

Isolation of Alkaloid from a Medical Plant (A Case Study of Morinda Lucida)

*¹Akpagu Francis C., ¹Nnamani Emmanuel V. And ²Eze Chukwuebuka G. I.

¹Applied chemistry option, department of science laboratory technology institute of Management and technology (imt), enugu, nigeria.

²Department of biochemistry, college of medicine, university of lagos, nigeria.

Abstract: The isolation and detection of alkaloids content of *Morinda lucida* (Ezeogwu) from Rubiaceae family, a medicinal plant was carried out using solvent extraction process. The dried powdered leaves of the plant were divided into batches. Different solvents were used on them. After 6 days of occasional shaking, it was filtered. The filtrates were used for testing the presence of alkaloids in *Morinda Lucida*. Mayer's reagents Wagner and Lugol's reagents and 5m sodium hydroxide were used as detecting reagents. Mayer's reagent yields cream precipitate in both acidic and alkaline extracts. Wagner and Lugol's reagents yield reddish brown precipitate in both acidic and alkaline extracts. 5ml sodium hydroxide gave white swirling precipitate. Other coloured precipitate like orange and pale orange was gotten as a result of difference in solvents used for isolation. The presence of the above precipitate indicates the presence of alkaloids in *Morinda Lucida*.

Keyword: *Morinda lucida*, Rubiaceae, alkaloids, isolation and detection.

I. Introduction

Alkaloids are large group of basic substances of natural origin usually plant which contain certain, hydrogen and one or more nitrogen and atom. The nitrogen and one or more nitrogen atoms. The nitrogen content is usually present in pyrrole, , pyrrolidine, quinolinine or isoquinoline. It can be naturally occurring chemical compounds containing basic nitrogen atoms (Awasthi and Aepita, 2007).

According to alkaloids are specified class of chemical compounds found in plants and occasionally animals. Many have long histories as poison, narcotics, hallucinogens and medicine agents. Generally, alkaloids are basic or alkaline substances i.e they neutralize acids. The basicity of alkaloids depends on the lone pair of electrons on their nitrogen atom thus forming salts with mineral acid such as HCl and H₂SO₄ and organic acids such as asatartaric acid or malic acid. Alkaloid must be active as a base in acid-base reaction. In principle, alkaloids could be classified either by the biological system in which they occur or by their structure. 3 said that most alkaloids contain oxygen but occasionally, as in nicotine, this element is absent. Such oxygen free compounds physically are usually volatile and liquids.

They vary widely in a substance and properties. Some are relatively simple with a few as five carbon e.g. xanthine, solanine is complicated with as many as 44 carbon atoms. The basic nature of alkaloids is not a constant. Many alkaloids have an oxidation state of -111 but some have quaternary nitrogen and oxidation state as high as + 111 e.g aristolachich acid 1 from Arisholachichia, these are acids. Many compounds are excluded from the group because it is simply practical to do so; purines, pyrimidine, pyraine, vitamins, antibiotics and amino sugars are all excluded. The objective of this work is aimed at isolating of alkaloids content of *Morinda Lucida* a medicinal plant and detecting it with some diodal.

II. Materials And Method

Materials

Drying broad, sample bottles, funnels, 250ml beaker, filter paper, analytical weighing balance, clamp, separating funnel, test tubes, water bath, measuring cylinder, masking tape, net, spatula, saturated sodium carbonate, methanol, Hexane, 1m hydrochloric acid, 5m sodium hydroxide, Mayer's reagent, Wagner and Lugol's reagent.

Sample Collection And Preparation

The plant material used was the leaf of *Morinda Lucida* (Ezeogwu). Fresh leaves of the above plant was obtained in sufficient quantity from secondary regrowth forest in Abali Amagu in Udi Local Government Area of Enugu State through the assistance of a plant taxonomist (Professor Jonathan Okafor) at 8.00am. the leave was sun-dried for some days under low temperature for easy pulverization, using a drying board. It was then grinded using grinding machine after it might have dried to powder form. The powdered material was sieved using net with little pores to remove the ungrounded fibre. 48g of the grined leave detriment that was weighed using analytical weighing balance and divided into 3 portions. Two out of 3 portions was moistened

with saturated sodium carbonate before 3 portions of the plant material were put into 1000ml bottles for extraction and was properly labeled.

