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STUDIES ON EXTRACTION, PHYTOCHEMICALS AND THIN LAYER CHROMATOGRAPHIC (TLC) ANALYSIS OF STEM BACK AND ROOT EXTRACTS OF *MORINDA LUCIDA* (NVUVU)

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Abstract: *The present study is aimed at qualitatively screening for phytochemicals in M. lucida stem bark and root to determine the major classes of compound present in them. This was done using three different solvents successively to extract. The effects of solvent-to-solid ratio on the percent yield of extracts were assessed. Phytochemical analysis was performed by simple chemical analysis including thin layer chromatographic (TLC) analysis which was carried out using n-hexane, ethyl acetate (3:1), n-hexane, ethyl acetate (3:2) and n-hexane, ethyl acetate, and methanol (2:1:1) solvent systems. The optimal percent yield as a factor of solvent-to-solid ratio of the extracts obtained is 10:1. The phytochemical analysis revealed the presence of saponins, tannins, anthracenes, flavonoids, alkaloids, carbohydrate, gum and Mucilages and protein. The TLC analysis of M. lucida stem back revealed the presence of 8 spots using n-hexane, ethyl acetate (3:1) solvent system and 7 spots using n-hexane, ethyl acetate (3:2) solvent system. The TLC results of the root extracts shows 10 spots using n-hexane, ethyl acetate (3:1) solvent system, 8 spots using n-hexane, ethyl acetate (3:2) solvent system and 2 spots using n-hexane, ethyl acetate, and methanol (2:1:1) solvent system. The present study provides evidence that solvent extract of M. lucida contains some possible medicinally important bioactive compounds and this justifies the use of the plant species as traditional medicine for treatment of various diseases.*

Keywords: *Phytochemicals, solvent-to-solid ratio, thin-layer chromatography, Morinda lucida, Successive extraction.*

Introduction

Phytochemicals are naturally occurring non-nutritive chemicals produced by plants for their protection, recent research demonstrate that they can also protect humans against diseases. That they have protective or disease preventive properties such as antioxidant, enzymes stimulants; have hormonal action, anti-bacterial activity and DNA replication action [Sowemimo et al., 2007] that

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acts as natural defense system for the host plants against bacteria, fungi and viruses [Njerua et al., 2013], [Abhijeet et al., 2018] and in addition provide colour, aroma and flavor [Sangeetha et al 2014], [Mace, 1963]. These chemicals called phytochemicals give the plant color (plant pigments), aroma and flavor. Some of the most important phytochemicals are carotenes, chlorophyll, and flavonoids and are naturally present in many foods [Olaniyan, 2016]. Phytochemicals help to protect our cells against oxidative damage and reduce the risk of developing certain types of cancer, reduce the risk of high blood pressure responsible for heart failure, and stimulate enzymes that make the estrogen less effective and could reduce the risk for breast cancer, prevent the multiplication of cancer cells by protecting DNA from carcinogens. They also have anti-bacterial properties, reduce the risk of urinary tract infections and will improve dental health, prevention and treatment of colorectal cancer, diabetes, heart disease, high cholesterol, gastrointestinal disorders and obesity. Some help to alleviate cholesterol levels in patients with cardiovascular complications, and may also improve dental health, help improve your overall health and boosts immune system [Olaniyan, 2016].

Materials and methods

Sample Collection and Preparation

The sample *Morinda lucida* (Nvuvu) stem back and roots were collected from Oyofo Oghe in Ezeagu Local Government Area on 29th June, 2019. Oyofo Oghe lies Northwest of Enugu Metropolis. This plant parts were collected by following leads supplied by local healers in Oghe communities where the plants are used in traditional medicine. The sample *M. lucida* stem back and roots were air dried in-doors to a constant weight at room temperature [Dilika et al., 1996], [Baris et al 2006] as differences in water content may affect solubility on subsequent separation by liquid-liquid extraction. The samples were ground into fine powder as this will increase the surface area for extraction thereby increasing the rate of extraction.

