

Phytochemical Screening and Antibacterial Activity of Three Medicinal Plants – *Lawsonia inermis*, *Mangifera indica* and *Piper betel*

Leela K and Anita RJ Singh*

Affiliated to the University of Madras Post Graduate and Research Department of Biotechnology, Women's Christian College, Chennai, Tamilnadu, India

ABSTRACT

Medicinal plants play a predominant role in our ecosystem due to the vast distribution of bioactive components such as alkaloids, phenols, tannins, flavanoids etc... within them and each with a specific function. A study on these bioactive compounds and their beneficial roles is of much interest and are of utmost importance these days due to their wide therapeutic properties. The present work has been carried out in order to investigate the presence of important bioactive components in different solvent extracts of *Lawsonia inermis* L., *Mangifera indica* L. and *Piper betel* L. leaf samples and thereby testing their antibacterial activity. Cold extraction method was employed for extraction of leaf samples using different solvents such as ethanol, chloroform, ethyl acetate and water (aqueous). The extracts were subjected to both qualitative and quantitative analysis to determine the presence and amount of bioactive compound in the sample. The extracts were also tested for their pathogen inhibitory activity (antibacterial activity) against both Gram +ve and Gram -ve bacteria such as *Staphylococcus aureus*, *Streptococcus mutans*, *Klebsiella pneumoniae*, *Salmonella typhi* and *Enterobacter spp.* Based on the results obtained ethanolic extract was found to be rich in majority of phytochemicals with increased bioactivity compared to other extracts. This further suggests that ethanolic extract of these samples could be used in future for isolation of various novel compounds for different pharmacological applications

KEY WORDS: ANTIBACTERIAL ACTIVITY, BIOACTIVE COMPONENTS, COLD EXTRACTION, MEDICINAL PLANTS, PHYTOCHEMICAL SCREENING, SOLVENT EXTRACTS.

INTRODUCTION

Nature has gifted us with enormous resources and one among them is the widely distributed, a therapeutically

valuable source termed as “Medicinal Plants”. These medicinal plants contain numerous beneficially active compounds that are highly under research worldwide for treatment of many diseases. India has a rich diversity of plant species and is known for its traditional system of medicine such as Siddha, Ayurveda and Unani (Pandey et al., 2013). Medicinal plants are used as a primary source in health care wherein all the parts such as root, leaf, flower, seed or even whole plant as such is used in the development of drug formulations. These natural or herbal drugs are widely preferred over synthetic medicines due to their easy availability, high efficiency and low toxicity (Patel DK.,2016). Phytochemicals are

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also referred to as “Secondary metabolites” and these are naturally occurring compounds found in plants which play an important role in protecting plant cells against various environmental conditions and apart from it they also impart health benefits to human beings in the form of dietary supplements and as medications (Nyamai et al., 2016). The nature and concentration of secondary compounds present also varies within plant species and each compound exhibits a specific activity. Based on their structure, solubility and biosynthetic pathway these compounds are classified into three groups: Phenolic compounds, Alkaloids and Terpenes. These compounds exert various biological activities and also participate in both the primary as well as secondary metabolism of plants (Irina Francesca Gonzalez Mera et al., 2019).

Some of the pharmaceutical applications of secondary metabolites involve antiviral, antitumor, antioxidant, immunosuppressant, anti-ageing, anti-inflammatory, anti-hyperglycemic activities etc (Vaishnavi and Demain., 2011).

Lawsonia inermis L. is a medicinal plant commonly referred to as “Henna” belonging to the family Lythraceae. They contain various bioactive components such as Lawsonsone, quinone, xanthone, gallic acid, triterpenoids which are found to exhibit anti-neoplastic, anti-inflammatory, anti-haemorrhagic, hypotensive activity etc. They are also used as astringents, in treating leprosy, nervous disorders, also for treating boils and burns (Ghodekar et al., 2019).

Mangifera indica L. commonly referred to as “Mango” belongs to the family Anacardiaceae. Some of the phytochemical components reported in the plant involve *Mangiferin, catechin, tannins, triterpenoids*. Different parts of the plant are used in the treatment of rheumatism, dysentery, haemorrhage, asthma and anaemia. They also possess anti-inflammatory, antiparasitic, anti-helminthic, anti-allergic, antipyretic, anti-HIV and hepatoprotective properties, (Shah et al., 2010). *Piper betel* L. commonly referred to as “Betel/Paan” belongs to the family Piperaceae. It is grown extensively in India, Malaysia, Sri Lanka and Thailand....It is an evergreen herb which has been studied from past for its pharmacological activities and also used in many ayurvedic preparations. They are used as antiseptics, as healing agents, mouth freshener and are also reported to possess antimicrobial, antioxidant and antileishmanial properties, (Datta et al., 2011).

Due to the high prevalence and emergence of new dreadful diseases, there is a need for development of new drug formulations in order to overcome these circumstances. Plant based secondary metabolites has drawn the interest of many researchers worldwide due to their low toxicity, easy availability and minimal side effects compared to the synthetic ones. Plants contain abundant secondary compounds most of which are yet to be completely studied. This study deals with the phytochemical screening and antibacterial activity of different solvent extract of three important medicinal

plants – *Lawsonia inermis* L., *Mangifera indica* L. and *Piper betel* L.

MATERIAL AND METHODS

Sample Preparation: The leaf samples of *Lawsonia inermis* L., *Mangifera indica* L. and *Piper betel* L. were collected from different localities. The collected samples were cleaned, washed thoroughly with distilled water and shade dried for about 3 weeks at room temperature. The samples were then powdered and stored in clean bottles until required.

Extraction & Determination of Percentage Yield: The powdered samples were extracted using solvents of different polarities and water (aqueous). For solvent extraction, about 10 grams of the powdered samples were kept immersed separately in 100ml of solvents such as ethanol, chloroform, ethyl acetate in the ratio of 1:10. The samples were covered and incubated in dark for about a week (Sivareddy et al., 2019).