Extraction

The extraction of active principles in the leaves of *Mirinda lucida* was carried out using solvent extraction process mechanically. 700ml of methanol was poured in the bottle containing plant material without sodium carbonate, 900ml of 1,1- dichloromethane was poured in the bottle containing plant material moistened with saturated sodium carbonate and 700ml of hexane was added to the third bottle. The content of the final bottle was shaken occasionally for 2days. After 2days the content was filtered to remove the solvent before adding 700ml of methanol. The 3 bottles was kept in a cool shelf and it was shaken occasionally for 6 days. After 6 days, the mixture was filtered with filter paper to remove the plant material from the extract. The bottle with muck of methanol only was added 400ml of hydrochloric acid in the molarity of 1, it was also filtered. The later filtrate was mixed with initial methanol filtrate. These mixture was heated with activated charcoal for purification and was filtered again of NaOH was added in sufficient quantity and the result observed. The extract from 1,1- dichloro methane and hexane with methanol was heated within the temperature range of the solvent to have the Goo. The Goo was then used for alkaloid test using diodal reagent.

Test For Alkaloid

The goo from each bottle was boiled with 2ml of 5% sulphuric acid in 50% ethanol about 10 minutes on a boiling water bath, it was cooled and filtered. Each filtrate was divided into two portions and was properly labeled, one portion from each was used directly for the following tests.

Mayer’s Test

3ml of each filtrate of the sample was poured into a test tube and a few drops of Mayer’s reagent added. The results were observed.

Wagner And Lugol’s Test

3ml of each filtrate of the sample was poured into a test tube and a few drops of Wagner and Lugol’s reagent added, the result were observed. The second part of each filtrate was transferred into a separating funnel and was made alkaline with dilute ammonia solution. The alkaline solution of each was then extracted with equal volume of chloroform. Then the lower chloroform layer was separated from the aqueous ammonia layer by running off through the tap. The aqueous layer of each sample was treated with the above diodal reagent that is Mayer and Wagner and Lugol. The result were observed.

III. Results And Discussion

Results

The research findings are determined and presented as follows:

Table 1: Result For GOO of 1,1 – dichloromethane.

TEST	OBSEVATION	INFERENCE
i. Initial acid extract + Mayer’s reagent + Wagner And Lugol’s reagent	Cream precipitate Reddish – brown precipitate	Alkaloid present Alkaloid present
ii. Initial Alkaline extract +Mayers reagent + Wagner and Lugol’s reagent	Cream precipitate Reddish brown precipitate	Alkaloid present Alkaloid present

Table 2: Result For GOO of Hexane and Methanol Extract

TEST	OBSERVATION	INFERENCE
Initial acid extract + Mayer’s reagent + Wanger and Lugol’s reagent	Cream precipitate Orange precipitate	Alkaloid present Alkaloid present
Initial Alkaline extract + Mayers reagent + Wanger and Lugol’s reagent	Cream precipitate Pale precipitate	Alkaloid present Alkaloid present

TEST	OBSERVATION	INFERENCE
Methanol extract + 1m hydrochloric acid extract of the same plant sample	Green – brown liquid	Alkaloid present
Green brown liquid heated with activated charcoal and filtered	Clear solution	Alkaloid absent
40ml of the filtrate +100ml of 5m NaOH	White precipitate which swirlson formation	Alkaloid present

IV. Discussion

Isolating of alkaloid content of any plant material can be done with many reagents which include hexan, methanol and chloromethane was used, hexan was used for removing fat, wax and terpenes content of one of the 3 portions used before adding methanol to the muck. The result was confirmed with the addition of ethanol to the filtrate. There is formation of two layers that on drying of the lower layer, fats were observed and when tested, translucency was seen.

The goo recovered from 1,1 – dichloromethane was higher than the one of methanol. Thus supporting the quote of Hess, (1998), who said that dichloromethane is a good solvent for alkaloid extraction. It lies on it non-toxicity and high volatility.

Morinda Lucida belong to rubiceae family and according to Bentley K, W, Roger M.F and Wink M, rubiceae family yields alkaloid in larger quantity than other plant family. The detecting of alkaloid content from the extract of *Morinda lucida* with diodal reagent like Mayers reagent and Wagner and Lugol's reagent has a larger yield. The precipitate formed in both the acidic and alkaline extract of this plant shows that it contains different alkaloid.

V. Recommendation

The separation and purification of the detection of alkaloid was not done and the structural activity relationship of the plant components is not contained in this write –up. It is then the wish of the writer that more specific analysis be carried out to identify various components which could lead to designing a method of synthesizing it or the analogue to enhance activity. Specific analysis like Infra Red (IR), Nuclear Magnetic Resonance (NMR), mass spectrometer, just to mention but a few could be used to discover the actual components to enhance elucidation of the structural components and its structural activity.