Successive extraction

Soxhlet extraction method was used in the extraction process. The solvent-to-sample ratio affects the quantity and quality of constituents obtained. In this studies solvent to sample ratios of 5:1, 10: 1, 15:1, 20:1 and 25:1 (v/w) solvent to dry weight ratio were used. Thirty grams of dried ground plant material (stem back and roots) were weighed and transferred into a 25 x 100 mm cotton extraction thimble and exhaustively extracted with 150, 300, 450, 600 mL analytical grade solvents of increasing polarity to ensure extraction of a wide polarity range of compounds. The extracts were concentrated to a constant weight and labeled n-hexane, ethyl acetate and methanol extracts of *M. lucida* stem back and roots respectively [Green, 2004], [Das et al ., 2010], [Odebiyi and Sofowara, 1978] - [Ogbuanu et al., 2014]

Screening for phytochemicals in the plant extracts

The extracts were subjected to a phytochemical screening using the following tests: the Wagner [35], Dragendorff [Sofowara, 1993], - [Trease and Evans, 1989] Mayer [Trease and Evans, 1989] and Kraint tests for alkaloids; the Ninhydrin [Yasuma and Ichikawa, 1953], [Devi et al., 2020], [Hemantkumar, 2017] Biuret [Sofowara, 1993],[Priyanga et al., 2014] and Millon [25] tests for amino acids and proteins; Fehling and Benedict tests for carbohydrates [Priyanga et al., 2014] and Molish [Sofowara, 1993] spot and saponification tests for Fixed oils and Fats [Kokate, 1999], Borntrager [Evans, 1997], Legal, Fehling and Ferric chloride [Sofowara, 1993], tests for glycosides; Born Traggers test for anthracenes [Sofowara, 1993], the ferric chloride [Mace, 1963] and gelatin [Evans, 1997] tests for tannins; the Shinoda, alkaline and lead acetate tests [20] tests for flavonoids; the Libermann-Burchard test for phytosterols [Olaniyan, 2016] the Rosenthaler test for saponins [Sofowara, 1993], [Kokate, 1999], the absolute alcohol test for gum and

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Mucilages [Sai et al., 2011], [Whistler and BeMiller, 1993].

Thin layer chromatographic analysis (TLC)

Thin layer chromatographic analysis is often used in evaluating medicinal plants material [WHO, 1998.]. The ascending technique was employed in the TLC analysis. A clean dry chromatographic tank with 50ml of the running solvent (mobile phase) made-up of n-hexane and ethyl acetate (3.1), (3.2) and n-hexane, ethyl acetate and methanol (2.1.1) respectively were used to develop the chromatograms. The spots on the TLC plate after development were visualized in iodine tank and the position of the spots marked with pencil. The retention factor (R_F values) for each spot was calculated for each extract.

Results and discussion

If the extraction is for general phytochemical analysis or screening then the larger the variety of compounds the solvent will extract the better, because there is a better chance that biologically active compounds will be present [Eloff, 1998]. The results showed that n-hexane is a better extracting solvent followed by ethyl acetate as they extract more variety of compounds respectively than methanol. Methanol extracted fewer secondary metabolites and probably will contain traces of residual solvent that is toxic and likely will interfere with the bioassay. Due to low toxicity, ease of evaporation at low heat and ease of subsequent handling of the extracts, n-hexane followed by ethyl acetate are recommended for extraction of secondary metabolites in *M. lucida* stem back and root extracts.

Fig. 1 shows plots for the percent maximum yield as a function of solvent-to-solid ratios ranging from 5:1 to 25:1 for both stem bark and root extractions.

Table 1: Effect of solvent to sample ratios on percent yield of *Morinda lucida* (Nvuvu) stem back and root extracts.

