Phytochemicals Quantification, TLC and Antimicrobial Assessment of the Leaves and Fruit Extracts of Lasimorpha Senegalensis (Schott) Araceae

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Abstract

Background: *Lasimorpha senegalensis* is a perennial herbaceous plant that grows in marshy places and produces a clump of leaves from a short, thick, strongly stoloniferous rhizome. It's been used to treat gonorrhea and dysentery, among other ailments. The leaves can also be used to treat coughs and uneasiness and agitation in bigger dosages. It's also given to pregnant women to help them deliver more quickly. The study aimed to conduct phytochemical screening and evaluate the antimicrobial properties of *Lasimorpha senegalensis*. The leaves and fruit parts *Lasimorpha senegalensis* (Schott) Araceae were collected at Korokorosei community in Bayelsa State, air-dried, pulverized and extracted using dichloromethane, ethanol, methanol and water. Flavonoids, saponins, tannins, ketones, and cardiac glycosides were all found to be present. Preliminary thin layer chromatographic screening of the extracts was also done, as well as the antibacterial evaluation of the different fractions using the agar diffusion technique.

Results: The phytochemical screening for tannins, cardiac glycoside, ketones, and flavonoids were positive. Inhibition zones for the aqueous fraction at 62.5, 125, 250 and 500 mg/ml for *E. coli* was 32, 22, 18 and 11 mm respectively, while 8, 9, 10 and 14 mm for *Pseudomnas aeruginosa*. For the methanolic fraction, at the above concentrations, 10, 11, 14 and 14 nm was observed as zones of inhibition on *Escherichia coli*, 14, 19, 15, and 20 nm for *Pseudomnas aeruginosa*, and 19, 20, 20, and 20 nm for *Staphylococcus aureus*, respectively. Furthermore, in the dichloromethane fraction, 13, 20, 19, and 15 nm were observed as zones of inhibition on *Escherichia coli*, 8, 10, 15, and 15 nm for *Pseudomnas aeruginosa*, and 20, 15, 20, and 18 nm for *Staphylococcus aureus*, respectively.

Conclusion: It is evident, from the results obtained that the leaf portion of the plants can be used in the treatment of infections caused by *E. col, Pseudomonas aeruginosa*, and *Staphylococcus aureus*. With the high amounts of flavonoids observed, it can possibly be employed as an antioxidant and a cardioprotective.

Background

A medicinal plant is any plant that contains chemicals that can be employed for therapeutic purposes or as precursors for the manufacture of effective pharmacotherapeutic drugs in one or more of its components (Da-Cheng, 2019). The synergistic bioactivity of phytochemicals in plant extracts is frequently regarded as a benefit that is difficult to duplicate with single synthesized conventional medications (Abdullahi et al., 2020). *Lasimorpha senegalensis* is a monocotyledonous flowering plant with blooms produced on a form of inflorescence spadix that belongs to the Araceae family. A spathe, or leaf-like bract, surrounds and protects the spadix (Bown *et al*., 2000). The plant is known as Swamp arum, with local names like Okwoo-bà (Ijaw/Izon/Korokorosei), Akasi-iyi (Ika), Ede mmiri (Igbo), etc., (Kay Williamson, 2012). *Lasimorpha senegalensis* is a perennial herbaceous plant that grows in marshy places and produces a clump of leaves from a short, thick, stoloniferous rhizome. It is found commonly in Senegal, Sierra Leone, Chad, Nigeria (Bayelsa), Central African Republic, DR Congo (VanderBurg WJ, 2004).

A short, thick, strongly stoloniferous rhizome creates a clump of leaves for the plant. Each leaf has an erect, spiny petiole up to 100cm long (up to 200cm) and a 50cm long (exceptionally up to 100cm) and 30cm wide arrow-shaped leaf blade (40cm). On a spiny, single peduncle up to 150cm (250cm) long, emerging from the leaves, the inflorescence is a cylindrical, purplish spadix up to 12cm long, encircled by a spathe up to 45cm long (Protabase, 2014). The plant usually forms large populations due to its strong development of underground suckers. The plant is found /occurs in swampy forest, along streams, in ditches and ponds and they are often very abundant (Govaerts *et al*., 2002). The plant is gathered in the wild for usage as food and medicine in the local community. In temperate climates, it might be used as an indoor pot plant, while in warmer climes, it could be used as a garden pond decorative. *Lasimorpha Senegalensis* is highly abundant in nature, especially in swampy environs (DeFilipps, & Krupnick, 2018). It is however potentially impacted by agricultural development, invasion by other species (*Cyperus papyrus*), and water pollution. It is classified as least concern in the International Union for Conservation of Nature (IUCN) red list of threatened species (Boos *et al*., 2003).

