International Journal of Modern Chemistry, 2020, 12(1): 1-12

Modern Scientific Press

International Journal of Modern Chemistry **ISSN: 2165-0128**

Journal homepage:www.ModernScientificPress.com/Journals/IJMChem.aspx *Florida, USA*

Article

Phytochemical and Antimicrobial Properties of Lavandula *angustifolia*

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Article history: Received 20 February 2020, Revised 1 April 2020, Accepted 1 April 2020, Published 20 April 2020.

Abstract: This present study deals with the quantitative and qualitative phytochemical screening, the antimicrobial properties and thin layer chromatography of *Lavandula angustifolia* leaves extracts. These were achieved using the cold maceration method of extraction involving hexane, water (aqueous) and ethanol, to ensure that a wide polarity range of components are extracted. Alkaloid, phenol, flavonoids, tannins, terpenes, glycosides and steroids were observed present in the leaves extract of this plant. Quantitative analysis was also conducted to determine the percentage composition of some of the secondary metabolites, and the antimicrobial sensitivity test was carried out using the well diffusion method to determine the susceptibility of the plant. It was interesting to note that the hexane extract of *L. angustifolia* have great potential of been used as a phytomedicine especially against the deadly *salmonella typhi* associated diseases e.g fever, malaria and typhoid at a better extent than the commercially available erythromycin drug which was used as control. This plant extracts were also discovered to be very active against pathogenic bacteria like *Escherichia coli* and *Pseudomonas aeruginosa* at great and encouraging degrees.

Keywords: *Lavandula angustifolia*; Phytochemical; Chromatography; Antimicrobial

1. Introduction

It is obvious that over the years, plant extracts and plant-derived medicines have contributed immensely to the overall health and well-being of human being (Anyanwu and Nwosu, 2014). The plant

kingdom has become a storehouse or treasury for potential drugs and in recent years, the importance of medicinal plants and its awareness has greatly increased (Yadav and Agarwala, 2011). The antimicrobial ability of these plant extracts and oils has established a platform for the processing and transformation of plant products into pharmaceuticals, preservatives and medicines. *L. angustifolia,* have been used as a folkloric medicine by the local inhabitant of Cross River state, particularly among the Bekwarra community. It is most commonly used for its medicinal care. *L. angustifolia* has been used traditionally to treat infections like fever, malaria, dermatitis and athletes foot. According to Krzyszt and Smigielski 2009, the oil is traditionally believed to be antibacterial, antifungal, carminative, sedative, anti-depressive and effective for burns and insects bites. According to (Graham, 2001) the plant kingdom still holds many species of plants containing substances of medicinal value which are yet to be discovered and hence the quest to explore them in this research. From the available literature and to the best of my knowledge, little work has been done on this selected plant in Bekwarra community of Cross River state, Nigeria. The purpose of this research is to provide a scientific rationale to validate the claimed therapeutic or medicinal properties of this plant and to identify and document traditional medicinal plants found in Bekwarra community of Cross River state, Nigeria.

2. Materials and Methods

2.1. Sample Collection and Preparation

The leaves of *Lavandula angustifolia* were collected from its natural habitat in Bekwarra Local Government Area of Cross River State, Nigeria. The sample was air-dried for two weeks under shade then milled into fine powder using a milling machine.

2.2. Extraction

The extraction was done using a solute-solvent ratio of 1:10 respectively. This was done by soaking 20 g of the samples in 200 ml of solvent (n-hexane, ethanol and water) for four (4) days with frequent agitation in order to obtain the leaves extract. The resulting mixture was filtered using a filter paper and concentrated by allowing the solvent for (n-hexane) mixture to evaporate in an open air, ethanol was concentrated using the rotary evaporator and water extract was concentrated in a water bath at 60 ºC. The extract was kept in a refrigerator until required for testing.