Extract	Stem back					Root				
	% Yield solvent to sample ratios					% Yield solvent to sample ratios				
	5:1	10:1	15:1	20:1	25:1	5:1	10:1	15:1	20:1	25:1
n-Hexane	0.248	0.484	0.484	0. 484	0.484	0.258	0.494	0.494	0.494	0.494
Ethyl acetate	0.239	0.468	0.468	0.468	0.468	0.301	0.478	0.478	0.478	0.478
Methanol	0.201	0.233	0.233	0.232	0.233	0.213	0.243	0.243	0.242	0.243

The data presented correspond to the maximum values extracted by each ratio. As expected the solvent-to- solid ratio was optimal at 10:1 ratio. In this studies (Table 1) solvent-to-dry sample ratios of 10: 1 (v/w) used yielded more and this agreed as reported as ideal by [Ncube et al 2008].

Out of 30 g of plant material, optimal yield obtained for stem bark and root was at a ratio of 10:1 solvent–to-solid for all the extracting solvent (Table 1). In this successive exhaustive extraction, methanol gave the least percent yield of extract. This probably is due to the fact that most compounds present in the plant samples are soluble in n-hexane and ethyl acetate.

The results of TLC analysis of *M. lucida* stem back (Table 3) shows a total of 8 spots using n-hexane, ethyl acetate (3:1) solvent system (3 spots for n-hexane extract, 5 spots for ethyl acetate extract and non for methanol extract respectively). For n-hexane and ethyl acetate (3:2) solvent

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system revealed a total of 7 spots (n-hexane and ethyl acetate extracts gave 4 spots and 3 each and non for methanol). Solvent system n-hexane, ethyl acetate and methanol (2:1:1) gave no spot for n-hexane, ethyl acetate and methanol extracts.

The presence of anthracene only in the root extract of *M. lucida* plant may be responsible for the use of the root in Oghe traditional medicine. This may probably be working in synergy with other secondary metabolites and probably justifying the use of root in traditional medicine.

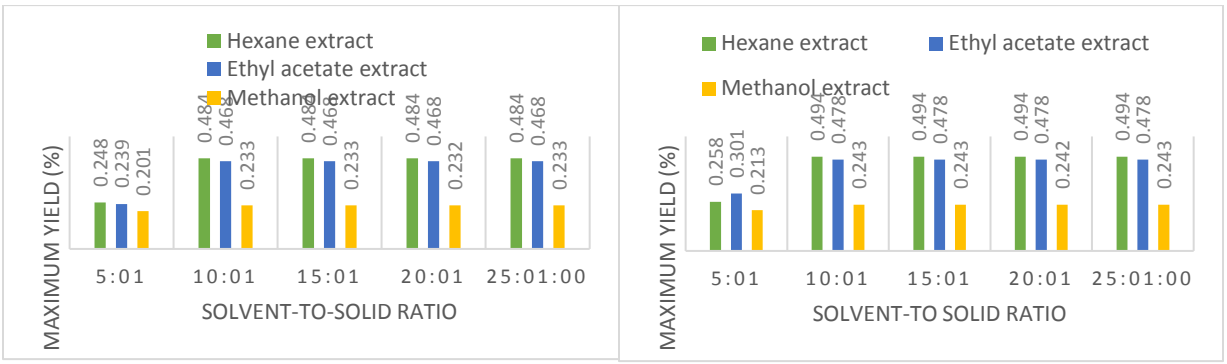

Fig. 1: Maximum percent extraction yield values of stem bark and root estimated with different solvent-to-solid ratio

The results of phytochemical analysis of the n-Hexane, ethyl acetate and methanol extracts of the stem bark and root of *M. lucida* plant were summarized in Table 2. The result showed that the plant is rich in saponins, tannins, anthracenes, flavonoids, alkaloids, carbohydrate and protein. The presence of these constituents in the investigated plant leaves account for their usefulness in traditional medicine. The degree of precipitation of secondary metabolites varies in solvents. This probably may be due to varying degree of solubility of different phytoconstituents in different solvents [Sangha]. n-Hexane (a non-polar solvent with dielectric constant of 1.9) stem bark extract shows the presence of saponins, hydrolysable tannins alkaloids, flavonoids, carbohydrate and proteins while the root contain saponins, tannins, anthracenes, alkaloids, flavonoids, carbohydrate and proteins.