For aqueous extraction, about 10 grams of the powdered samples were added to 100ml of double distilled water and heated on water bath for about 30mins (Al-Manhel and Niamah., 2015). The samples were filtered using whatmann no.1 filter paper and the filtrate obtained was concentrated or dried to yield the crude extracts. The percentage (%) yield of crude extracts obtained was calculated based on the formula: (Terblanche et al., 2017). Percentage yield (%) = Dry weight of extract (g) / Dry weight of sample (g) × 100

Qualitative analysis Phenol Estimation: Ethanol, Chloroform, Ethyl acetate and Aqueous (water) extracts of all the three samples were subjected to qualitative phytochemical analysis in order to identify the presence of bioactive components in the extracts. The analysis was carried out for about 23 different phytochemicals such as Phenol (Ferric chloride test), Tannins, Glycosides (Keller-Killani test), Cardiac glycoside, Alkaloid (Hagers test), Flavanoids (Lead acetate test), Terpenoids (Salkowski test), Diterpenes (Copper acetate test), Steroids, Phytosterol, Quinones, Saponin, Resin (Acetone water test), Coumarins (Sodium hydroxide test), Volatile oil, Fixed oil and fats (Stain test), Carboxylic acid, Phlobotannins, Carbohydrate (Benedict's and Fehling's test), Protein (Biuret and Xanthoproteic test), Amino acid (Ninhydrin test), Starch (Iodine test) and Lipids according to the standard procedure (Godghate et al., 2012; George., 2017; Kumar et al., 2013).

Quantitative analysis: Phenol estimation: Phenol estimation was carried out according to Folin-Ciocalteu method. To 0.5 ml of extract, 2.5ml of 10% Folin-Ciocalteu reagent and 2.5ml of 7.5% Sodium carbonate was added. The samples were then incubated at 45° C for about 45 minutes and the absorbance was determined at 765nm using UV-Visible Spectrophotometer. Gallic acid was used as the standard. Samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure

was repeated for gallic acid standard and calibration line was constructed. Total phenolic content was expressed in terms of Gallic acid equivalent (mg GAE/g extract), (Singleton et al., 1999).

Tannin estimation: To 0.5ml of extract, 8ml of distilled water was added followed by the addition of 0.5ml FC reagent and 1ml Sodium carbonate. The samples were then incubated for about 30 mins at room temperature and absorbance was measured at 725nm using UV-Visible Spectrophotometer. Tannic acid was used as the standard. Samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for tannic acid standard and calibration line was constructed. Total tannin content was expressed as mg TAE/g extract, (Anbukkarasi et al., 2017).

Flavanoid estimation: 10 gram of plant sample was extracted twice with 100ml of 80% methanol at room temperature. The whole solution was filtered and the filtrate obtained was transferred into a crucible and allowed to dry over water bath and weighed to a constant weight. Percentage flavanoid content was calculated using the formula: % Flavanoid content = Weight of dried extract / Weight of Sample \times 100 (Bohm and Kocipai., 1994).

Alkaloid estimation: About 200ml of 10 % acetic acid in ethanol was added to 5gram of plant sample taken in a conical flask, covered and incubated for about 4 hours. It was filtered and the filtrate was concentrated on water bath to one quarter of original volume. Concentrated ammonium hydroxide was added drop wise to the extract until precipitation was complete. The solution was allowed to settle and the precipitate was collected, washed with dilute ammonium hydroxide and filtered. The residue is alkaloid which was dried and weighed. Percentage alkaloid content was calculated using the formula: % Alkaloid content = Weight of alkaloid (residue) / Weight of sample \times 100 (Harborne, 1973).

Saponin estimation: About 20% of aqueous ethanol was added to 5gram of plant sample taken in a conical flask and heated at 55°C with continuous stirring for about 4 hours. The residue was again extracted with 100ml of 20% ethanol and heated at 90 °C in water bath. The concentrate was transferred into a separating funnel and to that 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered and about 60ml of butanol was added and extracted twice with 5% sodium chloride. The remaining solution was heated on water bath and after evaporation the sample was dried to a constant weight. Percentage saponin content was calculated using the formula: % Saponin content = Weight of Saponin / Weight of sample \times 100 (Obadoni and Ochuko., 2001).

Terpenoid estimation: 10 gram of the powdered sample was kept soaked in 100ml of ethanol for about 24 hours. It was filtered and the filtrate was extracted with 40ml petroleum ether using the separating funnel. Ether

extract was separated and allowed to dry. After drying the percentage terpenoid content was calculated using the formula: % Terpenoid content = Weight of terpenoid extract / Weight of Sample \times 100 (Ferguson, 1956).

Glycoside estimation: About 10ml of Baljets reagent (95ml of picric acid + 5ml of aqueous sodium hydroxide) was added to 1ml of extract and the sample was incubated for about 1hour at room temperature. It was then diluted with 20ml distilled water and mixed well. The absorbance was measured at 495nm using spectrophotometer. The samples were prepared in triplicate and mean value of absorbance was obtained. The values are expressed in Mean \pm Standard deviation, (El-Olemy et al., 1994).

Cardiac glycoside estimation: 1gram sample was taken and to that 2.5ml of 15% lead acetate was added and the mixture was filtered. To the filtrate 2ml chloroform was added and shaken vigorously. 3ml glacial acetic acid was added followed by the addition of 0.5ml of 5% ferric chloride and 0.05ml of concentrated sulphuric acid. The reaction mixture was incubated in dark for about 2hours and the absorbance was measured at 530nm. The samples were prepared in triplicate for each analysis and mean value of absorbance was obtained. The values are expressed in Mean \pm Standard deviation, (Ugwoke et al., 2017).