2.3. Phytochemical Screening of Extracts

A preliminary screening of each extract (aqueous extracts, n-hexane extracts and ethanol extracts) of *L. angustifolia* was performed following the standard phytochemical analysis protocol as described by (Ushie *et al*., 2016), Egwaikhide and Gimba 2007), and (Geetha*et al.,* 2014) the test was carried out as follows:

2.3.1. Detection of alkaloids

Wagner's test: 10 mg of the extract was taken and few drops of Wagner's reagent were added and the formation of a reddish brown precipitate indicates the presence of alkaloids.

2.3.2. Detection of saponins

Foam test: 0.5 mg of extract was diluted with 20 ml distilled water and shaken well in a graduated cylinder for 15 min. The formation of foam to a length of 1cm indicated the presence of saponins.

2.3.3. Detection of flavonoids

Lead acetate test: 10 mg of extract was taken and few drops of 10% lead acetate solution were added. Appearance of yellow colour precipitate indicates the presence of flavonoids.

2.3.4. Detection of tannins

Ferric chloride test: 5 mg of extract was taken and 0.5 ml of 5% ferric chloride was added. The development of dark bluish black color indicates the presence of tannins.

2.3.5. Detection of glycosides

Glycoside test: 0.5 mg of extract was dissolved in 1 ml of water and then aqueous NaOH solution was added. Formation of yellow color indicates the presence of glycosides.

2.3.6. Detection of steroids and sterols

Salkowski's test: 5 mg of extract was dissolved in 2 ml of chloroform and equal volume of concentrated sulphuric acid was added along the sides of the test tube. The upper layer turns red and lower layer turns yellow with green fluorescence, indicating the presence of the steroids and sterols compound, in the extract.

2.3.7. Detection of anthraquinones

Borntragers test: About 0.5 g of the extract was boiled with 2 ml of 10% HCl for few minutes in a water bath. The resultant solution was filtered and allowed to cool. Equal volume of chloroform was added to the filtrate. Few drops of 10% NH_3 solution was added to the mixture and heated. Formation of rose-pink colour indicated the presence of anthraquinones on both extracts.

2.3.8. Detection of phenols

Sodium hydroxide test: 5 mg of extract was dissolved in 0.5ml of 20% sulphuric acid solution. Followed by addition of few drops of aqueous sodium hydroxide solution, it turns blue which indicates the presence of phenols.

2.3.9. Detection of terpenoids

Salkowski's test: 0.2g of the extract was mixed with 2 ml of chloroform and 3 ml of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown interface was formed which indicates a positive result for the presence of terpenoids

2.4. Quantitative Determination of Phytochemicals

2.4.1. Determination of total alkaloids

5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed according to Dey and Chaudhuri (2012), Obadoni and Ochuko (2001),

Percentage yield of alkaloid= $\frac{W2}{W1}\ X\ 100$…….. eqn(1)

Where, W_2 = Weight of dried end product; W_1= Weight of powdered sample taken for the analysis

2.4.2. Determination of total tlavonoids

5 grams of plant sample was repeatedly extracted with 100 ml of 80% aqueous methanol at room temperature. The mixture was filtered through a Whatman No. 1 filter paper into a pre weighed 250 ml beaker. The filtrate was transferred into a water bath and allowed to evaporate to dryness and weighed according to Krishnaiah*et al.*, 2009, and (Harborne, 1983), the percentage yield was calculated using eqn(1)

2.4.3. Determination of saponin

Estimation of saponin was done according to slightly modified standard method by Van-Burden and Robinton (1981). Ten grams of powder sample was taken in 250 ml conical flask and 100 ml of 20% ethanol was added to it. The mixture was heated in a hot water bath of 55^0C for 5 hours with continuous stirring. The mixture was filtrated through Whatman paper number 1 and the supernatant liquid was separated. The solid residue was mixed with 20% ethanol and heated in a similar way for about 5 hours. The solution was filtered and mixed with previously filtered solution. The combined filtered solution was placed on a hot water bath of 90^0C and heated still the volume was reduced to 20% of its initial volume. The concentrated sample was transferred into a 250 ml separating funnel and 10 ml of diethyl ether was added to it and shaken vigorously. The aqueous layer was separated carefully after setting down the solution. The purification process repeated again. 60 ml of n-butanol extracts were washed

twice with 10 ml of 5% aqueous NaCl solution. The remaining solution was heated in a water bath at 50^0C until the solvent evaporates and the solution turns into semi dried form. The sample was then dried in an oven. This saponin content was calculated by the following equation:
The percentage yield was also calculated using eqn(1)

