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Phytochemical Screening and Antimicrobial Study on the Leaves of *Morinda lucida* (Rubiaceae)

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Abstract
An ethno medicinal study was conducted through the means of an informal interview with an herbalist at Akim-Tafo, in the Eastern region of Ghana. Preliminary phytochemical tests carried out on the leaves of *Morinda lucida* indicated the presence of saponins, anthraquinones, cardenolides, alkaloids, sterols and tannins. Thin Layer Chromatography and Column Chromatography method were used in the laboratory for the analysis of a crude extract from *Morinda lucida*. A total of nine components were isolated from *Morinda lucida* using a solvent system containing hexane and ethyl acetate (2:1) as the mobile phase and a stationary phase of silica gel. The crude leaf extract of *Morinda lucida* was investigated for inhibitory activity on *Salmonella typhi*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* in vitro using the agar well diffusion method. *Morinda lucida* showed inhibitory activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* with no activity against *Salmonella typhi* at a concentration of 10mg/ml.
Keywords: *Morinda lucida*, thin layer chromatography, column chromatography, antimicrobial activity.

1. Introduction

Plants play significant roles in the lives of both humans and animals; from providing basic needs such as food, shelter and clothing to being used as herbal medications. Medicinal plants contain many useful substances some of which are biologically active and are known as secondary metabolites. These substances can be used for therapeutic purposes and have been applied for several decades to serve as precursors for the synthesis of new drugs (Evans, 2002).

The problem of microbial resistance is becoming persistent and proactive studies to develop new drugs, using natural product screening must be taken to reduce this problem. Screening of natural products used to treat bacterial infections in traditional medicine is important in establishing their effect in treating bacterial diseases. Due to the renewed interest in plant medicine, conscious efforts are now directed at the screening of medicinal plants with a view of isolating phyto-constituents that will serve as templates for the total synthesis of compounds with enhanced similar structure-activity relationship.

The plant *Morinda lucida* falls under the family Rubiaceae known to have wide usage in traditional medicine (Karou et al, 2011). *Morinda lucida* is a tropical West African tree of medium-size about 18–25 m tall, the bark is grey to brown in colour, flowers are white in colour, the fruit is a drupe, seed is ellipsoid, yellowish and soft (Zimudzi & Cardon, 2005). *Morinda lucida* which commonly known as the Brimstone tree, is known in Ghana as Kon kroma in Twi (Addo-Fodjour et al, 2008) while in Nigeria it is known as Nfia in Igbo (Nweze, 2011).

Morinda lucida is used as an astringent and antiseptic for ulcerating abscess, exudate is rubbed on affected area (Adomi and Umukoro, 2010). The crude ethanolic extract of the leaves is said to contain alkaloids, cardenolides and saponins (Adomi and Umukoro, 2010). Ogundare and Onifade (2009) worked exclusively and extensively on the effect of the leaves of *Morinda lucida* on Escherichia coli, and reported that it has potent inhibitory activity both *in vitro and in vivo*.

This research work aims at isolating the phyto-constituents present in *Morinda lucida* and providing scientific evidence of its antimicrobial activity that may account for its use in traditional medicine.

2. Materials and Methods

2.1 Ethnomedicinal Survey

The plant was selected for this study based on its ethno-medicinal use. The background data were collected through organized semi-structured interviews with a local herbalist at Akim-Tafo in the Eastern region of Ghana.

2.2 Collection of plant material

Fresh leaves of the plant *Morinda lucida* were taken from Adenta, a suburb in Accra, Ghana. The plant was identified by taxonomists at the Botany Department (University of Ghana) who identified the correct species.

2.3 Test Organisms

The test organisms were *Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella typhi* obtained from the Microbiology Laboratory of Central University College, Miotso.

2.4 Preparation of plant extract

The plant was separated from other extraneous material which was gathered during collection. The leaves of the plant were air-dried at room temperature for eight weeks and milled into fine powder using a milling machine. Two and a half liters of absolute ethanol was then added to the 500g of the powdered sample to cold macerate. The flask was then covered tightly and the mixture was shaken for about four hours on a mini orbital shaker set at 150rpm every day for three days. The mixture was filtered off into a Buchner flask using a sintered glass filter with the aid of a vacuum pump. The filtrate was collected in a round bottom flask and was concentrated using a rotary evaporator.

A dark-greenish ethanolic concentrate obtained was poured into an evaporating dish and air-dried. The crude extract was dissolved in 1000ml of dichloromethane and this was put in a separatory funnel and extracted with equal volume of distilled water. The mixture was shaken for some time for the organic component in the aqueous solution to be extracted into the dichloromethane. The shaking was stopped and the mixture was allowed to settle. It separated into two layers with the organic layer below the aqueous layer.