Several ethnomedicinal uses of *Lasimorpha Senegalensis* have been reported, include the management of gonorrhea and dysentery. In southern Nigeria, the fruits are reported to be part of ingredients for many remedies (Anumudu *et al*., 2019). In Sierra Leone, the young leaves are eaten as famine food and as an ingredient of palaver sauce, while, the young leaves are eaten as vegetables and the rhizomes used to treat ulcers in Gabon (Adamu et al., 2005). Also, in Congo, the leaves are taken to cure cough and in larger doses to treat nervousness and agitation. It is also given to women during childbirth to accelerate delivery (Adamu et al., 2005). Furthermore, the leaf sap has been taken orally against hiccups in Côte d'Ivoire and in the Eastern part of Nigeria (Igbos). Hepatitis and feverish diseases have also been treated with it. (VanderBurg WJ, 2004; Dalziel *et al*., 1937). *Lasimorpha senegalensis* has been reported to contain calcium oxalate crystals which are toxic if consumed raw. However, the calcium oxalate can easily be broken down by

thoroughly cooking the plant or by fully drying it. Moreover, caution should be taken when including this plant in the diet of people suffering from rheumatism, arthritis, gout, kidney stones and hyperacidity as it could induce adverse side effect which can lead to death (VanderBurg WJ, 2004; Dalziel *et al*., 1937).

There are previous reports on *Lasimorpha senegalensis*, Araceae, regarding its geographical distribution, edible and medicinal uses. Various Araceae species are used to cure malaria and its symptoms throughout the world's tropical regions (Frausin et al., 2015). Antimalarial species belonging to the genus *Amorphophallus Blume*, *Culcasia, Homalomena*, and others have been discovered in the African countries of Ivory Coast, Kenya, Gabon, Benin, and Togo, according to reports. The biggest number of Araceae plants are utilized as antimalarials in the Amazon region (Pedralli G, 2002; Frausin et al., 2015). The Amerindian ethnic groups employ the Neotropical genera; *Philodendron* Schott and *Anthurium* Schott: *Yanomami* (Brazil), *Tirios, Waypi* (French), *Makuna* and *Miraa* (Colombia), as well as *Secoya* and *Tacana* (Pacific Coast, Colombia) (Frausin et al., 2015). Surprisingly, no antimalarial Araceae genus was apparently employed on both the African and American continents, indicating that the species used had a regional range (Ayoola, 2008). Decoction was the method of extraction most cited for antimalarial remedies for species of Araceae. Leaves and the tubercles were the parts most often cited (Ayoola, 2008).

Another study examined *L. senegalensis* (Schott) leaf extract's antioxidant and hepatoprotective properties. The findings revealed that the leaf extract contained significant levels of bioactive phytochemicals as well as free radical scavenging activities. The extract also increased endogenous antioxidants and lowered lipid peroxidase and liver enzymes considerably (Chinyere *et al*., 2020).

Traditional medicine, despite being an old practice in illness prevention and treatment, is still widely used around the world to treat a variety of human ailments. Methanolic and aqueous extracts of *L. senegalensis* were tested for antibacterial activity against human pathogens, *Escherichia coli* and *Staphylococcus aureus*, in a study conducted between 2018 and 2019. (Anumudu *et al*., 2019). The efficacy of L. senegalensis against the test organisms at various concentrations was determined using the agar well diffusion method.

A tetrazolium chloride microtiter dilution experiment was used to establish the minimum inhibitory concentration (MIC). The inhibitory zone widths for both test organisms employing plant extracts ranged from 0 to 14 mm, which was less than the control (Trimethoprim / Sulfamethoxazole and chloramphenicol) which ranged from 0 to 26 mm. The MIC was 62.5 mg/ml to 500 mg/ml. Methanolic stem extract yielded the lowest MIC (Karunanidhi et al., 2013).

Preliminary phytochemical screening revealed the presence of flavonoids responsible for the antibacterial activity. As a result, *L. senegalensis* is deemed medicinally important because it contains physiologically active chemicals with anti-infectious disease potential (Anumudu et al., 2019). Hence, the current study was aimed to conduct phytochemical screening of the leaves and fruits of *Lasimorpha senegalensis* (Schott) using different solvents, determine the possible fractions present by thin-layer chromatography and screen for its potential antimicrobial properties using clinical isolates of *Neisseria gonorrhea, E. coli, S. aurous, amoeba histolitica* and *candida albican*.