Coumarin estimation: To 0.5ml of extract, 2ml of distilled water and 0.5ml of lead acetate solution was added and shaken. To this 7ml distilled water was added and mixed well. 2ml of this solution was taken in a test tube and to that 8ml of 0.1M Hydrochloric acid solution was added and incubated at room temperature for about 30 minutes and the absorbance was measured at 320nm using UV-Visible spectrophotometer. The samples were prepared in triplicate for each analysis and mean value of absorbance was obtained. The values are expressed in Mean \pm Standard deviation, (Osorio and Martins., 2004).

Steroid estimation: About 0.5ml of extract was taken separately in a test tube and similarly different concentrations (20-100 μ g/ml) of standard were added to a series of test tubes labelled S1-S5. Cholesterol was used as the standard. To this 5ml of ferric chloride diluting reagent was added followed by the addition of 4ml of concentrated sulphuric acid. The reaction mixture was incubated at room temperature for about 30mins and the absorbance was measured at 540nm in colorimeter. Standard graph was plotted from which the steroid content was determined. The concentration of steroid was expressed in mg/100ml (Zak, 1954).

Analysis of Nutritional components Carbohydrate estimation: It was performed according to Phenol-sulphuric acid method. Glucose was used as the standard. Different concentrations of standard glucose (20-100 μ g/ml) and about 0.5ml of extract were taken separately into a series of test tube. Volume in all test tubes were made upto 1ml using distilled water. 1ml phenol and 5ml of sulphuric acid was added, shaken for 10 minutes

and incubated in boiling water bath at 25 - 30°C for 20 minutes. Green colour developed was read at 490nm in colorimeter. Standard graph was plotted from which the carbohydrate content was determined. The concentration was expressed in mg/100ml, (Dubois et al., 1956).

Protein estimation: It was performed according to Lowry's method, Bovine serum albumin was used as the standard. Different concentrations of standard (BSA) (50-250µg/ml) and 0.5ml of extract were taken separately into a series of test tube. Volume was made upto 2.5ml in all test tubes with distilled water. 5ml of Reagent I (48ml of Reagent A- 2% sodium carbonate in 0.1N sodium hydroxide + 1ml of Reagent B - 1% Potassium sodium tartarate + 1ml of Reagent C - 0.5% Copper sulphate) was added to each tube and incubated for about 10minutes. After 10 minutes, 0.5ml of Reagent II (Folin - Ciocalteu in the ratio of 1:2) was added, mixed and incubated at room temperature for about 30minutes. Blue colour developed was read at 660nm in colorimeter. Standard graph was plotted from which the protein content was determined and concentration was expressed in mg/100ml (Lowry et al., 1951).

Aminoacid estimation: It was performed according to Ninhydrin method. Leucine was used as the standard. Different concentrations of standard Leucine (20-100µg/ml) and 0.5ml of extract was taken separately into a series of test tube. Volume was made upto 2ml in all tubes with distilled water. To this 2ml of ninhydrin reagent was added, vortexed and incubated in water bath for about 15 minutes and cooled. About 3ml of 50% ethanol was added to all tubes and mixed well. The absorbance was measured at 570nm in colorimeter. Standard graph was plotted from which the aminoacid content was determined and the concentration was expressed in mg/100ml. (Moore, S and Stein, W.H., 1948)

Lipid estimation: It was carried out according to Folch method. 1gram of sample was kept immersed in 20ml solvent mixture comprising Chloroform: methanol in the ratio of 2:1. It was agitated for about 15-20 minutes in shaker and the solution was filtered. About 0.9% sodium chloride solution was added to the filtrate and centrifuged at 2,000 rpm for 10 minutes. Upon centrifugation 2 layers got seperated wherein the upper phase is methanol while lower chloroform phase contains lipid. Lipid was collected, allowed to dry and weighed. Weight of lipid is expressed as mg lipid per 100mg of dry material, (Folch, 1957).

Antibacterial activity: The extracts were screened for their antibacterial activity against both Gram positive and Gram negative bacteria such as *Staphylococcus aureus*, *Streptococcus mutans*, *Klebsiella pneumoniae*, *Salmonella typhi* and *Enterobacter spp* by means of Agar well diffusion method. About 150ml of Muller Hinton agar medium was prepared, poured into the petriplates and allowed to solidify. Once solidified the bacterial cultures were swabbed onto the agar medium using a sterile cotton swab .The wells were punctured using a

sterile cork borer and the extracts were dispensed into each well using a micropipette. The petriplates were then incubated at 37°C for 24 hours and observed for the zone of inhibition. The diameter was measured in mm (Saif et al., 2017).

RESULTS AND DISCUSSION

Extraction: In this study, cold extraction method was employed for extraction of plant leaf samples (Figure

Figure 1: *Lawsonia inermis* L

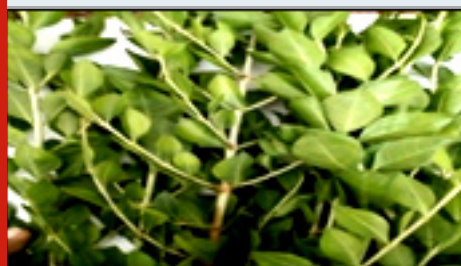


Figure 2: *Mangifera indica* L



Figure 3: *Piper betel* L.



Figure 4: Extraction of *Lawsonia inermis* L., *Mangifera indica* L. and *Piper betel* L. Using Different Solvents and Water

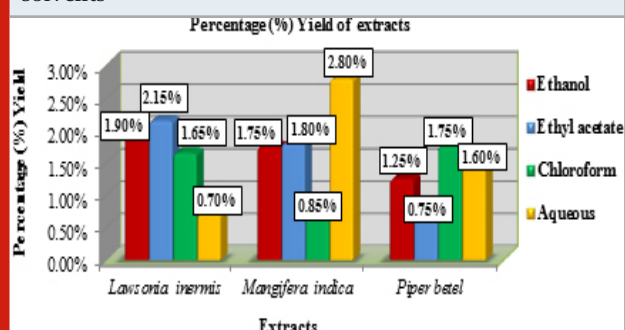


1-3) using various solvents such as ethanol, ethyl acetate, chloroform and aqueous (Figure 4). This method of extraction is found to be advantageous over hot extraction method wherein the chance for loss of required volatile compounds in the plant is low.