The organic layer was collected into a reagent bottle and 4g of MgSO4 was added to absorb any traces of water present in the organic layer. After twelve hours, it was filtered using a sintered glass filter and the filtrate was collected in a round bottom flask and was concentrated using a rotary evaporator. A dark-greenish precipitate was obtained which was poured into an evaporating dish and placed in a desiccator to aid the dichloromethane in drying up.

2.5 Phytochemical Screening

The crude dichloromethane and ethanolic extracts of the plant were screened using standard phytochemical procedures to confirm the presence of phytochemicals.

Classical methods described in the Trease and Evans Pharmacognosy (Evans, 2002) was used to identify some of the phytochemical groups. Other tests used include; Wagner's test and Dragendorff's test for Alkaloids, froth test for saponins, Salkowski test for Terpenoids, Liebermann-Burchard (LB) test for Sterols and triterpenes and Keller-Killiani test for Cardiac Glycosides.

2.6 Determination of TLC Solvent System for Extracts and Fraction

Thin layer chromatography (TLC) was used to resolve the extracts and fractions following a standard procedure (Matthews, 2003). Ten already coated glass chromatographic plates were used. The TLC plates were of designation Adamant UV 254 with the following specifications; dimension of 10cm × 5cm, coated with silica gel 60 (pore size= 60), thickness of 0.25mm and fluorescent indicator of UV 254nm. Different solvent systems were tried and the best one was selected as a good solvent to run the column chromatography. This was done by comparing the R_f values of the various solvent systems, solvent systems used included Pet. Ether: Ethyl acetate, Dichloromethane: Ethyl acetate and Hexane: Ethyl acetate. Method of visualization used was anisaldehyde-sulphuric acid spray. The solvent system was allowed to travel a predetermined distance of 8 cm from the origin. The possible spots were marked and the distance travelled by the spot from the base was measured with the corresponding distance travelled by solvent. The R_f values were calculated for all the spots. The method used for the column chromatography was according to that described in the works of (Matthews, 2003).

2.7 In vitro determination of Antimicrobial activity

Using a loop under aseptic methods, streaks of the cultivated strains of the bacteria were each suspended in 5ml of sterile water. 0.2ml of the suspended microorganism was transferred to a test tube containing exactly 15ml of

sterile molten nutrient agar. This was later poured into the sterile petri dish. The agar was allowed to set and holes were bored into the plate using sterile cork borer of 20mm in diameter aseptically. 0.3ml of the aqueous crude extract of concentration 10mg/ml was used to fill each of the wells bored on the plate using a sterile syringe and needle. These were then allowed a diffusion time of 1 hour. A control experiment was set up the same way, however instead of the extract; sterile water was each introduced into the hole bored. The Petri dishes were incubated at 37oC for 24 hours. At the end of incubation, zones of inhibition around the wells were observed, measured using a meter rule and recorded in millimeters.

3. Results and Discussion

3.1 Phytochemical tests

Preliminary qualitative tests for presence of phytochemicals were carried out on both the ethanol and dichloromethane extract. The results from classical phytochemical test on *Morinda lucida* intimated the presence of saponins, terpenoids, tannins, anthraquinones and alkaloids. This is similar to the reports of Adomi and Umukoro (2010) who reported presence of saponins, cardenolides, anthraquinones and alkaloids but absence of tannins. Differences such as this may be attributed to, the chronological age of the plant, percentage humidity of the harvested material, situation and time of harvest, and whether the method of extraction was a possible source of variation for the chemical composition, toxicity and bioactivity of the extracts (Felix, 1982).

3.2 Isolation of Phyto-constituents

The best solvent system was Hexane/ Ethyl acetate (2:1) based on the criteria that it gave a good separation of components during the T.L.C analysis and gave a maximum number of components. In the thin layer chromatography, varying ratios of different solvents were tried on the compound using stationary phase of silica gel 0.25mm thick pre-coated on a glass plate. The addition of more of hexane and less of ethyl acetate restricted movement of the compounds. However, further increase retarded movement of the compounds. This indicated the presence of non-polar compounds. Hexane and ethyl acetate (2:1) was found to give the best resolution with six spots and was used as mobile phase in performing the column chromatography.

Table 1. Result of column chromatography carried out on *Morinda lucida* crude extract.