Table 2: Results of phytochemical screening of *Morinda lucida* (Nvuvu) plant stem back and roots extracts

Parameters	**Stem back**			**Root**		
	A	**B**	**C**	**A**	**B**	**C**
Saponin	+	+	+	+	+	+
Saponin Glycoside	+	+	+	-	-	-
Tannins	-	-	-	-	-	-
Hydrolysable Tannins	+	-	-	+	+	-
Pseudo Tannin	-	-	-	-	-	-

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Test of Tannin (Using Gelatin)	-	-	-	+	-	-
Digital Glycoside	-	-	+	-	-	-
Glycoside General	-	-	-	-	-	-
Anthracene (Born Tragger Test)	-	-	-	+	-	-
Resins	-	-	-	-	-	-
Steroid and Triterpenoids (Libemann Buchard) Test	-	-	+	-	-	-
Volatile Oil Test	-	-	-	-	-	-
Alkaloid: (a). Draggendoff Test	-	-	-	-	-	-
(b). Wagner's Test	-	-	-	-	-	-
(c). Mayer's Test	-	-	-	-	-	-
(d). Kraint's Test	+	+	+	+	+	+
Flavonoid Test (a) Magnesium ribbon test				-	-	+
(b). Alkaline test	+	-	-	-	-	-
(c). Lead Acetate test	-	+	-	-	+	+
Carbohydrate Test	+	-	+	+	-	+
Protein	+	-	+	+	+	+
Gum and mucilages	-	-	-	-	+	-

+ = positive; - = negative; A = n-Hexane; B = Ethyl acetate; C = Methanol

Ethyl acetate (slightly polar solvent with dielectric constant of 6.4) stem bark extract gave saponins, hydrolysable tannins, alkaloids and flavonoids while the root extract gave saponins, hydrolysable tannins, alkaloids, flavonoids and protein. Methanol (polar solvent with dielectric constant of 31.0) stem bark extract gave saponin, saponin glycoside, alkaloids, carbohydrate and protein while the root extract contain saponin, alkaloids, flavonoids, carbohydrate and protein.

The TLC results of the root extracts shows a total of 10 spots (6 spots for n-hexane and 4 spots for ethyl acetate extracts while non for methanol extract) using n-hexane, ethyl acetate (3:1) solvent system. The results also using n-hexane, ethyl acetate (3:2) solvent system revealed a total of 8 spots (4 spots each for n-hexane and ethyl acetate extracts) while non for methanol extract. Hexane, ethyl acetate, and methanol (2:1:1) solvent system gave 0ne spot each for n-hexane and ethyl acetate extracts respectively and non for methanol extract.

TABLE 3: The number of spots for TLC analysis of *Morinda lucida* (Nvuvu) stem back and root extracts.

Solvent system	**Stem back**			**Root**		
	A	**B**	**C**	**A**	**B**	**C**
n-hexane, ethyl acetate (3:1)	3	5	-	6	4	-

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n-hexane, ethyl acetate (3:2)	4	3	-	4	4	-
n-hexane, ethyl acetate, methanol (2:1:1)	-	-	-	1	1	-

A = n-Hexane; B = Ethyl acetate; C = Methanol

Conclusion

The solvent-to-solid ratio was optimal at 10:1 ratio, meaning that more extract will be obtained at this ratio. The results obtained in the present study indicated *M. lucida* stem bark and root as a rich source of bioactive components. The results of TLC analysis of *M. lucida* stem back shows a total of 8 spots using n-hexane, ethyl acetate (3:1) solvent system and 7 spots using n-hexane, ethyl acetate (3:2) solvent system. Solvent system n-hexane, ethyl acetate and methanol (2:1:1) gave no spot for n-hexane, ethyl acetate and methanol extracts. The n-hexane, ethyl acetate (3:1) is a better solvent system followed by n-hexane, ethyl acetate (3:2). While n-hexane, ethyl acetate and methanol (2:1:1) solvent system could not partition the phytochemical and as such not a good solvent system.

