible and larger amounts can usually be collected, making them ideal for natural product discovery. One of the earliest success stories of a terrestrial based natural product is penicillin G which was isolated and identified from the *Penicillium* bacterium in 1928("American Chemical Society International Historic Chemical Landmarks. Discovery and Development of Penicillin,"). Taxol, an approved anti-cancer drug, from the bark of *Taxus brevifolia*, was isolated in its pure form in 1967, but difficulty in determining it identity meant that the complete structure was not reported until 1971 [8]. Silybin, is an example of a plant derived natural product currently undergoing clinical trials as an anti-cancer agent [9-11].

Infectious diseases are the world's leading cause of premature deaths, killing almost 50 000 people every day. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world [12-17]. However, the situation is alarming in developing as well as developed countries due to indiscriminate use of antibiotics. The drug-resistant bacteria and fungal pathogens have further complicated the treatment of infectious diseases in immune compromised, AIDS and cancer patients [18-19]. In the present scenario of emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for new antimicrobial substances from other sources including plants.

India has a rich flora that is widely distributed throughout the country. Herbal medicines have been the basis of treatment and cure for various diseases and physiological conditions in traditional methods practiced such as Ayurveda, Unani and Siddha. Several plant species has been used by many ethnic groups for the treatment of various ailments ranging from minor infections to dysentery, skin diseases, asthma, malaria and a horde of other indications [2-23]. The past three decades have seen a dramatic increase in microbial resistance to antimicrobial agents [24- 25] that lead to repeated use of antibiotics and insufficient control of the disease [26].

In this present study the extraction, screening, characterization and biological activity of the *Adhatoda Vasica (AV), Azadirachta Indica (AI), Cassia Auriculata (CA), Ficus Benghalensis (FG), Ficus Religiosa (FR), Leucas Aspera (LA), Murraya Koenigii(MK), Punica Granatum (PG), Tinospora Cordifolia (TC), and AV, AI, CA, FB, FG, FR, LA, MK, PG, TC,* with lemon using solvent like water has been carried out.

2. Materials and methods

2.1 Plant collection

The ten medicinal plants were collected from the rural area of Jawvathu hills. These plant materials were collected by the knowledge of the ancient people from that area. Fresh plants bought and washed over running tap water, it has been dried under dark and shade. The well dried plant materials were homogenized to fine powder using blender and stored in airtight polythene bags and refrigerated at 4ºC. Similarly the lemon peel has been derived from the fresh lemon and it was also dried under shade, this powder was made from using mortar and pestle.

2.2 Preparation of plant extract

Decoction method

Plant powders has been extracted by using distilled water by decoction method. Over all for the extraction, water was used as solvents. About 50gm of plant material was weighed and transferred into the beaker, and extracted with 500 ml of distilled water at 80ºC. The process of extraction was continued for 24 hours or till the extracts completion. 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.

2.3 Experimental

2.3.1 Phytochemical qualitative analysis

Alkaloids

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

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

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 flavonol 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 flavonol.

Test for Tannin

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

Test for Tannin

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

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

Test for Amino acid and Protein

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

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

Test for Terpenoids

Salkowski Test: To 0.5g of each of extracts was added 2ml of chloroform and 3ml of concentrated sulphuric acid was added carefully to form layer. Deep red color appearred 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 24h. 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 1 ml 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, including 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 were 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
3.1 Phytochemical Qualitative Analysis
Some medicinal plants have analyzed and shown in the table Table 3.1. Alkaloids, flavonoids, tannin, carbohydrate, amino acids and terpenoids. Alkaloids were present in AV, AZ, PG, LA and it shows same results in lemon peel combination. Flavonols also presented some plants such as AV, CA, FR, PG, CQ with lemon. Tannin compounds were present only in FB (+L), LA (+L). Carbohydrate were presents in all plants. Amino acids were absent may be present in on TC(+L).

