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Chromatographic characterization of bioactive chemical groups of *Syzygium lineatum (DC.) Merr. & L.M. Perry* (Malibado) Extracts

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Abstract
Medicinal plants are a source of great economic value all over the world. Medicinal value of plants lies in some bioactive compounds that produce a definite physiological action on the human body. The biological active compounds that are present in plants referred as phytochemicals. These phytochemicals derived from various parts of plants such as leaves, barks, seed, seed coat, blossoms, roots and pulps and consequently utilized as wellsprings of direct therapeutic specialists. Phytochemistry depicts the enormous number of secondary metabolic compounds present in the plants. The plants are the stores of normally happening substance mixes and of fundamentally differing bioactive particles. The present investigation was aimed to characterize the bioactive compounds of *Syzygium lineatum (DC.) Merr. & L.M. Perry* plant extracts (hexane, carbon tetrachloride and chloroform) were used in various analyses of thin layer chromatography, column chromatography and UV-visible spectroscopy. The extraction of bioactive compounds from the plants and their quantitative and qualitative estimation is significant for investigation of new biomolecules to be utilized by pharmaceutical and agrochemical industry directly or can be utilized as a lead molecule to synthesize more potent medicines.

Keywords: *S. lineatum*, chromatographic characterization, solvent- solvent partitioning, thin layer chromatography, column chromatography, spectrophotometric analysis

1. Introduction

The search for novel foods is a relevant practice worldwide. Nearly 80% of the world's population relies on traditional medicines for primary health care, most of which involve the use of plant extracts [8]. Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries [7]. The constituents present in the plant play a significant role in the identification of crude drugs [5]. Phytochemical screening is very important in identifying new sources of therapeutically and industrially important compounds like alkaloids, flavonoids, phenolic compounds, saponins, steroids, tannins, terpenoids, etc [2].

Syzygium lineatum (D.C.) *Merr. & L.M. Perry* locally known as malibado and lubeg in some parts of the region, is one of the fruits that the Philippines can call its very own. This plant is native to Northern Luzon, originating from primary forests at low to medium elevation [1]. *S. lineatum* fruits are believed to contain antioxidants which give fruits their vibrant color. Khoo, *et al*. [4] mentioned that it is also an excellent source of Vitamin C, a very good source of dietary fiber. Likewise, this plant could be a good source of the essential dietary mineral and may contain active constituents that brings varieties of health benefits and may serve as starting material for the preparation of functional foods. Functional foods offer health benefits in addition to the nutritional value inherent in their chemical compositions, and they may have potential roles in reducing the risk of chronic degenerative diseases [3].

Information on the bioactive chemical groups of *S. lineatum*, would promote its sustainable utilization and its protection since it is becoming endangered. The extraction and identification of bioactive chemical groups from this plant material is the first step in its utilization as functional food ingredients, additives to food, pharmaceutical, and cosmetic products, hence this study.

2. Materials and methods

2.1. Extraction of Bioactive Constituents

Seeds, pulp, leaves and bark samples of *S. lineatum* were collected and subjected to preliminary processing. The samples were air dried separately then cut into small pieces and grinded. The resulting powdered samples were exhaustively extracted with 95% ethyl alcohol. These were filtered and the resulting filtrates were subjected to vacuum concentration (Stuart rotary evaporator), at temperatures between $40^{O}C$ and $45^{O}C$ until sticky residue were obtained. These were kept in a flask wrapped with carbon paper, labeled properly and stored in the refrigerator for subsequent chemical analysis.

2.2. Solvent partitioning of the ethanolic crude extracts

Solvent- solvent partitioning was done using the protocol designed by Kupchan and Tsou [6] and modified version of Wagenen *et al* [9]. A portion of the crude extracts were triturated separately with 95% ethanol, and successively fractionated using three solvents of increasing polarity. The resulting fractions were evaporated to dryness and kept in air tight containers for further analysis.

2.3. Optimization of TLC solvent system.

Different solvent systems were evaluated for optimization of Thin Layer Chromatography (TLC). Neat solvents were applied on pre-coated TLC plates previously spotted with concentrated fractions and evaluated. Solvents that gave good separation were used in establishing the solvent system to be used for the chromatographic evaluation.

2.4. Characterization of bioactive constituents
The resulting fractions were subjected to chromatographic techniques in order to characterize the bioactive chemical groups that are present.

2.4.1. Thin Layer Chromatography
Certain volume of the crude extract and the resulting fractions were applied as a spot at the origin of the TLC plate [Silica gel G60 F254 A1 she*et al*uminum plates (Merck, Germany)]. The spots were dried between application by the flow of cool air to allow application to be as thin as possible and improve separation. The plates were developed in glass tanks (CAMAG) which were previously saturated with solvent vapors, reducing the time for development. Two different mobile phases were established. For non-polar compounds, mobile phase used consist of chloroform: methanol in a ratio of 9:1. With the more polar compounds, separation was achieved by using mobile phase consisting of chloroform: methanol in a ratio of 16:4.
In the location of separated compounds, the plates were viewed under daylight and UV light (254nm and 366nm) to locate the UV active compounds.