2.5. Antimicrobial Analysis

2.5.1. Test micro-organism

The organisms*: Pseudomonas aeroginosa*, *Salmonella typhi* and *Escherichia coli*, were gotten from the Microbiology laboratory, Federal University Wukari, Taraba state Nigeria.

2.5.2. Sterilization of materials

All glass wares used in this study were washed with detergent, rinsed and sterilized in a dry ventilated oven at 160 ºC for 2 hours. All media were sterilized by autoclaving at a temperature of 121ºC and 15 psi for 15 minutes. The scalpel, cork borer, inoculating needle were sterilized by dipping them into 70% ethanol and passing them over a Bunsen burner flame until red hot.

2.5.3. Media preparation

The medium used was Mueller Hinton Agar (MHA) prepared according to the manufacturer's instruction and as described by Cheesebrough (2004). About 38 g of powdered MHA medium was dissolved in 1 L of sterile distilled water and sterilized by autoclaving at 121ºC at 15 psi for 15 min and allowed to cool before pouring carefully into 100 sterile Petri dishes. The Petri dishes that contained the medium were incubated for 24 hours at room temperature (37 ºC) to check for sterility before use.

2.5.4. Antimicrobial activity

Antimicrobial susceptibility testing was done using the well diffusion method to detect the presence of anti-bacterial activities of the plant samples (Perez *et al*., 1990). A sterile swab was used to evenly distribute bacterial culture over the appropriate medium (Mueller Hinton Agar). Mueller Hinton agar was prepared as per the instructions by the manufacturer. The plates were allowed to dry for 15 minutes before use in the test. Once the media solidified then it was then inoculated with the bacteria species. The media was then punched with 6 mm diameter hole and was filled with extract; a pipette was used to place 30 µl of the extract into the well. A total of two extracts was used on a particular bacterial species; with a total of three plates used for each extract including. The positive control was the same on all isolate. The plates were incubated at 37ºC for 24 hours after which they were examined for inhibition zones. A ruler was used to measure the inhibition zones

3. Results and Discussions

3.1. Phytochemical Analysis

Table 1 shows the results of the preliminary phytochemical screening carried out on the leaves extract of *L. angustifolia*. The results provide evidence for the presence of alkaloid, tannins, glycosides, steroids, phenols, terpenes, flavonoids, saponins and anthraquinones in selected extracts. Flavonoid was absent in the hexane and water extracts of *lavandula angustifolia* except in ethanol extract which was detected present. Anthraquinones was only present in hexane and ethanol extracts of *lavandula angustifolia.* Hence *L. angustifolia*can be used as an analgesic or anaesthetic due to the presence of alkaloids, this is in accord with the report by harborne (1988). Alkaloids has contributed to the majority of the poisons, neurotoxins and traditional Psychedelics and social drugs (e.g nicotine, caffeine, methamphetamine ephedrine cocaine, and opiates) by humans (Zenk and Juenger 2007).The presence of tannins in all the plant extracts could attribute to the use of such plant as anti-septic and as astringents against diarrhea (Fujiki*et al*., 2012). Glycoside found in this plant encourages the use of the plant in the treatment of arrow poison pharmacologically (Trease and Evans 1989). *Lavandula angustifolia*can be used as anti-inflammatory, antiplasmodic and diuretic properties which can be attributed to high flavonoid, steroids, glycosides and saponnis (Savithramma 2011). These phytochemicals are biologically active and can be responsible for the antimicrobial activity of the plants (Oyama*et al*., 2016).