Table 3.1: Qualitative analysis of Crude drugs

S. No	Phytoconstituents	Tests	Name of plants																			
			AV	AV+L	AZ	AZ +L	CA	CA+L	FB	FB+L	FR	FR+L	MK	MK+L	PG	PG+L	TC	TC+L	CQ	CQ +L	LA	LA+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 AL, AS, ASL shown in figure 3.1,3.2, and 3.3 respectively as shown in below.

Absorption value of AL = 1.8
Absorption value of AS = 2.7
Absorption value of ASL = 2.2

It could be concluded that the range is almost nearest to presence of alkaloids.

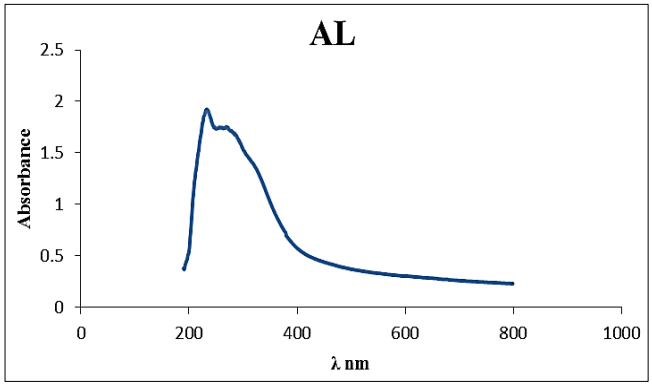


Fig 3.1: UV Vis Spectrum of AL

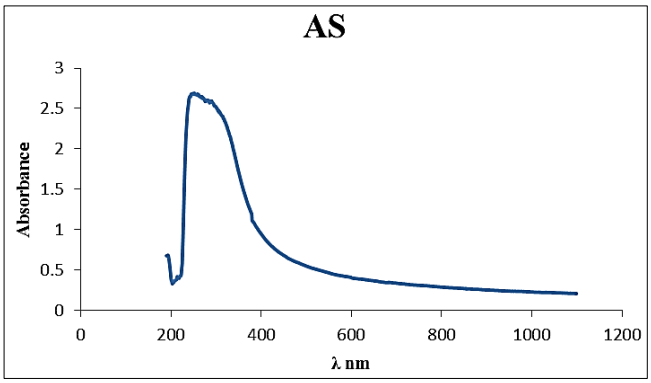


Fig 3.2: UV Vis Spectrum of AS

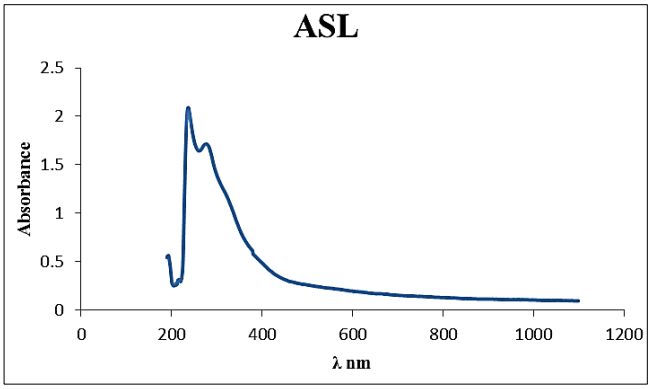


Fig 3.3: UV Vis Spectrum of ASL

3.3 Ft-IR Spectrum

FT-IR Spectrum of AL, AS, ASL 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 AL, AS, ASL. 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 alkaloids in the extracts. Some common functional groups stretching frequency are given below.