2.4.2. Column chromatography
The samples from the three fractions were subjected to column chromatography. Normal column chromatography was done using a glass column packed with silica gel (flash column) by wet packing method. The required amount of stationary phase (silica gel) was poured to form silica column and soaked with hexane. Then about 5 ml of the chloroform soluble residue was poured on top of the silica column taking care not to disturb the top of the column. A piece of filter paper circle was then place on top and solvent was poured over it, the column was then eluted with gradient of dichloromethane and methanol. Eluates were collected every after 5ml resulting to a total of 60 eluates. The eluates were subjected to TLC and those with common Rf values were pooled together. The pooled eluates were then left undisturbed allowing the excess solvents to evaporate resulting to crystal formations. The crystals will then be subjected to further evaluation.
The same procedure was applied to the carbon tetrachloride and hexane fractions, using gradient of appropriate solvents.

2.5. Spectrophotometric analysis
Sample of the crude extract, the fractions of hexane, chloroform and carbon tetrachloride were centrifuged at 3000rpm for 10 minutes and filtered. The solutions were then diluted 10x with the same solvent for elution. The resulting solutions were transferred to the cuvettes and for their absorption using a spectrophotometer (Agilent T60) for proper evaluation of the bioactive chemical groups present in the sample. The extract was examined under visible and UV light in the wavelength ranging from 220-1100nm.

3. Results and Discussions
3.1. Extraction of bioactive chemical groups
Table 1 summarizes the result of the solvent extraction of the different parts of *S. lineatum*. The extracting solvent is 95% ethanol. The variation in the color of the resulting crude extracts indicates the presence of different chemical groups present in the sample.

Table 1: Result of the extraction process on *S. lineatum* plant parts.

Sample	Dry Weight (grams)	Volume of Solvent	Volume of Crude Extract (mL)	Concentration of the extract (grams of dried plant material/mL of extract)	Color of Crude Extracts
Seeds	640	450	65.3	9.8	Light brown
Pulp	381.5	1200	74.32	5.13	Light Violet
Leaves	650	3,250	126.97	5.12	Green
Bark	221.6	1200	43.17	5.13	Dark brown

3.2 Solvent system evaluation
A total of 10 neat solvents were evaluated with the result showing twelve promising solvents. These were further evaluated and their polarities were assessed through literature search. The end results served as the bases for use in the chromatographic separation.

3.3 Liquid Partitioning/Fractionation of the Extracts
Liquid partitioning resulted to three fractions namely hexane fraction, carbon tetrachloride fraction and chloroform fraction. The amount of extracts recovered from partitioning is shown in table 2.

Table 2: Result of liquid partitioning of the ethanolic extracts from *S. lineatum* plant parts.

Sample	Dry Weight (grams)	Weight of Crude Extract (grams)	Weight of Derived Fractions(grams)		
			n-Hexane	Carbon tetrachloride	Chloroform
Seeds	640.0	10.35	1.6974	1.1385	1.3765
Pulp	381.5	6.33	1.0381	0.7680	0.8419
Leaves	650.0	9.42	1.5449	1.0362	1.2530
Bark	532.6	10.65	1.5975	1.1715	1.4165

3.4 Chromatographic analysis of the different fractions of *S. lineatum* extracts
a. TLC monitoring of the bioactive chemical groups. Screening of bioactive chemical groups of the three fractions of *S. lineatum* was done by applying thin layer chromatography. The results are shown in table 3.

Table 3a: TLC monitoring of bioactive chemical groups present in pulp

Nature of Fraction	No. of Spots developed		
	Daylight	254nm	366nm
Non polar	4	4	1
Semi polar	3	3	2
Polar	2	1	3

Table 3b: TLC monitoring of bioactive chemical groups present in seed extract

Nature of Fraction	No. of Spots developed		
	Daylight	254nm	366nm
Non polar	7	-	4
Semi polar	-	4	4
Polar	-	7	4

Table 3c: TLC monitoring of bioactive chemical groups present in the leaf extract

Nature of Fraction	No. of Spots developed		
	Daylight	254nm	366nm
Non polar	7		4
Semi polar	-	4	4
Polar	3	7	4

Table 3d: TLC monitoring of bioactive chemical groups present in the bark extract

Nature of Fraction	No. of Spots developed		
	Daylight	254nm	366nm
Non polar	5		4
Semi polar		4	4
Polar		7	4