Table 1: Phytochemical Screening of *lavandulaangustifolia*leaves extract

Phytochemicals	***Aqueous extract***	***Hexane extract***	***Ethanol extract***
Alkaloids	+ +	+	+
Saponins	+	+	_
Flavonoids	_	_	+
Tannins	++	+	+
Glycosides	+	+	+
Steroids	+	+	+
Anthraquinones	+	_	+
Phenols	+	+	+
Terpenes	+	+	+

+ = present, - = Not present, ++=intensively present

3.2. Thin Layer Chromatography

Chromatographic profiles of crude extracts obtained through different solvents systems were similar. A critical look at the chromatographic profiles for each extraction technique and solvent used enables one to evaluate the qualitative and quantitative variations in secondary metabolite content

(Cristiane, 2009). In addition, these data present compound profiles related to the biological effects and medicinal use. As shown in tables 2, the solvent system with the best component resolution is a mixture of hexane and ethyl acetate in a ratio of 7:3 because it enabled the resolution of more number of components and so can also be used as an alternative extraction mixture.

Table 2: Thin Layer Chromatography Analysis

Samples	Solvent system	Number of component	Retention factor
aqueous(water) extract	n-hexane:ethyl acetate (1:1)	6	0.21, 0.27, 0.45, 0.64, 0.91
	n-hexane:ethyl acetate (5:1)	5	0.19, 0.25, 0.30, 0.53, 0.69
	n-hexane:ethyl acetate (7:3)	8	0.11, 0.17, 0.23, 0.40, 0.49, 0.61, 0.80, 0.90
Hexane extract	n-hexane:ethyl acetate (1:1)	2	0.50, 0.72
	n-hexane:ethyl acetate (5:1)	5	0.11, 0.40, 0.53, 0.76, 0.96
	n-hexane:ethyl acetate (7:3)	7	0.10, 0.18, 0.57, 0.75, 0.81, 0.90, 0.96

3.3. Quantification

A quantitative examination was carried out and the result shown in Table 3 and fig.1 is a plot showing the quantities of the phytochemicals which includes alkaloid, flavonoid and saponins. The result showed that saponin was more abundant in the plant (56.14% saponin). The abundant nature of saponins in this plant could be responsible for the phytoanticipins or phytoprotectant nature of the plant alongside the antimicrobial activity observed in these plants as backed up by (Lacaille-Dubois *et al*., 2000) and (Yoshiki*et al*., 1998).The flavonoid content in *L. angustifolia* could be responsible for the plant's special ability to inhibit the growth of *salmonella typhi*as recorded from the result of the antimicrobial analysis in agreement with (Sarithramma 2011).the appreciable quantity of alkaloid in the plant makes it a good analgesic or anesthetic (harborne, 1988). The quantitative determinations of the phytochemicals or secondary metabolites are presented on the chart (fig.1).

Table 3: Quantitative Phytochemical analysis of*Lavandulaangustifolia* in percentages

S/No	*phytochemicals*	*Percentage composition*
1	*Alkaloids*	*4.90*
2	*Flavonoids*	*28.54*
3	*Saponins*	*56.14*