References

1. Abhijeet, K., Archana, G. Nikita, K., Shubhangi, K. and Snehal, W. (2018). Phytochemical screening of Achyranthes aspera L., Amaranthus spinosus L., Parthenium hysterophorus L., Argemone mexicana L., Portulaca oleracea L. and Euphorbia geniculata Ortega. From Kolhapur district of Maharashtra. *International Journal of Herbal Medicine*, 6(3): 05-08.
2. Baris, O., Gulluce, M., Sahin, F., Ozer, H., Kilic, H., Ozkan, H., Sokmen, M. and Ozbek, T. (2006). Biological activities of the essential oil and methanol extract of *Achillea Biebersteinii* Afan. (Asteraceae). Turk.J. Biol., 30: 65-73.
3. Das, K., Tiwari, R.K.S. and Shrivastava, D.K. (2010). Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. *Journal of Medicinal Plants Research*, 4(2): 104-11.
4. Devi, B.N., Salma, S. and Lavanya, V. (2020). Phytochemical Evaluation and In vitro Antidiabetic Activity of Ethanolic Extract of Viscum articulatum. *Int. J. Pharm. Sci. Rev. Res*., 60(1): 99-104.
5. Dilika, F., Afolayan, A.J. and Meyer, J.J.M. (1996). Comparative antibacterial activity of two *Helichrysum* species used in male circumcision in South Africa. *S. Afr. J. Bot*. 63(3): 158-159.
6. Eloff, J.N. (1998a). A sensitive and quick microplate method to determine the minimum inhibitory concentration of plant extracts for bacteria. *Plant. Med*., 64: 711-713.
7. Evans, W.C. (1997). "Trease and Evans Pharmacognosy", Harcourt Brace and company. Asia pvt. Ltd.Singapore.
8. Evans, W.C. (1997). Pharmacology. Harcount Brace and Company, Asia, Singapore, Pp. 226 – 228.
9. Finar, I.L. (1986). "Stereo Chemistry and the Chemistry of Natural Products" Longman, vol. 2.
10. Gahan, P.B. (1984). "Plant Histo chemistry and Cytochemistry: *An Introduction*. Academic Press, Florida, U.S.A.
11. Green, R.J. (2004). Antioxidant Activity of Peanut Plant Tissues. Master's Thesis. North Carolina State University. USA.
12. Harborne, J.B. (1998). "Phytochemical Methods: *A Guide to Modern Techniques of Plant Analysis*." 2nd Edition, Chapmann and Hall Publishers, London.
13. Hemantkumar, A.T. (2017). Phytochemical Screening and Antibacterial, Antifungal activity of Pinda concanensis (Dalzell) P.K. Mukh. &

Constance (Apiaceae) an endemic plant from Nasik District (M.S.) India. *Annals of Plant Sciences*, 6(12): 1886-1892.