Table 3.2: FT-IR peak values

S. No.	Types of bond	Functional group	Wave number(cm^{-1})
1	C-H C-H(Methyl) C-H(Bend)	Alkenes Alkanes Alkanes Alkenes Aromatic	>3000 2850-3000 1360 900-1000 3000
2	C-C(Stretch)	Alkanes	800-1200
3	C=C	Conjucated diene Aromatic	1600-1650 1543
4	Triple bond	Alkynes	2346-2369
5	C=O C=O(Stretch)	Acid Aldehyde Ketone Ether	1612 1730 1721 1730
6	C-O(Stretch) C-O(Stretch)	Alcohol Ether	1000-1200 1000-1300
7	O-H(Stretch) O-H O-H(Stretch)	Alcohol/Phenol Acid Alcohol	3430 2346-2967 3430
8	N-H	Amine	3430
9	N-O	Nitro compound	1415

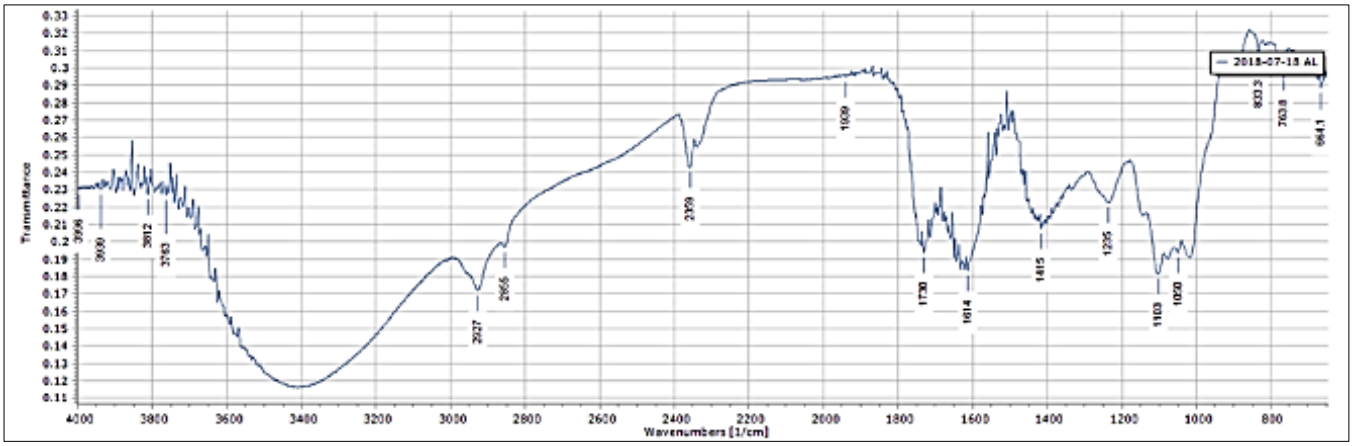


Fig 3.4: FT-IR Spectrum of AL

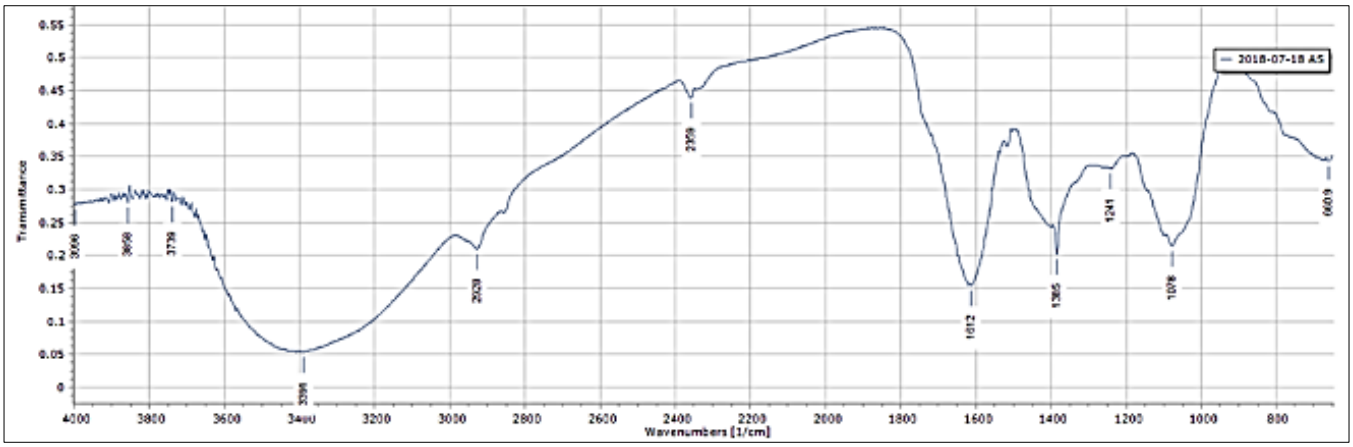


Fig 3.5: FT-IR Spectrum of AS

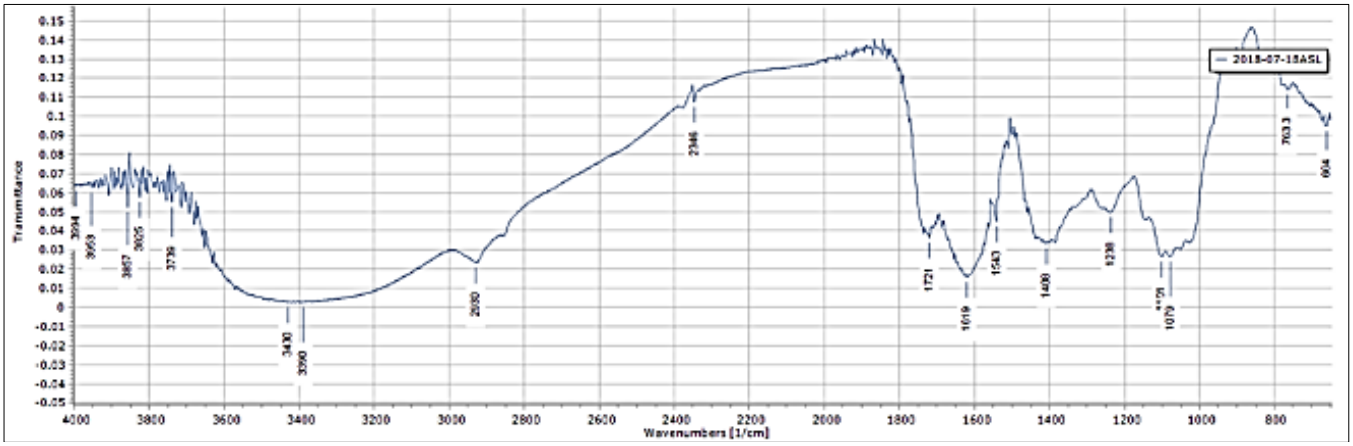


Fig 3.6: FT-IR Spectrum of ASL

3.4 Antimicrobial Activity

Anti-bacterial activity of AS, AL and ASL compounds

The various medicinal plants were investigated against the two human pathogens. We have used two organism *Bacillus subtilis* and *Candida albicans.* Antibacterial activity of the samples mentioned in Table 3.3. From the results comparatively ASL exits the higher Zone of Inhibition than others (AS, AL) increasing the concentration the inhibition efficiency also increased. Antifungal activity also same as the bacterial activity. Table 3.4 shows the antifungal activity, where ASL compound exhibit the higher efficiency.

Compound AS

This compound at 25µL concentration was observed with 8 mm size. Followed by 50µL, 75µL and 100µL concentrations were observed with 14, 15 and 16mm respectively, When compared with positive control zone of inhibition 23 mm, this compound AS shows less activity against *Bacillus subtilis*.