Method

Reagents

Ethanol, methanol, distilled water, dichloromethane, 10% ammonia, concentrated sulfuric acid, resorcinol, concentrated hydrochloric acid, ferric chloride, acetic acid, n-Hexane, 5% ferric chloride, normal saline, distil water, Amoxicillin (25ug) disc.

Location of Plant Collection

The leaves and fruits of *Lasimorpha senegalensis* (Schott), Araceae, were collected at Korokorosei kingdom; latitude: 4.750752, [4°45'02.7"N], longitude: 6.006715 [6°00'24.2"E], Olodiama clan, in Southern Ijaw Local Government Area, Bayelsa State, Nigeria. The Korokorosei Kingdom boarders with other communities including Ikienghebiri, Ikebiri I, Olugbobiri, Ondewari, Okpotuwari and other settlements, all surrounded by aquatic environ. The plant was authenticated by a botanist at the Pharmacognosy and Herbal Medicine department, Faculty of Pharmacy, Niger Delta University, Wilberforce Island.

Plant Extraction

The leaves and fruitss of *Lasimorpha senegalensis* (Schott) Araceae were collected, air-dried and pulverized to coarse powder using a mechanical blender. About 903g of the coarse leaf powder was transferred into Winchester bottle and cold-macerated for five days with about 3.8L of methanol. About 801 g of the coarse fruit powder was also transferred into Winchester bottles and 3.8 L of methanol was used to extract using cold maceration technique for a week. The powder was filtered and the filtrate was concentrated using water bath at 44°C. Tthe concentrated extracts were allowed to cool and they were stored safely.

Phytochemical Screening

Flavonoids, Saponins, Tannins, Ketones, and Steroids (Cardiac glycosides) were screened for phytochemicals and secondary metabolites using conventional techniques (Auwal et al., 2014; Nwankwo et al., 2021).

Saponins can be detected using the following test: In a test tube, 0.5g of the plant extract was added to 10ml of distil water and briskly shaken. After strong shaking, no foaming forms, indicating the absence of Saponins in the leaf and fruit.

For Cardiac glycoside, 0.5g of extract was dissolved in 5ml chloroform and then filtered. In the test tube, 1 mL of acetic acid was added. To build a layer underneath, 1ml concentrated sulfuric acid was slowly introduced through the test tube's side. The presence of steroidal nucleus is shown by a shift in color from violet to green in the leaf and fruit extracts.

For flavonoids, 5 mL of 10% ammonia solution was added to a part of the extract's aqueous filtrate, followed by a concentration and equal volume of conc. sulfuric acid. The presence of flavonoids in the leaf and fruit is indicated by the yellow hue.

For Tannins: 10ml distilled water was added to the extract. After which, a couple of drops of ferric chloride were added. The presence of tannins in the leaf and fruit is indicated by the brownish-green coloring. 2ml conc. HCl plus a few drops of resorcinol. The presence of ketones in the plant's leaf and fruit is indicated by the rose coloration.

Fractionation of the Extracts

Three factions were prepared for the leaf including aqueous, methanol and dichloromethane fractions, while aqueous, ethanol, and dichloromethane fractions where prepared for the fruit.

Thin Layer Chromatography (TLC)

The different fractions were spotted using a capillary tube on the plate. A saturated chamber containing the solvent system n-hexane and Ethyl acetate in a ratio of 5:2, and 9:1, respectively were developed. The TLC plate was then transferred into the chamber. After 15 minutes, the plate was removed from the chamber when the solvent had gotten to the solvent front. The plate was allowed to dry then viewed under ultra Violet Lamp and the spots seen were noted with a sharp pencil. It was stained with anisaldehyde and sulfuric acid and allowed to dry, after which the spots became visible. The distance moved by the solute and the distance moved by the solvent front was noted and the retardation factor calculated.