14. Kokate, C.K. (1999). "Practical pharmacognosy" 4 th edition, Vallabh Prakashan Publication, New Delhi, India
15. Mace, M.D. (1963). "Histichemical localization of phenols in healthy and diseased tomato roots", *Phytopathology*, 16: 915-925.
16. Ncube, N. S., Afolayan, A. J. and Okoh, A. I. (2008). Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *African Journal of Biotechnology*, 7(12): 1797-1806.
17. Njerua, S.N., Matasyoh, J., Mwaniki, C.G., Mwendia, C.M. and Kobia, G.K. (2013). A Review of some Phytochemicals commonly found in Medicinal Plants. *International Journal of Medicinal plants Photon.* 105: 135-140.
18. Odebiyi, O. and Sofowara, E. A. (1978). Phytochemical screening. Nigeria medical plants. Loydia, 41: 231–234.
19. Odebiyi, O.O. and Sofowora, E. A. (1979). Antimicrobial alkaloids from a Nigerian chewing stick (Fagara zanthoxyloides). *Planta Med.*, 36(3): 204–207.
20. Ogbuanu, C.C., Ehiri, R.C. and Ogah, S.P.I. (2014). Phytochemical Screening and Preliminary TLC Characterization of Alkaloids in *Sabicea brevies* Root. Research Journal of Phytochemistry, 8: 1-8.
21. Olaniyan, M.F. (2016).Health Promoting Bioactivities of Phytochemicals: *Research Findings*. Lap Lambert Academic Publishing, Germany.
22. Priyanga, S., Hemmalakshmi, S. and Devaki, K. (2014). Comparative Phytochemical Investigation of Leaf, Stem, Flower And Seed Extracts Of Macrotyloma Uniflorum L. Indo. *American Journal of Pharm Research*. 4(11): 5415-5420.
23. Sai Koteswar Sarma D, Venkata Suresh Babu A. (2011). Pharmacognostic and phytochemical studies of *Thespesia populnea* Linn *J. Chem. Pharm. Res.* 3(4):237-244.
24. Sangeetha, V.S., Michael, B. and Beena, L. (2014). Phytochemical analysis of Annona reticulate L. leaves extracts. *Int. Res J Pharm. App Sci*. 4(5) 4-8.
25. Sangha, S. R. B., Gayatri, M.C. and Rajanna, L. (2015). Antimicrobial Activity of Isolated Flavonoid Fractions from Drypetes Roxburghii (Wall.) Huresawa and Its Phytochemical Fingerprinting. *International Journal of Innovative Research in Science, Engineering and Technology*, 4(7): 6214-6224.
26. Sofowora, A. (1993). Screening Plants for Bioactive Agents: *Medicinal Plants and Traditional Medicine in Africa*. 2nd Edn. Spectrum Books Ltd., Ibadan, Niger,ia, Pp. 134-156.
27. Sowemimo, A.A., Fakoya, F.A., Awopetu, I. Omobuwajo, O.R and Adesanya, S.A. (2007).. Toxicity and mutagenic activity of some selected Nigerian plants. *J Ethnopharmacol*, 113(3): 427–432.
28. Trease, G.E. and Evans, W.C. (1978). A Text Book of Pharmacognosy. 11th Edn., Bailliere Tindall, London, pp. 60-75.
29. Trease, G.E. and Evans, W.C. (1989). Pharmacognosy. 13th Edn., ELBS/Bailliere Tindall, London, UK. Pp. 345-346, 535-536, 772-773.
30. Trease, G.E. and Evans, W.C. (1989). Pharmacognosy. 13th Edn. ELBS/Bailliere Tindall, London, UK, pp. 345-346, 535-536, 772-773.

31. Wagner, H. (1993). Pharmaceutical Biologic. 5 (Eds). AUFI 15 BN 3-437-20 498-X. Gustav Fisher Vwelag, Stuttgart, Germany.
32. Whistler, R.L. and BeMiller, J.N. (1993). Industrial Gums; Polysaccharides and their Derivatives, Academic Press, London.
33. WHO (1998). Quality control methods for medicinal plant materials World Health Organization Geneva,
34. WHO (2002). The World Health Report: *Reducing Risks, Promoting Healthy Life. World Health Organization, Geneva*.
35. Yasuma, A. and Ichikawa. (1953). "Ninhydrin-Schiff and alloxan- Schiff staining. A new histochemical staining methods for proteins", *J. Lab clin Med*, 41: 296-299, 1953.