Organic solvents are generally used for extraction of bioactive components from plant sample. However, among the solvents ethanol is most commonly used in

medicinal preparations due to its safety and low toxic nature (Wendakoon et al., 2012). The choice of solvent used for extraction also varies depending on the type of compound to be isolated. Ethanol can be used for extraction of Polyphenol, flavanols, tannins, alkaloids, steroids etc... Water can extract compounds such as saponins, tannins, starch, polypeptides, while chloroform is used for extracting flavanoids and terpenoids (Pandey and Tripathi., 2014).

Figure 5: Shows Percentage yield of extracts in different solvents



Percentage Yield of extracts: The percentage yield of extracts obtained using different solvents like ethanol, chloroform, ethyl acetate and aqueous were calculated using the formula: % Yield = Dry weight of extract (g) / Dry weight of sample (g) × 100. *Lawsonia inermis* L. showed higher yield in ethyl acetate extract with 2.15% and lower yield in aqueous extract with 0.7%. *Mangifera indica* L. showed higher yield in aqueous extract with 2.8 % and lower yield in chloroform extract with 0.85%. *Piper betel* L. showed higher yield in chloroform extract with 1.75% and lower yield in ethyl acetate extract with 0.75% (Figure 5). Among the solvents ethanol showed considerably better % yield in all the three samples.

Table 1. Shows Qualitative Phytochemical Results for Ethanol (ET), Ethyl Acetate (EA), Chloroform (CH) and Aqueous (AQ) Extracts of *Lawsonia inermis* L., *Mangifera indica* L. and *Piper betel* L.

S.no	Phytochemicals	Results											
		<i>Lawsonia inermis</i> L.				<i>Mangifera indica</i> L.				<i>Piper betel</i> L.			
		ET	EA	CH	AQ	ET	EA	CH	AQ	ET	EA	CH	AQ
1	Alkaloid	+	+	+	+	+	+	+	+	+	+	+	+
2	Phenol	+	-	-	+	+	+	-	+	+	-	-	+
3	Tannin	+	+	-	+	+	+	-	+	+	-	-	+
4	Flavanoid	+	+	-	+	+	+	+	+	+	+	+	+
5	Glycoside	+	+	+	+	+	+	+	+	+	+	+	+
6	Cardiac glycoside	+	+	+	+	+	+	+	+	+	+	+	+
7	Terpenoids	+	+	+	+	+	+	+	+	+	+	+	+
8	Diterpenes	+	+	+	+	+	+	-	+	+	+	+	+
9	Coumarins	+	+	-	+	+	+	-	+	+	+	-	+
10	Lipids	+	+	+	+	+	+	+	+	+	+	+	+
11	Saponin	+	+	-	+	+	-	-	+	+	-	-	+
12	Steroid	+	+	+	+	+	+	+	+	+	+	+	+
13	Phytosterol	+	+	+	+	+	-	+	+	+	+	+	+
14	Quinones	+	-	-	+	+	-	-	-	+	+	+	+
15	Resin	+	-	+	-	+	-	+	+	-	-	+	+
16	Volatile oil	+	+	-	-	+	+	-	+	+	+	-	-
17	Fixed oil and Fats	+	+	+	-	+	+	+	-	+	+	+	-
18	Carboxylic acid	+	+	+	+	+	+	+	+	+	+	+	+
19	Phlobotannins	+	-	-	+	+	-	-	+	+	-	-	+
20	Carbohydrate	+	+	+	+	+	+	+	+	+	+	+	+
21	Protein	+	-	-	+	+	-	-	+	+	-	-	+
22	Aminoacid	+	-	-	+	+	-	-	+	+	-	-	+
23	Starch	-	+	+	-	-	+	+	-	-	-	-	-

A study by Mohammed et al., 2016 has reported the use of about 250ml of ethanol, chloroform and 500ml water for extraction of 50 grams of *Mangifera indica* L. leaf by percolation method. The % yield

reported was 13.24% for aqueous extract, 6.76% for ethanolic extract and 2.46% for chloroform extract. The extract yield also varies depending upon the factors such as solvent choice, pH, solvent to sample

ratio, temperature, time and the technique followed (Terblanche et al., 2017).

as antimutagenic properties. They are also used in the treatment of ailments such as cardiovascular problems, inflammation, stroke (Ozcan et al., 2014).

Figure 6: Total Phenolic Content (mg GAE/g Extract)

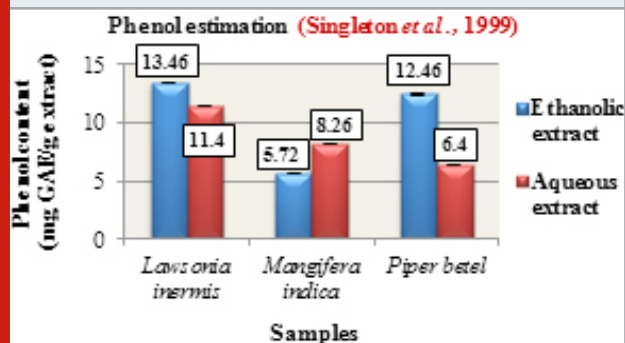


Figure 8: Flavanoid Estimation

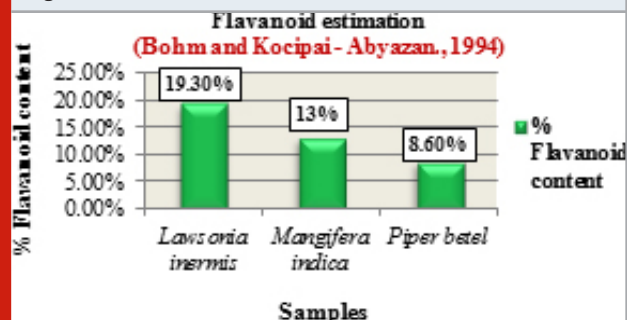


Figure 7: Total Tannin Content (mg TAE/ g extract)