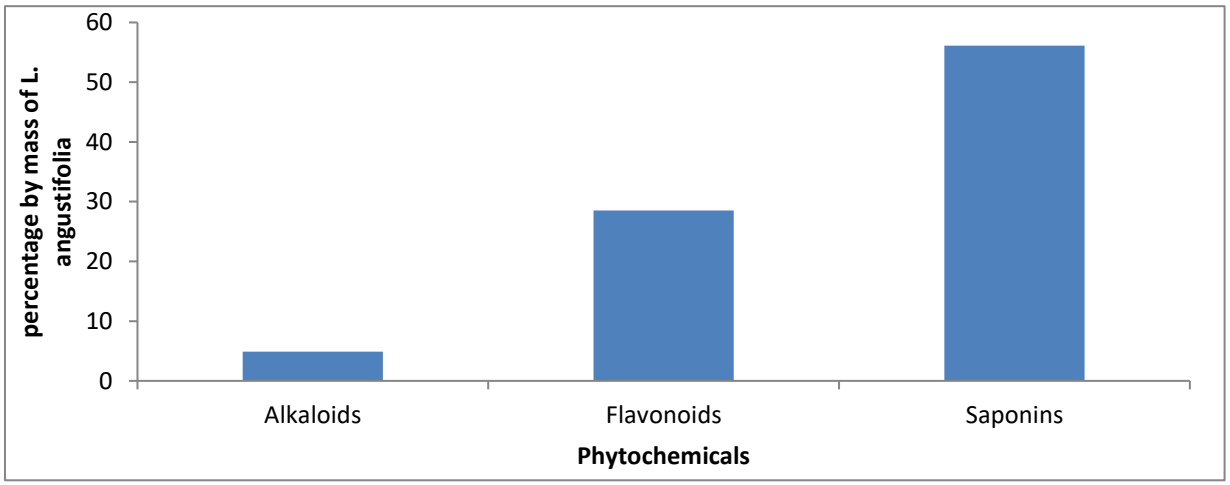

Figure 1: A Chart for the quantification of secondary metabolites in *L. angustifolia*

3.4. Antimicrobial Activity

The agar well diffusion method was used to study the antimicrobial properties. The extracts from *L. angustifolia* presented appreciable antimicrobial activity/properties to most of the tested microorganisms. As presented in Table 4 and Fig. 2, the various extracts were studied against three pathogenic bacteria strains which include *Escherichia coli*, salmonella typhi and pseudomonas aeruginosa. During the antimicrobial analysis, A positive control drug erythromycin was used to compare the results of the plants extracts, erythromycin control showed a zone of inhibition ranging from *15.67±1.15*, *16.00±1.00* and *18.33±0.58* against Escherichia Coli, Salmonella tylphi and pseudomonas aeruginosa respectively.

Table 4: Antimicrobial Screening of *Lavandula angustifolia* extracts

TEST ORGANISMS	WATER	HEXANE	ETHANOL	*Erythromycin(control)*
E.coli	0.00	*5.33±0.58*	0.00	*15.67±1.15*
S.typhi	0.00	*20.67±1.15*	0.00	*16.00±1.00*
P. aeroginosa	0.00	*8.00±2.52*	*9.33±2.08*	*18.33±0.58*

Value represents mean±Standard deviation of three replicates.
There is significant difference between means at P(< 0.05)

Salmonella typhi recorded the highest diameter of zones of inhibition with hexane extract of *L. angustifolia*(20.67±1.15 mm) than that by erythromycin control of 16.00±1.00mm, this finding indicates a possible use of such extracts as an alternative remedy for salmonella typhi associated diseases such as

typhoid, fever, especially the hexane extract of *L. angustifolia*. This finding supports the report by oyama*et al*., 2016.