Fractions	No of spots	Colour	R_f value	Test/Remarks
FR 1-2	1	Green	0.829	Chlorophyll
FR 3-4	1	Light green	0.800	------
FR 5	2	Green	0.683	-----
FR 6-8	2	Green	0.615	------
FR 9-12	1	Pale yellow	0.567	-----
FR 13-17	2	Green	0.520	-----
FR 18	1	Amber	0.474	Sterols
FR 19	1	Deep green	0.372	Terpenoids
FR 20-22	1	Yellow	0.352	-----

Key: FR = Fraction ------- = no conclusion drawn

The result of the column chromatography carried out on *Morinda lucida* crude extract is shown in Table 1.

A total of nine components were isolated from the crude leaf extract. The R_f values of these compounds were compared to literature values. Remarks were made on the probable presence of chlorophyll (R_f = 0.829), terpenoids (R_f = 0.372) and β-sitosterol (R_f = 0.474). Compound A may be identified as chlorophyll due to its characteristic green colour and high yield. The high R_f value of 0.829 showed that it had a high polarity. Leaves are known to contain high levels of chlorophyll which gives it a characteristic green colour. Nweze (2011) reported on the presence of β-sitosterol (a phyto-sterol) in *Morinda lucida* using NMR analysis, the said compound was also found to have low values. Sterols test were carried out on the isolated samples with low R_f values from the column chromatography. Compound G gave a positive test for sterols with R_f value of 0.474.

3.3 Antimicrobial activity

The Crude aqueous extract of *Morinda lucida* inhibited the *in vitro* growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* while it showed no inhibitory activity against *Salmonella typhi* (Table 2). Results obtained on the antibacterial activity of the crude *M. lucida* leaf extract (Table 3.9) showed that at a concentration of 10mg/ml, it had inhibitory activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

but none against *Salmonella typhi*. This inhibitory activity is a confirmation of the earlier report of its antibacterial activity by
Karou et al (2011). It also validates its application as a plant used in traditional medicine. According to reports by Adomi and Umukoro (2010), the ethanolic crude root bark extract of *M. lucida* at a concentration of 1000mg/ml showed antimicrobial activity against *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas aeruginosa*. However in this study, there is no evidence of activity against *Salmonella typhi*.

Table 2. Antimicrobial activity of *Morinda lucida* on test organisms.

Test Organisms	**Crude extract of *Morinda lucida* (10mg/ml)**				
	Zone of inhibition (mm)			Mean	S.D
	Plate 1	Plate 2	Plate 3		
Staphylococcus aureus	3	1	1	1.7	±1.2
Pseudomonas aeruginosa	1	5	3	3	±2.0
Salmonella typhi.	0	0	0	0	±0

The antibacterial activity in the *Morinda lucida* leaf may be indicative of the presence of some metabolic toxins or compounds such as aromatic phenols. Aromatic phenolic compounds have been found to have antimicrobial properties (Alma et al, 2003). Karou et al, 2011 reported that Phenolic compounds are the main chemical group responsible for the antimicrobial activity of plants including Rubiaceae. There could be a relationship between chemical structure of aromatic phenols and antimicrobial activity. From the preliminary phytochemical tests carried out, tannins were found to be present.
In reports from Adomi (2008) where the aqueous extract of *Morinda lucida* was screened against various gram positive and gram negative bacteria; the recorded inhibition zone diameters with 1000 mg mL-1 extract varied from 14 to 25 mm. In the results obtained from Table 3.9, inhibition zone diameters varied from 0 to 5 mm. The difference in activity could be due to different factors such as method used for antimicrobial assay, solvent used and concentration of *Morinda lucida*.
The agar diffusion assay used in this study is efficient for the quantification of the antimicrobial activity; however, the solubility and the diffusion of some extract in the agar medium can be a limiting factor. The concentration used was just 10mg/ml as opposed to 1000mg/ml which was used by Adomi (2008). Probably at higher concentrations >10mg/ml, higher zones of inhibitions could be obtained and it could inhibit the growth of *Salmonella typhi*.
Water was the solvent used to perform the antimicrobial study. According to reports by Adomi and Umukoro (2010), the ethanolic extract of *Morinda lucida* showed activity against a wide range of bacteria. Alcohols such as ethanol are known to possess antimicrobial properties and their use as solvent in antimicrobial assay could affect results. The use of different solvents such as ethanol and water can be responsible for the different results obtained.

4. Conclusion

Saponins, tannins, cardenolides, carotenes, sterols, triterpenes, anthraquinones, cardiac glycosides and alkaloids may be present in the leaves of *M. lucida*. It is observed that *Morinda lucida* inhibited the in vitro growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Thus, they can be used in the treatment of infectious diseases caused by these resistant microbes. The study also validates the significance of *M. lucida* as a valuable source of new leads for drug development.

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