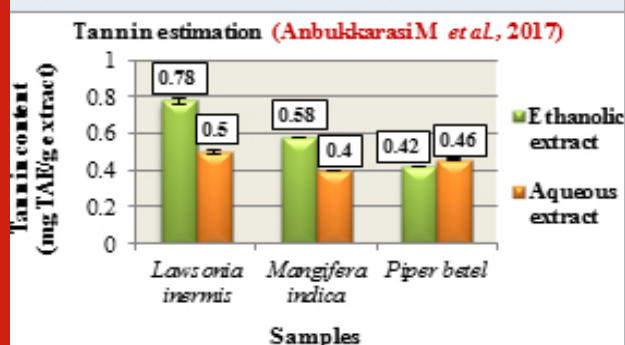
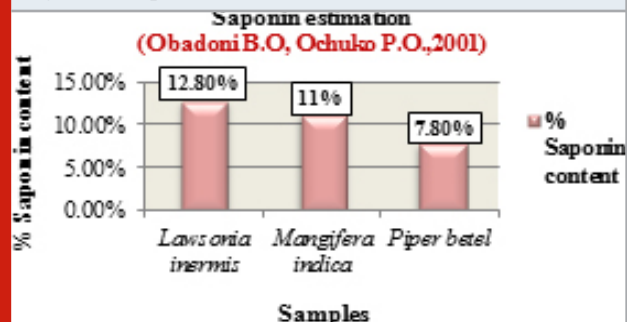


Figure 9: Saponin Estimation



Qualitative phytochemical analysis: Preliminary phytochemical screening was carried out for the extracts of all three samples and the results obtained were tabulated (Table 1). Among the extracts, ethanolic and aqueous extract of all the three samples showed positive result for majority of the phytochemicals such as phenol, tannin, flavanoid, alkaloid, saponin, terpenoids, diterpenes, glycoside, cardiac glycoside, coumarins, steroids, phlobotannins, carbohydrate, protein, aminoacid and lipids etc... compared to ethyl acetate and chloroform extracts. Similar results were found to have been reported by Sharma et al., (2018) for ethyl acetate extracts of *Lawsonia inermis* L. and by Forhad Uddin et al., (2015) for ethanol extract of *Piper betel* L.

Quantitative phytochemical analysis: Based on the qualitative results, ethanolic and aqueous extracts of all the three samples were chosen for further quantification studies. Phenol estimation was carried out according to Folin-Ciocalteu method and the total phenolic content is expressed in mg GAE/ g extract. The phenolic content was found to be higher in the ethanolic extracts of *Lawsonia inermis* L. with 13.46 mg GAE/g and *Piper betel* L. with 12.46 mg GAE/g while *Mangifera indica* L. showed higher phenolic content in its aqueous extract with 8.26 mg GAE/g. Comparatively among the three samples, *Lawsonia inermis* L. showed higher phenolic content in both its ethanolic and aqueous extracts followed by *Piper betel* L. and *Mangifera indica* L. (Figure 6). Phenols are reported to possess anti-cancer, antioxidant as well

Tannin estimation was carried out according to Folin method and the total tannin content was expressed in mg TAE/ g extract. The tannin content was found to be higher in the ethanolic extracts of *Lawsonia inermis* L. with 0.78 mg TAE/g and *Mangifera indica* L. with 0.58 mg TAE/g while *Piper betel* L. showed higher tannin content in its aqueous extract with 0.46 mg TAE/g. Comparatively *Lawsonia inermis* L. showed higher tannin content in both its ethanolic and aqueous extracts followed by *Mangifera indica* L. and *Piper betel* L. (Figure 7). Tannins are polyphenolic compounds which are reported to possess antiviral, antiparasitic, anti-oxidant, anti-inflammatory activities. They are also used in the treatment of hemorrhoids, infections, skin ulcer, (Praveen and Upadhyaya., 2012).

The % Flavanoid content was determined based on the method by Bohm and Kocipai-Abyazan.,1994. Among the samples, *Lawsonia inermis* L. showed higher flavanoid content of 19.30% followed by *Mangifera indica* L. with 13% and *Piper betel* L. with 8.60% (Figure 8). Flavanoids are pharmacologically active compounds that are loaded with numerous health benefits and are found to be potential candidates in drug production with reported activities such as antioxidant, antiageing & anticancer properties, (Hayat et al., 2017). The % Saponin content was determined based on the method by Obadoni B.O and Ochuko P.O., 2001. Among the samples, *Lawsonia inermis* L. showed higher saponin content of 12.80% followed

by *Mangifera indica* L. with 11% and *Piper betel* L. with 7.80% (Figure 9). Saponins exhibit numerous medicinal properties and are widely used in the treatment of cancer, against microbial infections, as anti-inflammatory and hepatoprotective drugs. Apart from health care they are also used in industries for preparation of detergents, cosmetics, soap due to their surfactant properties (Moghimpour and Handali, 2015).