Compound AL

This compound at 25 µL concentration was observed with 10 mm zone size. Followed by 50 µL, 75 µL and 100 µL concentrations were observed with 12, 14 and 17mm respectively. When compared with positive control zone of inhibition 25mm, this compound AL show prominent activity against *Bacillus subtilis*

Compound ASL

This compound at 25µL concentration was observed with negligible zone size 6mm. Followed by 50µL, 75µL and 100µL concentrations were observed with 10, 15 and 18mm respectively. When compared with positive control zone of inhibition 24mm, this compound ASL shows prominent activity against *Bacillus subtilis*

Antibacterial activity (*Bacillus subtilis*)

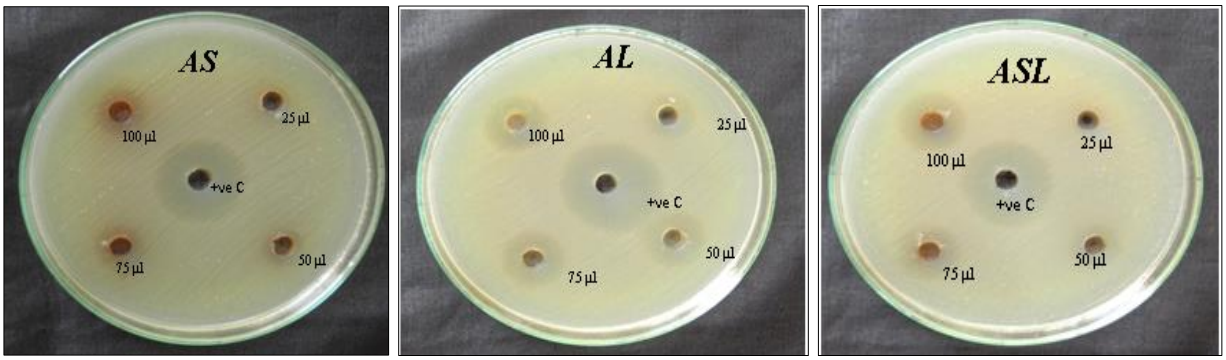
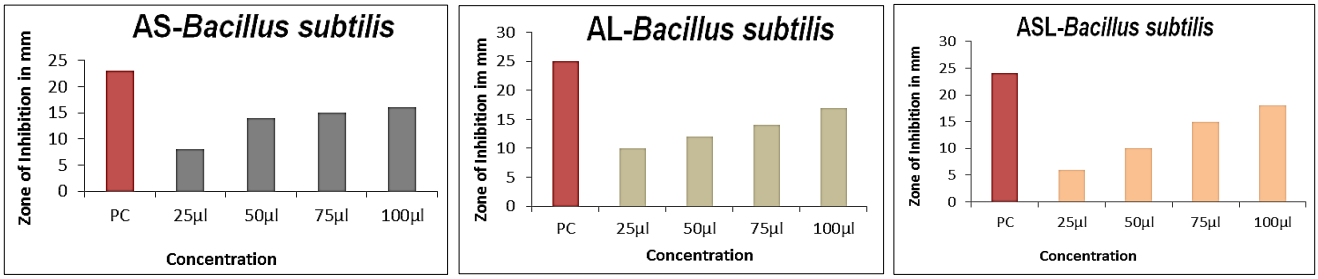


Fig 3.7: Antibacterial activity of sample AS, AL and ASL

Table 3.3: Antibacterial activity of AS, AL, ASL (*Bacillus subtilis*)

S. No.	Sample	Positive Control	Zone of Inhibition (mm)			
			25µl	50µl	75µl	100µl
1.	AS	23	08	14	15	16
2.	AL	25	10	12	14	17
3.	ASL	24	06	10	15	18



(a) **(b)** **(c)**

Fig 3.8(a, b, c): Anti bacterial activity of AS, AL, ASL (*Bacillus sub tilis*)

5 Antifungal activity of AS, AL and ASL

Candida albicans were treated with different samples (AS, AL, ASL) respect with various concentration. Higher concentration shows the higher inhibition efficiency.