Antimicrobial Screening

Preparation of Stock solution and serious dilution: 0.5g of the extract in 0.5ml of 0.5% DMSO and was shaken properly to dissolve the extract. Then 4.5ml of distilled water was added to the solution. Five concentrations (500mg/ml, 250mg/ml, 125mg/ml, and 62.5mg/ml) were prepared for each fractions (Aqueous fraction, DCM fraction, Methanol fraction). And for the fruit, five concentrations were prepared using the different fractions. Antimicrobial screening was carried out against the following organisms: *Staphylococcus areus, Pseudomonas eruginosa, Echerichia coli, Protis*. All these organisms were collected from the laboratory stock stored in the refrigerator. The test organisms were cultured from the stored of a molten agar plate and incubated at 37°C for 24hours. The various bacteria culture or isolates were standardized using sterile bottle containing 5ml of sterile water and the turbidity was adjusted and compared to McFarland's standard, (A 0.5ml of McFarland's standard was prepared by mixing 0.05ml of 1.175% barium chloride dehydrate ($BaCL_2.2H_2O$) with 9.95ml of 1% sulfuric acid (H_2SO_4).

Agar Preparation and Susceptibility Testing for Anti-microbial

The weight if the agar-agar was calculated based on the amount in milliliters (40ml) of agar needed and this was transferred into a beaker containing distilled water and autoclaved at 121°C for 15minutes. The agar was then poured into the petri dishes allowed to cool and set and transferred into a hot air oven for final drying. These processes were carried out under aseptic conditions to avoid contamination. The standardized bacteria suspension, was poured into the Muller Hinton Agar plates and the excess fluid was poured

into a beaker containing sodium hypochlorite. In other to ensure a uniform and confluent growth, the suspension was poured twice over the entire surface by repeating the procedure, taken care the second time to turn the plate through 60°. The inoculum on the agar plate was allowed to dry for 5-15minutes. A sterile cork-borer was used to prepare four holes of 10milliliter in diameter, in each agar plate aseptically at a distance of 15mm apart. Using a sterile pasture pipette, two drops of molten agar was used to seal each hole. Concentration of 500mg/ml, 250mg/ml, 125mg/ml, and 62.5mg/ml of the test sample extract were introduced into the various hole respectively and allowed to stand for 1hour for sufficient pre diffusion of the extracts to occur. This method was used to test each fractions (Aqueous fraction, methanol fraction, and dichloromethane fraction) against each of the test organisms (*Escherichia coli*, *Pseudomonas aeruginosa, Staphylococcus areus, Proteus vulgaris*) separately and incubated at 37°c for 24 hrs and inhibition zone measured and recorded appropriately.

Results

Table 1
Phytochemical and Secondary Metabolites screening

Phytochemicals	Positive indicator	Results	
		Leaf	Fruit
Saponins	Frothing which persist on warming	-	–
Flavonoids	A yellow coloration	++	+++
Cardiac glycoside	A change in color from violet to green.	+++	+
Ketones	A rose coloration	+++	+++
Tannins	A brownish-green	++	+

Key: +++ = densely present, + = present, – = absent

Table 2
TLC Analysis

Sample	Solvent System (n-Hex:ETA)	Spots	Rf value	Solvent System (n-Hex:ETA)	Spots	Rf value
Leaf	9:1	1	0.8	5:2	1	0.1
(Methanol)	9:1	2	0.2	5:2	2	0.2
	9:1	3	0.3	5:2	3	0.4
	9:1	4	0.4	5:2	4	0.6
	9:1	5	0.6	5:2	5	0.6
	9:1	6	0.7	5:2	6	0.8
	9:1			5:2	7	0.9
Fruit (Methanol)	9:1	1	0.1	5:2	1	0.3
	9:1	2	0.8	5:2	2	0.8
Leaf (DCM)	9:1	1	0.59	5:2	1	0.1
	9:1	2	0.7	5:2	2	0.2
	9:1	3	0.8	5:2	3	0.4
	9:1	4	0.9	5:2	4	0.6
	9:1	0		5:2	5	0.7
	9:1	0		5:2	6	0.8
	9:1	0		5:2	7	0.9
Fruit (DCM)	9:1	1	0.9	5:2	1	0.9
Leaf (Aq)	9:1	1	0.8	5:2	0	
Fruit (Aq)	9:1	0		5:2	0	
Key: *Aq = Aqueous, n-Hex = n-hexane, ETA = Ethyl acetate, Rf = Retention factor*						

Table 3
Showing antimicrobial activity of the leaf *Lasimorpha senegalensis* (Schott) Araceae