Figure 10: Alkaloid Estimation

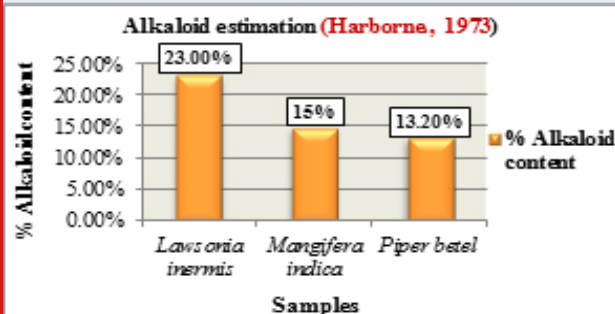
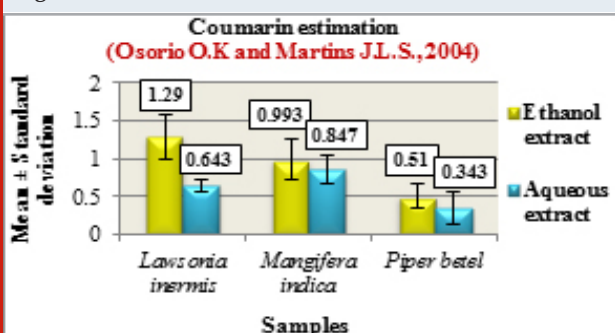


Figure 11: Coumarin Estimation



Alkaloid estimation was carried out according to the method given by Harborne., 1973. Comparatively *Lawsonia inermis* L. showed higher alkaloid content with 23% followed by *Mangifera indica* L. with 15% and *Piper betel* L. with 13.20% (Figure 10). Alkaloids play a protective role in plants against insects and herbivores due to their bitter tasting property. They exhibit antiarrhythmic, antihypertensive activities and are also used as analgesic & antimalarial drugs due to their stimulant properties, (Saxena et al., 2013).

Coumarin estimation was carried out based on the method by Osorio O.K and Martins J.L.S., 2004. The results are expressed in Mean ± Standard deviation. The ethanolic extracts of all the three samples showed higher coumarin content compared to the aqueous extracts. Among the samples, *Lawsonia inermis* L. showed increased coumarin content followed by *Mangifera indica* L. and *Piper betel* L. (Figure 11). Coumarins are reported to possess anticancer, anti-aging as well as anticoagulant properties and are also used as enhancing agents in cosmetic products and as food additives, (Rohini K and Srikumar PS., 2014).

Figure 12: Glycoside Estimation

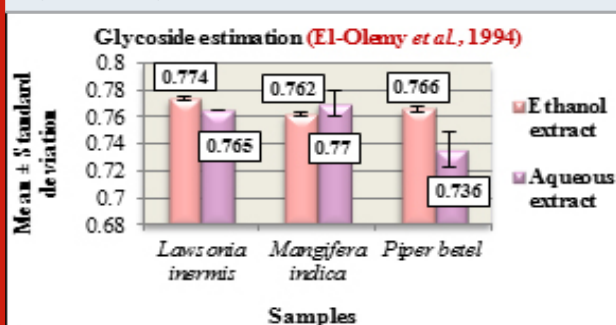


Figure 13: Cardiac Glycoside Estimation

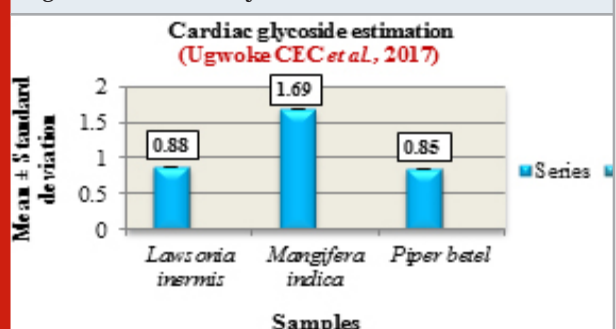


Figure 14: Steroid Estimation

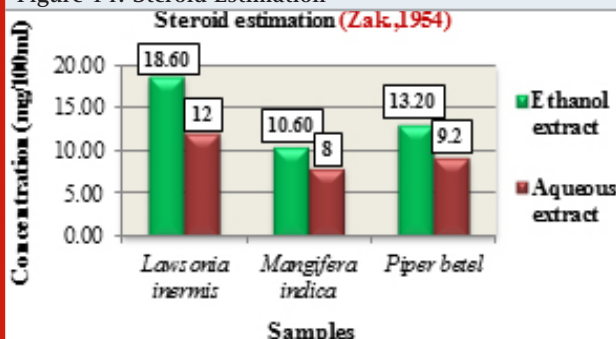
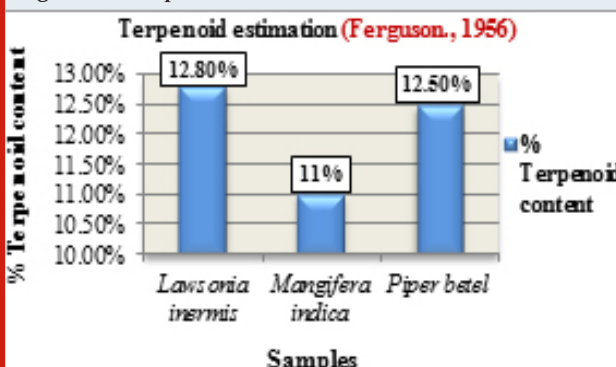