Compound AS

There was observed with 8mm at 25µL concentration. Followed by 50µL, 75µL and 100µL concentrations are found with negligible zone of inhibition *viz.*, 9, 11 and 13mm respectively. When compared to control zone size 19mm compound AS showed less activity against *Candida albicans* fungal strain.

Compound AL

There was 9mm zone observed at 25µL concentration. Followed by 50µL, 75µL and 100µL concentrations are found with negligible zone of inhibition *viz.*, 13, 16 and 19 mm respectively. When compared to control zone size 25mm compound AL showed less activity against *Candida albicans* fungal strain.

Compound ASL

There was 6 mm zone observed at 25µL concentration. Followed by 50µL, 75µL and 100µL concentrations are found with negligible zone of inhibition *viz.*, 14, 16 and 19mm respectively. When compared to control zone size 24mm compound ASL showed good activity against *Candida albicans* fungal strain.

Antifungal activity (*Candida albicans*)

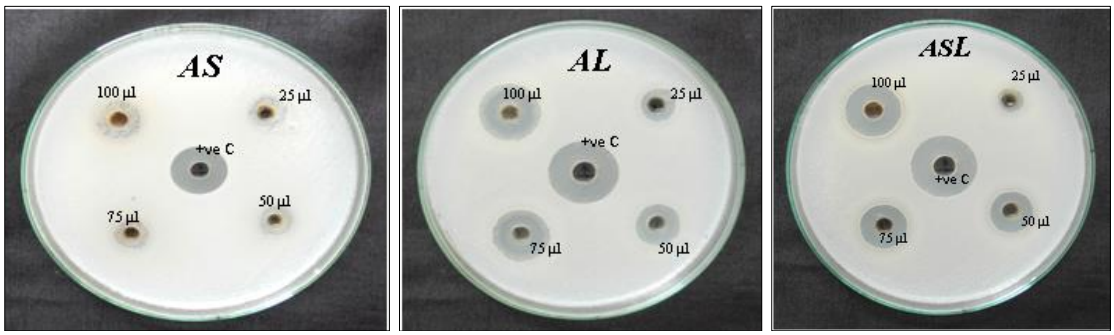
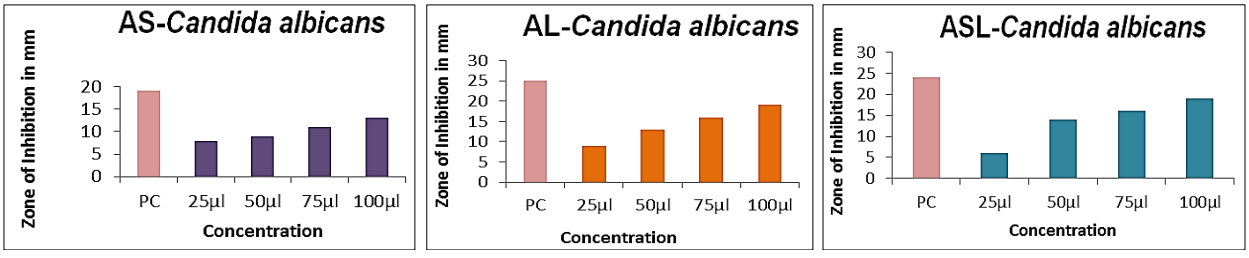


Fig 3.9: Antifungal activity of sample AS, AL and ASL

Table 3.4: Anti fungal activity of AS, AL and ASL compounds (*Candida albicans*)

S. No.	Sample	Positive Control	Zone of Inhibition (mm)			
			25µl	50µl	75µl	100µl
1.	AS	19	08	09	11	13
2.	AL	25	09	13	16	19
3.	ASL	24	06	14	16	19



(a) **(b)** **(c)**

Fig 3.10: Antifungal activity of AS, AL and ASL (*Candida albicans*)