Organisms	Aqueous fraction (mg/ml)				Methanol fraction (mg/ml)				Dichloromethane fraction (mg/ml)				(H_2O)	Amoxicillin
Conc (mg/ml)	500	250	125	62.5	500	250	125	62.5	500	250	125	62.5	(0.3ml)	(25µ)
E. coli (mm)	11	18	22	32	14	14	11	10	15	19	20	13	0	37
Pseudomonas aeruginosa (mm)	14	10	9	8	20	15	19	14	15	15	10	8	0	27
Staphylococcus aureus (mm)	0	0	0	0	20	20	20	19	18	20	15	20	0	28
Proteus vulgaris (mm)	0	0	0	0	0	0	0	0	0	0	0	0	0	10
Key: *mm = millimeter, mg/ml – milligram per milliliter, µ - microgram*														

Discussion

The extraction of the leaf with methanol yielded 2.2%w/w and the extraction of fruit with ethanol yielded 1.9%w/w. The results of the phytochemical screening for tannins, cardiac glycoside, ketones, and flavonoids were positive. While phytochemical screening for saponins was negative for the leaves and fruits of *Lasimorpha senegalensis* (Fig. 1 and Table 1). The TLC showed that the leaf aqueous fraction with the solvent ratio of n-hexane and ethyl acetate (5:2) showed the presence of one (1) spot, dichlormethane fraction showed seven (7) spots, and the methanol fraction showed six (6) spots (Table 2). This specifies that, the plants has several chemical constituents with the each spot indicating a particular chemical entity as was obtained from the retention factors values. For the second solvent ratio of n-hexane and ethyl acetate (9:1), the aqueous fraction showed one (1) spot, seven (7) spots for dichloromethane and six (6) spots for the methanol fraction, depicting the presence of several chemical constituents (Fig. 2). The antimicrobial susceptibility test conducted using the leaf extracts showed some activities against *Escherichia coli, Pseudomonas aeruginosa*, and *Staphylococcus arues* except aqueous fraction that did not show any antimicrobial activity against *Staphylococcus aureus* and *Proteus vulgaris*. All the faction showed no activity against *Proteus vulgaris*. Comparing with the standard drug, (Amoxicillin), the zones of inhibition for the aqueous fraction at 62.5, 125, 250 and 500 mg/ml for *E. coli* was 32, 22, 18 and 11 mm respectively, while 8, 9, 10 and 14 mm for *Pseudomnas aeruginosa* at same concentration. No inhibition was observed for *Staphylococcus aureus* and *Proteus vulgaris.* A dose dependent response was observed from the above activities, for the E. coli, as dose was decreased, higher zone of inhibition (antimicrobial activity) was obtained, while the reverse was seen in the case of *Pseudomnas aeruginosa* (Table 3).

For the methanolic fraction, at the above concentrations, 10, 11, 14 and 14 nm was observed as zones of inhibition on *Escherichia coli*, 14, 19, 15, and 20 nm for *Pseudomnas aeruginosa*, and 19, 20, 20, and 20 nm for *Staphylococcus aureus*, respectively. Thus, more activities was seen against *Staphylococcus aureus* (a gram positive organism) and *Pseudomnas aeruginosa* (a gram negative organism). Furthermore, in the dichloromethane fraction, 13, 20, 19, and 15 nm was observed as zones of inhibition on *Escherichia coli*, 8, 10, 15, and 15 nm for *Pseudomnas aeruginosa*, and 20, 15, 20, and 18 nm for *Staphylococcus aureus*, respectively (Table 3). These findings are similar to a previous findings on antimicrobial properties evaluation *of Lasimorpha senegalensis*, (Anumudu *et al.*, 2019). The leaf section of the plants can also be utilized to treat disorders or infections caused by *E. coli*, urinary tract infections, gastroenteritis, neonatal meningitis, hemorrhagic colitis, and Crohn's disease, according to the findings (Todar K., 2007); *Pseudomonas aeruginosa*, such as bronchopneumonia, septic shock, urinary tract infection, GIT infection, skin and soft tissue infections (Todar, 2004); and *Staphylococcus aureus* (bacteremia, urinary tract infections, abscess (boils), cellulitis, meningitis, soft tissue infections, pneumonia and septicemia, as well as food poisoning (Kuehnert et al., 2005; Tong et al., 2015 Woodson J., 2017).

Conclusion

The results obtained from the antimicrobial susceptibility test showed activities against *Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus*, but no efficacy on *Proteus vulgaris*. Thus, it is a potential antimicrobial plant that can be explored in the development of use antibacterial agents. From the results obtained, it shows that the plant leaves, when standardized can be used as supplement in heart ailments as it contains a high level of cadiotonic steroids. The plant contains a high amount of flavonoids, hence, it can employed as an antioxidant, cardioprotective, etc. The leaf also showed the presence of tannins which have been reported to be present in most medicinal formulations used in the management of sexual dysfunction, diabetes, urinary and respiratory tract infections. Therefore, further is required for its standardization and isolation of active constituents.