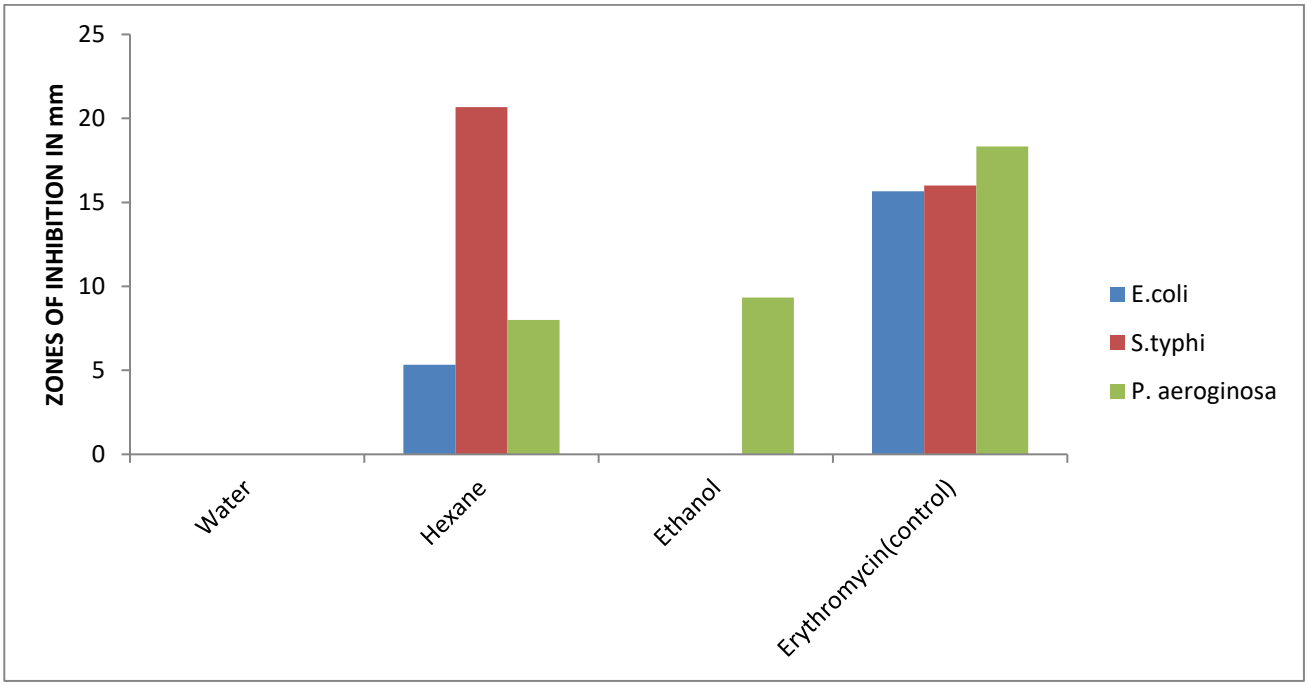

Figure 2: A Chart showing the zones of inhibition by*L.angustifolia*

Pseudomonas aeruginosa was appreciably inhibited by the Ethanolic extract of *Lavandula angustifolia* with an inhibition zone of 9.33±2.08 mm followed by the hexane extract with a zone of inhibition of 8.00±2.52 mm meanwhile, the water extract shows no activity against p. *aeroginosa*. For this test organism, Erythromycin control showed an activity with 18.33±0.58 mm zone of inhibition. The activity of the plant extracts against Escherichia coli was not well marked with only hexane extract showing a zone of inhibition of 5.33±0.58 mm and the rest presenting no activity.

All the aqueous extract shows no activity against the test organisms. The inability of most of the aqueous extract to show no antimicrobial activity could be attributed to soluble flavonoids (mostly anthocyanins) which have no antimicrobial significance and water soluble phenolics only important as antioxidant compound. Though, traditional healers use primarily water but plant extracts from organic solvents give more consistent antimicrobial activity compared to water extracts just as reported by (Tiwari *et al*., 2011).

4. Conclusions

The phytochemical analysis reveals the presence of alkaloid, flavonoids tannins, glycosides, steroids, phenols, terpenes, saponins, anthraquinones in the plant extracts used with alkaloid, flavonoid

and saponins present in a considerable amount. Hence, *L. angustifolia* can be used for the treatment of inflamed surfaces of the body, neurotoxins, astringents against diarrhea, antibacterial and as antiplasmodic.

The result obtained from this study indicates that *Lavandula angustifolia* have a high potential to act as useful drug especially against *Salmonella typhi* associated diseases since the extract showed more activity than the commercial drug (erythromycin) which was used as a control. Thus, this study indicates that the plant show much promise in the development of phytomedicines due to their antibacterial properties.

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