3.6 XRD

XRD analysis were carried out for the two sample which was named as AS and ASL. These two power samples were investigate under the XRD. This method is bulk method, and reveals information on the bulk structure of the powder. There appeared three strong peaks both AS and ASL. Fig.3.11 shows the three strong peaks at 2ɵ=28.55 º,40.73 º,23.07º, and also Fig.3.12 shows the 2ɵ=21.73 º,8.73 º,38.24º, first peak, at the low 2ɵ range, is associated with the carbon atom supported. Fig 3.11 & 3.12 shows the XRD pattern of AS and ASL. The shifts in 2ɵ correspond to decreased lattice constant due to incorporation of metal atoms. f.c.c. reflexions showed a tail, which can be attributed to a low composition homogeneity. The mineral analysis have been done by using X-ray, the value shows the favored results with AS and ASL, this was sorted by D_1 using 1.54056 – $CuKa_1$ for 2θ. The minerals were obtained such as Cu (100), Fe (101), Ca (202), Co (100), Mg (100).

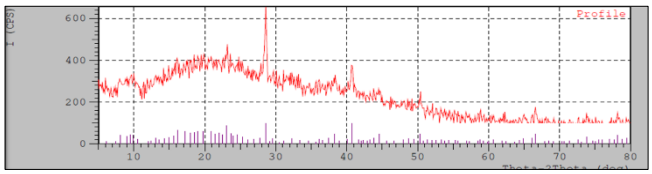


Fig 3.11: XRD pattern of AS

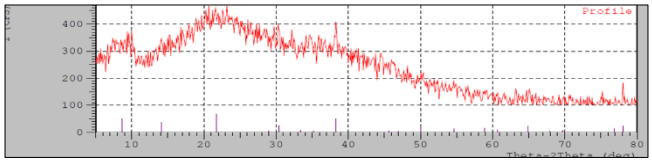


Fig 3.12: XRD pattern of ASL

4. Conclusion

The following conclusions were made from the investigation It could be concluded that AV, AI, CA, FB, FG, FR, LA, MK, PG, TC, with and without lemon contain a number of pharmaceutically important phytochemicals like alkaloids, saponin. Phytosterols, terpenes, carbohydrates, tannins, flavonoids, amino acid, glycosides, quinine and 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 AS (Cu) and ASL (Fe, Ca, Co, Mg, As and Al). The anti-bacterial and antifungal potential of water extracts of the plants with Lemon showed the inhibition on Bacillus subtilis and Candida albicans