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Figures

Figure 1

Leaves (aerial parts) and fruit of Lasimorpha senegalensis

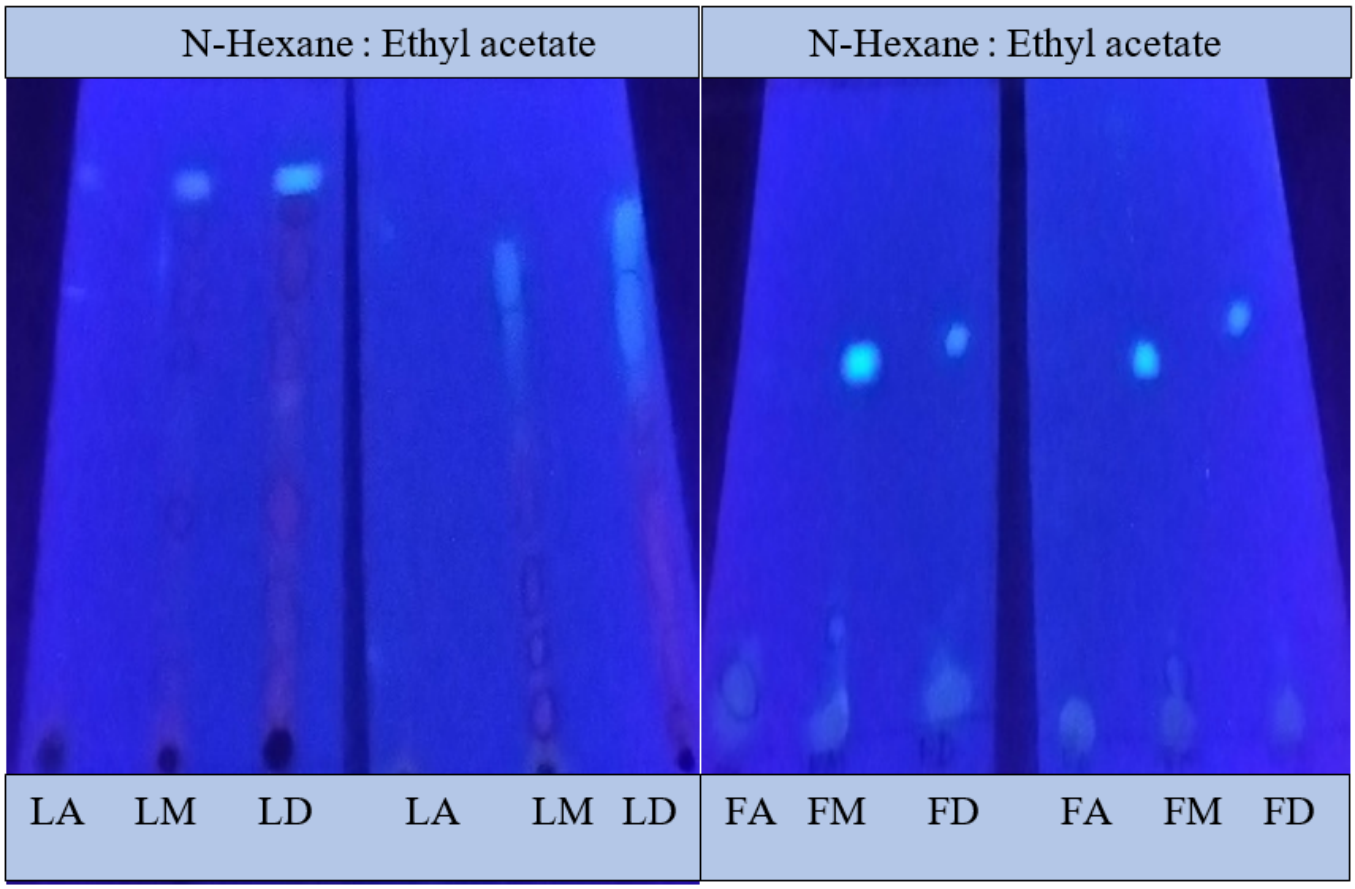

Figure 2

TLC plates of the leaf and fruit extract under UV lamb: (L – Leaf, F – Fruit, A – Aqueous, M – Methanol, D – Dichloromethane)

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