Figure 15: Terpenoid Estimation



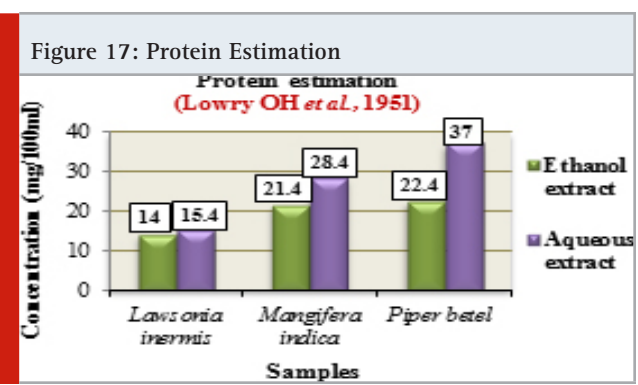
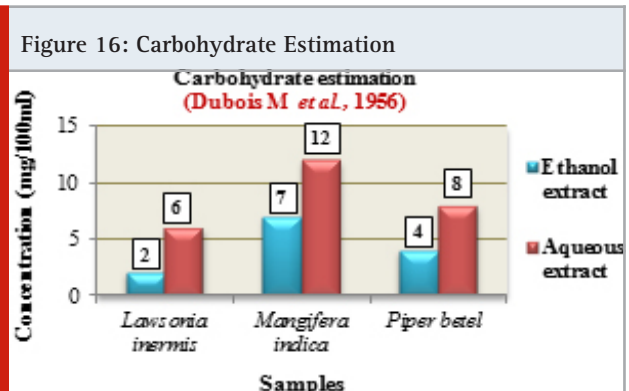
Glycoside estimation was carried out according to Baljets method. The results are expressed in Mean ± Standard deviation. The glycoside content in the ethanolic and aqueous extracts of all the three samples were determined. Based on the estimation, *Lawsonia inermis*

L. and *Piper betel* L. showed higher glycoside content in the ethanolic extracts while *Mangifera indica* L. showed higher glycoside content in its aqueous extract (Figure 12). Glycosides play a predominant role in pharmaceutical industry due to their innate biocompatibility and are also used as analgesic, antipyretic, anti-inflammatory agents, (Saawarn et al., 2015).

Cardiac glycoside estimation was carried out according to method by Ugwoke CEC et al., 2017. The results are expressed in Mean \pm Standard deviation. Among the samples, *Mangifera indica* L. showed higher cardiac glycoside content followed by *Lawsonia inermis* L. and *Piper betel* L. (Figure 13). Cardiac glycosides are compounds produced by plants in very small amounts. Cardiac glycoside such as peruvoside are used as a promising source in the control of cancer mainly ovarian cancer and leukemia (Patel., 2016). They are also used in the treatment of congestive heart failure (Morsy., 2017).

Steroid estimation was carried out according to method by Zak.,1954. The total steroid content was expressed in concentration(mg/100ml). *Lawsonia inermis* L. showed higher steroid content in its ethanolic extract with 18.60mg than aqueous extracts with 12mg. *Mangifera indica* L. also showed increased steroid content in its ethanolic extract with 10.60mg than aqueous extract with 8mg while *Piper betel* L. showed higher steroid content in its ethanolic extract with 13.20mg than aqueous extract with 9.2mg. Comparatively among the three samples, *Lawsonia inermis* L. showed higher steroid concentration followed by *Piper betel* L. and *Mangifera indica* L. (Figure 14).

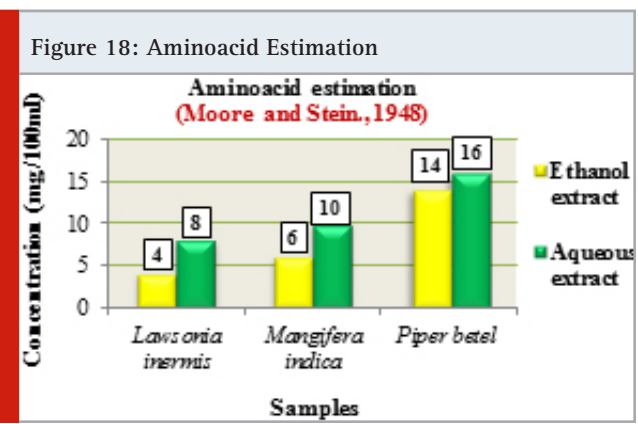
Steroids exhibit different biological activities such as regulation of cell proliferation, tissue differentiation and in cell membrane stabilization. They are also utilized as carbon and energy sources by various bacteria and also participate in cellular signalling mechanisms (Cabezon et al., 2018). Terpenoid estimation was carried out based on method by Ferguson.,1956. Among the samples, *Lawsonia inermis* L. showed higher terpenoid content with 12.80% followed by *Piper betel* L. with 12.50% and *Mangifera indica* L. with 11% (Figure 15). Terpenoids are the largest class of compounds produced



by plants. They exhibit antiviral, anti-inflammatory, antimicrobial, antioxidant activities and are also used as food additives and as fragrances in perfumes, (Priyanka P.Brahmkshatriya and Pathik S. Brahmkshatriya.,2013).

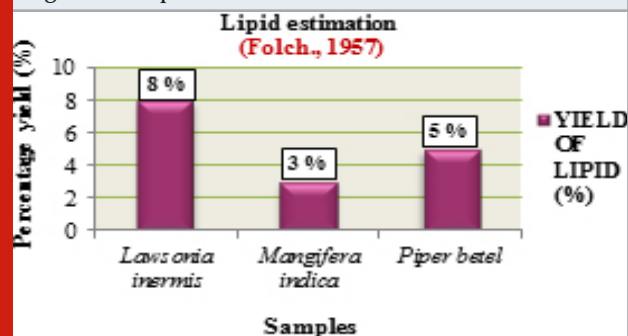
Analysis of nutritional components: Essential nutritional components such as carbohydrate, protein, amino acid and lipid content in the ethanolic and aqueous extracts of *Lawsonia inermis* L., *Mangifera indica* L. and *Piper betel* L. were determined.

Carbohydrate estimation was carried out according to Phenol sulphuric acid method. The results were expressed in mg/100ml. Carbohydrate concentration was found to be higher in the aqueous extracts of all the three samples compared to that of the ethanolic extracts. Among the three samples, *Mangifera indica* L. showed higher carbohydrate content with 12mg in aqueous and 7mg in ethanolic extract followed by *Piper betel* L. with 8mg in aqueous and 4mg in ethanolic extract and *Lawsonia inermis* L. with 6mg in aqueous and 2mg in ethanolic extract (Figure 16). Carbohydrates are essential compounds produced by plants which serve as energy reserves and are widely used in pharmaceutical industry, textile, paper, food, agricultural as well as in chemical industries, (Bemiller J.N., 2004).