3. Column chromatographic analysis of the leaf extracts

1. Hexane fraction

Column: Silica gel F,
Eluant: gradient of Pet ether: Ethyl acetate:methanol
TLC monitoring**:**
Solvent system: $CHCl_3$: MeOH (19:1)
Solvent front: 44mm
Crystals were derived from eluate no. 1-10, 16-17, 18-19, 24-27
Status: for further analysis
Solvent system: $CHCl_3$: MeOH (16:4)
Solvent front: 44mm
Crystals were derived from eluate no. 28-29
Status: for further analysis

2. Chloroform fraction

Column: Silica gel F
Eluant: gradient of n-hexane: dichloromethane: methanol
No. of eluates: 50
TLC monitoring:
Solvent system: $CHCl_3$: MeOH (19:1)
Solvent front: 44mm
Crystals were derived from eluate no.23-28; 42-50
Status: for further analysis
Solvent system: $CHCl_3$: MeOH (16:4)
Solvent front: 44mm
Crystals were derived from eluate no. 29-42; 43-53
Status: for further analysis

3. Carbon tetrachloride fraction

Column: Silica gel F
Eluant: gradient of n-hexane: dichloromethane: methanol
No. of eluates: 50
TLC monitoring:
Solvent system: $CHCl_3$: MeOH (19:1)
Solvent front: 44mm
Crystals were derived from eluate no. 3, 4, 6,8,12, 13; 7, 9, 15-18, 26-28, 21
Status: for further analysis
Solvent system: $CHCl_3$: MeOH (16:4)
Solvent front: 44mm
Crystals were derived from eluate no. 26, 27, 28
Status: for further analysis

4. UV-VIS Profile of the Malibado Extracts

The UV-VIS profile of *S. lineatum* extracts were selected from 220 – 1100 nm and the absorbance ranges from 0.000 to 2.55 to detect the characteristic wavelength of the plant extract. Agilent T60 spectrophotometer was used.

Table 4: Result of UV VIS analysis of different fractions of *S. lineatum*

Sample	Wavelength (nm)	Absorbance
Leaves		
Polar	390	0.154
Nonpolar	420	2.549
Semi-polar	436	0.137
Bark		
Polar	895	0.125
Nonpolar	877	0.147
Semi-polar	853	0.127
Pulp		
Polar	797	0.114
Nonpolar	763	0.111
Semi-polar	680	0.110
Seeds		
Polar	1071	0.143
Non-polar	1027	0.146
Semi-polar	975	0.129

4. Conclusion

The result of the study will serve as reference material for studies on natural products and will support future undertakings involving utilization of *S. lineatum*, a Philippine berry considered as indigenous plants to our region.

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6. References

1. Arbor A. *Syzygium lineatum* (DC.) Merr. & L.M. Perry, Arbor, 1938, 19:109.
2. Akindele AJ, Adeyemi OO. Anti-inflammatory activity of the aqueous leaf extract of Byrosocarpuscoccineus. Fitoterapia, 2007; 78:25-28.
3. Kaur S, Das M. Functional Foods: An Overview. August 2011. Food science and biotechnology. 2011; 20(4):861-875. DOI: 10.1007/s10068-011-0121-7
4. Khoo H, Azlan A, Tang S, Lim S. Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. Food Nutr Res. 2017; 61(1):1361779. Published online 2017 Aug 13. doi: 10.1080/16546628.2017.1361779
5. Koparde A, Doijad R, Magdum C. Natural Products in Drug Discovery, Pharmacognosy - Medicinal Plants, Shagufta Perveen and Areej Al-Taweel, Intech Open, 2019. DOI: 10.5772/intechopen.82860. Available from:

https://www.intechopen.com/books/pharmacognosy-medicinal-plants/natural-products-in-drug-discovery
6. Kupchan SM, Tsou G, Sigel CW. Datiscacin, a novel cytotoxic cucurbitacin 20-acetate from *Datisca glomerata*. J Org Chem. 1973; 38(7):1420-1. doi: 10.1021/jo00947a041.
7. Musthafa M, Nastaran A, Marikar F, Rajandram D, Ali Ahmed A. Phytochemical, Pharmaceutical and Biochemical Activites of Selected Climber Plants: A Review. Annual Research & Review in Biology. 2017; 16(5):1-22. 2017; Article no.ARRB.34796 ISSN: 2347-565X, NLM ID: 101632869
8. Sandhya B, Thimas S, Isabel W. Ethnomedical plants used by the Valaiyan community of Piranmalai Hills (Reserved Forest), Tamilnadu, India—a pilot study. African Journal of Traditional, Complementary and Alternative Medicines, 2006, 3(1). DOI: 10.4314/ajtcam.v3i1.31145
9. Van Wagenen BC, Larsen R, Cardellina JH, II Ran dazzo D, Lidert ZC, Swithenbank C, *et al*. Ulosantoin, a potent insecticide from the sponge Ulosaruetzleri. J Org. Chem, 1993; 58:335-337.