References

1. Bennett Richard N, Wallsgrove Roger M. Secondary metabolites in plant defence mechanisms. New Phytologist 1994;127(4):617-633.
2. Pawlik Joseph R, Chanas Brian, Toonen Robert J, Fenical William. Defences of Caribbean sponges against predatory reef fish. I. Chemical deterrency. Marine Ecology Progress Series 1995;127:183-194.
3. Hay Mark E. Marine chemical ecology: chemical signals and cues structure marine populations, communities, and ecosystems. Annual Review of Marine Science 2009;1:193-212.
4. Puglisi Melany P, Sneed Jennifer M, Sharp Koty H, Ritson-Williams Raphael, Paul Valerie J. Marine chemical ecology in benthic environments. Natural Product Reports 2014;31(11):1510-1553.
5. Newman David J, Cragg Gordon M. Natural products as sources of new drugs over the 30 years from 1981 to 2010. Journal of Natural Products 2012;75(3):311-335.
6. Giamarellou Helen, Poulakou Garyphallia. Multidrug-resistant gram-negative infections: what are the treatment options? Drugs 2009;69(14):1879-1901.
7. Igbinosa Etinosa O, Odjadjare Emmanuel E, Igbinosa Isoken H, Orhue Phillips O, Omoigberale May NO, Amhanre Napoleon I. Antibiotic synergy interaction against multidrug-resistant *Pseudomonas aeruginosa* isolated from an abattoir effluent enviroment. Sci. World J 2012.
8. Wani Mansukhlal C, Taylor Harold Lawrence, Wall Monroe E, Coggon Philip, McPhail Andrew T. Plant Antitumor agents. VI. Isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. Journal of the American Chemical Society 1971;93(9):2325-2327.
9. Butler Mark S. Natural products to drugs: natural product-derived compounds in clinical trials. Natural Product Reports 2008;25(3):475-516.
10. Provinciali Mauro, Papalini Francesca, Orlando Fiorenza, Pierpaoli Sara, Donnini Alessia, Morazzoni Paolo, *et al*. Effect of the silybinphosphatidylcholine complex (IdB 1016) on the development of mammary tumors in HER-2/neu transgenic mice. Cancer Research 2007;67(5):2022-2029.
11. Young-Won C, Marcy JB, Hee BC, Kinghom AD. Drug Discovery from Natural Sources. The AAPS Journal 2006;8(2) Article 28 (httn://M'\vw. aupsi. ora)
12. Zevanovic A. Marine Natural Products Isolation, Screening and Analoque Synthesis. PhD Thesis of chemistry, University of Wollongong. Research online 2012: http://ro.uow.edu.au./theses/3692.
13. Piddock KJV, Wise R. Mechanisms of resistance to quinolones and clinical perspective. Journal of Antimicrobial Chemotherapy 1989;23:475-483.
14. Singh M, Chaudhry MA, Yadava JNS, Sanyal SC. The spectrum of antibiotic resistance in human and veterinary isolates of Escherichia coli collected from 1984-1986 in Northern India. Journal of Antimicrobial Chemotherapy 1992;29:159-168.
15. Mulligen ME, Murry-Leisure KA, Ribner BS, Standiford HC, John JF, Karvick JA *et al*., Methicillin resistant Staphylococcus aureus. American Journal of Medicine 1993;94:313-328.
16. Davis J. Inactivation of antibiotic and the dissemination of resistance genes. Science 1994;264:375-382.
17. Robin EH, Anril W, Alexander M, Loeto M, Keith K. Nasopharyngeal carriage and antimicrobial resistance in isolates of Streptococcus pneumoniae and Heamophilus influenzae Type b in children under 5 years of age in Botswana. International Journal of Infectious Dis eases 1998;3(1):18-25.
18. Rinaldi MG. Problems in the diagnosis of invasive fungal diseases. Review of Infectious Diseases 1991;13:493-495.
19. Diamond RD. The growing problem of mycoses in patients infested with human immunodeficiency virus. Review of Infectious Diseases 1993;13:480-486.
20. Dhar LM, Dhar MM, Dhawan BN, Mehrotra BN, Ray C. Screening of Indian plants for biological activity. Part I. Indian Journal of Experimental Biology 1968;6:232-247.

21. Perumal Samy R, Ignacimuthu S. Screening of 34 Indian medicinal plants for antibacterial properties. Journal of Ethnopharmacology 1998;62:173-178.
22. Perumal Samy R, Ignacimuthu S. Antibacterial activity of some folklore medicinal plants used by tribals in Western Ghats of India. Journal of Ethnopharmacology 2000;69:63-71.
23. Dahanukar SA, Kulkarni RA, Rege NN. Pharmacology of medicinal plants and natural products. Indian Journal of Pharmacology 2000;32:S81-S118.
24. Chopra I, Hodgson J, Metcalf B, Poste G. New approaches to the control of infections caused by antibiotic resistant bacteria. An industry perspective. Journal of the American Medical Association 1996;275:401-403.
25. Baquero F. Gram-positive resistance: Challenges for the development of new antibiotics. Journal of Antimicrobial Chemotherapy 1997;39:1-6.
26. National Center for Infectious Diseases (NCID). Campaign to prevent antimicrobial resistance in healthcare settings. Centre for Disease Control and Prevention 2002. Available online at http://www.cdc.gov/drugresistacne/ healthcare/problem.htm.