Protein estimation was carried out according to Lowry's method. The results were expressed in mg/100ml. The aqueous extracts showed higher protein concentration than the ethanolic extracts in all the three samples. Comparatively among the three samples, *Piper betel* L.

Figure 19: Lipid Estimation



showed higher protein content with 37mg in aqueous and 22.4mg in ethanolic extracts followed by *Mangifera indica* L. with 28.4mg in aqueous and 21.4mg in ethanolic extract and *Lawsonia inermis* L. with 15.4mg in aqueous and 14mg in ethanolic extract (Figure 17). Proteins are organic compounds distributed in plant tissues and are widely used in clarification of wine, in

vaccines, tissue engineering and controlled drug delivery (Wadhve et al., 2014).

Aminoacid estimation was carried out according to Ninhydrin method. Results were expressed in mg/100ml. The aqueous extracts showed higher aminoacid concentration than ethanolic extracts in all the three samples. Aminoacid content in *Lawsonia inermis* L. was found to be 8mg in aqueous and 4mg in ethanolic extracts. *Mangifera indica* L. had aminoacid content of about 10mg in the aqueous and 6mg in ethanolic extracts while *Piper betel* L. showed aminoacid content of 16mg in aqueous and 14mg in ethanolic extracts. Comparatively *Piper betel* L. showed higher aminoacid content followed by *Mangifera indica* L. and *Lawsonia inermis* L. (Figure 18). Amino acids play an important role in Pharma industry in the form of precursor in antibiotic production, in transfusion, in the treatment of heart failure, peptic ulcer etc. They are also utilized as flavor enhancers, food additives, in the manufacture of artificial sweetners (Ivanov et al., 2016).

Table 2: Antibacterial Activity of Ethanol (ET), Ethyl Acetate (EA), Chloroform (CH) and Aqueous (AQ) Extracts of *Lawsonia inermis* L., *Mangifera indica* L. and *Piper betel* L.

S.no	Organisms	Extracts – Zone of inhibition (in mm)											
		<i>Lawsonia inermis</i> L.				<i>Mangifera indica</i> L.				<i>Piper betel</i> L.			
		ET	EA	CH	AQ	ET	EA	CH	AQ	ET	EA	CH	AQ
1	<i>Staphylococcus aureus</i>	8	5	4	2	16	15	-	3	9	7	3	1
2	<i>Streptococcus mutans</i>	5	3	7	1	2	-	1	6	6	5	2	1
3	<i>Klebsiella pneumoniae</i>	6	3	3	3	3	5	2	5	6	3	2	1
4	<i>Salmonella typhi</i>	6	4	4	2	10	9	6	5	7	8	3	-
5	<i>Enterobacter spp.</i>	8	3	4	1	7	5	-	10	4	5	2	1

Lipid estimation was carried out according to method given by Folch, 1957. Among the samples, *Lawsonia inermis* L. showed higher lipid content of 8% followed by *Piper betel* L. with 5% and *Mangifera indica* L. with 3% (Figure 19). Lipids are widely utilized in the preparation of cosmetics, in paints and varnishes, in detergents, also in pharmaceuticals and diagnosis, (Alvarez and Rodriguez., 2000).

Antibacterial activity of extracts:

Antibacterial activity was determined for the ethanol, ethyl acetate, chloroform and aqueous extracts of *Lawsonia inermis* L., *Mangifera indica* L. and *Piper betel* L. against 5 Pathogenic organisms such as *Staphylococcus aureus*, *Streptococcus mutans*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Enterobacter spp* (Figure 20).

Figure 20: Antibacterial Activity of *Lawsonia Inermis* L., *Mangifera Indica* L. and *Piper Betel* L. Extracts

Among the three samples *Lawsonia inermis* L. showed good inhibition against all organisms followed by *Piper betel* L. and *Mangifera indica* L. The ethanolic extract from all the three samples were found to be more active in inhibiting bacterial pathogens while aqueous extracts from *Lawsonia inermis* L. & *Piper betel* L. and chloroform extract from *Mangifera indica* L. displayed less inhibition activity. Chloroform extract of *Mangifera indica* L. displayed no inhibition against *Staphylococcus aureus* and *Enterobacter spp* while ethyl acetate extracts didn't show any inhibition against *Streptococcus mutans*. In case of *Piper betel* L. only aqueous extract was found not to be active against *Salmonella typhi* (Table 2). Bangash et al., (2012) have also reported that ethanolic extract was the most effective against bacterial pathogens than chloroform extract which is least effective.

CONCLUSION

The present work deals with the phytochemical studies and antibacterial activity of leaf samples of Medicinal plants *Lawsonia inermis* L., *Mangifera indica* L. and *Piper betel* L. Phytochemical analysis is generally carried out in order to analyze the presence or absence as well to determine the quantity of a specific compound in the

plant extract. The nature and distribution of bioactive compound varies in different plant species based on their growth and environmental conditions. In this study phytochemical analysis (Qualitative & Quantitative study) was carried out for the leaf extracts of *Lawsonia inermis* L., *Mangifera indica* L. and *Piper betel* L. obtained using different solvents and their antibacterial activity was also determined against 5 bacterial pathogens such as *Staphylococcus aureus*, *Streptococcus mutans*, *Klebsiella pneumoniae*, *Salmonella typhi* and *Enterobacter spp.* Among the tested extracts, ethanolic extract showed good yield as well as increased activity against all bacterial pathogens and based on the results obtained further studies will be carried out in future on the isolation and purification of compounds for different pharmacological applications.

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Conflict of interest: None

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