The need for us to enhance or harness the health knowledge of our local herberlist could be a source of increasing the pharmaceutical raw material resource, since they were able to solve one problem or the other medicinally without knowing the actual quantity or dose to use or the quantity of the material. The source of chemistry is then an advantage to harness our naturally abundant materials to resuscitate our industries. Furthermore, instruments for the analysis should be provided to the researcher to enable them run all the analysis required at a cheap rate as well as getting familiar with the use and functional operations of these equipments.

References

- [1]. Awasthi, A. and Aepita, V. (2007): "Phenyl Kyalamines as Calcium Channels Blockers". *Journal of chemistry* 199(5): 565-570.
- [2]. Bentley, K. W (1999): "The Chemistry of Natural Products". 5th ed. Interscience Fattorusso, E, Tag Lialatela, S and Orazio, V(2008): "Modern Alkaloids Structure, Isolation, Synthesis and Biology". 2nd ed., Wiley VCH:USA, 81-431
- [3]. Finar, I.L. (2007). "Stereochemistry and Natural Product". 5th ed. Thearson Education: India, 710-782.
- [4]. George, R.K. and Kelvine, M.C. (2009). "Isolation of alkaloids from plant". Plenum Press, New York, 52-101
- [5]. Hesse, M. (2005): "Alkaloid Nature's Curse or Blessing", 1st ed. Plenum Press: New York, 11-14
- [6]. Holland, M.; Heber, L.; Jian-Xian, G.; Francisco, O.; Mercedes, C., Paula, F. and Andrew, J.W. (1999): Enanto Selective Synthesis and Pharmacological Evaluation". *Journal of Pharmaceutical Research*, 16, 281-287.
- [7]. Keay, R.W. ; Onochie, A. and Stanfield, D.P (1998). "Useful Plants of West Africa", Federal Department of Forest Research: Ibadan, 27-30.
- [8]. Kuznestsova, O.F. ; Mosevich, I.K.; Korsakov, M. C.; Fedorova, O.S and Krasokowa, R.N. (2002): "Asymmetric Synthesis of Dihydropheny Alamine" *Radiochemistry*, 44 (6): 582-587.
- [9]. Kyristiana, J. and Monica, B. (2007): "Detection of Alkaloids from a plant Extract", 5th ed. Eastern Press: Indian, 70-81.
- [10]. Maduka, H.C.C. and Okoye, Z. S.C. (2002): "Elemental Composition of *Morinda Lucida* a Nigerian Medicinal Plant", *Journal of Biological Science* 5, 88-108.
- [11]. Manfred, S.K. (1981); "Alkaloid and its Chemistry". John Wiley and Sons Inc: Canada and USA, 440- 421.
- [12]. Pelletier, S.W. (1997): "Chemistry of the Alkaloids", 2nd ed: Van Nost and Reinhold Company : New York,15-25.
- [13]. Raven, P.H.; Ray, F.E. and Susan,E.E. (2005): *Biology of Plant*". 5th ed. W.H Freeman and Company: New York , 89-90.
- [14]. Richler, H.G. and Dall Witz, M.J. (2000). "Commercial Timers", *Journal of Forest Research*, 2,27-30.
- [15]. Rogers, M.F. and Wink, M . (1998) "Alkaloids Biochemistry, Ecology and Medicinal Application". Plenum Press: New York, 12-31.
- [16]. Samy, R.P. and Ignacimuthu, S. (2000): "Antibacterial Activity of Some Folklore Medicinal Plants used by Tribals in Western Ghats of India". *Journal Ethanopharmacol* 19 (2): 201-212.
- [17]. Sasique, J.; Chandra, T,T. and Elango,V. (1987): "Biochemical Modes of Action of *Morinda lucida* and *Cassiva Occidentalis* in Inflammation". *Journal Ethanopharmacol* 19 (2):201-212.
- [18]. Shaman, M.and Moniot, L. (1998): "Nomenclature and Research on 180 Quinoline Alkaloids". Plenum Press: New York, 72-77.
- [19]. Skoog, D.A; West, D.M; Holles, J.F. and Crouch, S.R.(2004): "The Fundamentals of Analytical Chemistry", 8th Ed Eastern Press: India. 714 – 737.
- [20]. Tona, L.; Cimanga, R.K.; Messia, K.; Musuamba, C.T.; DeBruyune, T.; DeBruyune, T.; Appers, S., Hemans, N; Van Merit, S.; Pieters, L., Tolre, J and Vlien Tinck, A.J. (2004) "In Vitro Anti Plasmodial Activity of Extracts And Fractions from Seven Medical Plant Used In The Democratic Republic of Congo" *Journal Enthano Pharmacol* 93 (1): 20-32.