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Phytochemical, antimicrobial and nutritional properties of *Morinda lucida* benth and *Nauclea latifolia* leaf extracts

Stephen Amadin Enabulele *, Oluwatosin Amusa, Eseosa Uwadiae

Benson Idahosa University, Benin City, Edo State, Nigeria
**Corresponding author E-mail:senabulele@biu.edu.ng*

Abstract

The leaves of two medicinal plants namely; *Morinda lucida* and *Nauclea latifolia* were investigated for their antibacterial, mineral and phytochemical properties. Results of the study showed that the aqueous and ethanolic extract of the two leaves had significant antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Listeria monocytogenes* and *Proteus mirabilis.* The biologically active constituents present in the extracts were cardiac glycosides, tannins, saponin, coumarins, reducing sugar, terpenoids, alkaloids, steroids and flavonoids. The percentage yield extracts of the respective plants were *Morinda lucida* leaves 12.9% for ethanol and 9.0% for aqueous extracts while *Nauclea latifolia* had 12.1% for ethanol and 8.4% for aqueous extracts. Results of the antibacterial activity analysis revealed that the ethanolic extracts of the two medicinal plants at different concentrations were more active against the test organisms named above than the aqueous extracts. The MIC values for the ethanolic extracts ranged between 25.0 and 100.0 mg/ml while that of aqueous extract ranged between 50.0 and 100.0 mg/ml. The MBC values for ethanolic extract ranged between 50.0 and 100mg/ml while that of aqueous extracts also ranged between 50.0 and 100mg/ml. Analysis of the mineral content of the plant leaves shows that they were rich in potassium and calcium. This study confirms that the leaf extracts have some reasonable level of antimicrobial activity and appreciable amounts of mineral content which could be beneficial to human health. The findings from this work can be further exploited for isolation and characterization of novel Phytochemical compounds which could be used in the treatment of infectious diseases especially in light of the emergence of drug-resistant microorganisms and the need to produce new effective antimicrobial agents.

Keywords: *Morinda lucida; Nauclea latifolia; Antimicrobials; Phytochemicals; Medicinal Plants.*

1. Introduction

Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world. In rural areas of developing countries, they continue to be used as the primary source(s) of medicine [7]. About 80% of the people in developing countries are reported to use traditional medicines for their health care and as such renewed interest in plant medicine, is been developed by a lot of scientists and conscious efforts are now directed at the screening of medicinal plants with a view of screening secondary metabolites and mineral that will serve as templates for the total synthesis of compounds with enhanced similar structure activity relationship [6][17].

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans. These compounds known as secondary plant metabolites are known to have active biological properties and their traditional usage in curing illnesses has deep root and is documented among the Nigerian native people [1].Plants are used and still being used in treating ailments such as malaria, diarrhoea, burns, gonorrhoea, stomach disorders and other infectious diseases especially in tropical Africa and in less privileged societies of the world [10]. *Morinda lucida* benth (Rubiaceae) which is a tropical West Africa rainforest tree and also called Brimestone is one of such plant. Stem, bark, roots and leaves infusion of *Morinda lucida* benth, are known to be locally used as an anti-malarial, anti-diabetic, antimicrobial and in the treatment of jaundice [19]. The plant is reputed to be one of the most used traditional medicines against fever in sub Saharan Africa [4].

Nauclea latifolia commonly known as pin cushion tree and belonging to the family Rubiaceae is also one of such plants. The leaves of *Nauclea latifolia* is used in the treatment of ailments such as malaria, gastrointestinal tract disorders, sleeping sickness, prolong menstrual flow, hypertension and as a chewing stick [4]. The plant is also documented to be used as a tonic and fever medicine, for toothaches, dental caries, septic mouth and diarrhoea [18].

Several drugs including some antibiotics are no longer active against targeted organisms. It has been reported that the effective lifespan of these therapeutics agents are limited [16]. Therefore, we experience antibiotic resistant organisms, and ineffective synthetic drugs. More so, majority of the orthodox drugs are both expensive and display dangerous side effects in the users. Hence, discovering and identifying new safe drugs using medicinal plants without severe side effects has become an important goal of research in biological science. It is in this context that the aqueous and ethanolic extracts of leaves of *Morinda lucida* (fig.1) and *Nauclea latifolia* (fig.2), were screened for their Phytochemical, antimicrobial and nutritional properties.

Fig. 1: *Morinda lucida* Leaves [4].

Fig. 2: *Nauclea latifolia* Leaves and Fruits [4].

2. Materials and methods

2.1. Collection of samples

Leaves of *Morinda lucida* (Brimestone tree), and *Nauclea latifolia* (Pin cushion tree) were collected from, Ogan, Ifo, Ogun State and were identified by Dr. O. Olorunfemi of the Department of Botany, University of Benin, Edo State.

2.2. Preparation of samples

The leaves were air dried for five days, after which they were milled into powder with a dry sterilized Panasonic blender model MX-JI20P.The powdered leaves were then sieved through a 2.0mm filter and subsequently stored in an air tight sterile container until it was used.

2.3. Leaf extracts preparation

The leaf extracts were obtained by using the method of [19]. One hundred grams (100g) of the powdered samples were soaked in 400ml of solvent in different sterile conical flasks and plugged with cotton wool. It was then wrapped with aluminium foil and shaken vigorously. The mixture was left to stand for 24h in a shaking water bath maintained at 37^0C. The mixtures were then filtered using a clean muslin cloth and Whatman No.1 filter paper. Thereafter the filtrate was evaporated to dryness by means of a rotary evaporator model SM- 52CS-1 attached to a vacuum pump. The percentage yield of each of the crude extracts were determined for each solvent and estimated as dry weight (extract)/dry sample weight x 100. The extracts were stored in an airtight container and kept in refrigerator until needed for further analysis.

2.4. Phytochemical screening

The Phytochemical analysis of the leaf extracts were done according to the method of [19].They were tested for the presence of flavonoids, cardiac glycosides, reducing sugars, steroids, phenolics, essential oils, tannins, saponins. terpenoids, flavonoids and steroids.

2.5. Sources of microorganisms

The organisms used for the investigation were clinical isolates obtained from Faith Mediplex hospital, Benin City, Edo State. The bacterial species used were *Stapylococcus aureus*, *Listeria monocytogenes*, *Esherichia coli*, *Proteus mirabilis* and *Pseudomonas aeuruginosa*. The isolates were re-identified by biochemical tests according to methods outlined by [8].

2.6. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The method of [14] was adapted in determining the MIC and MBC of respective extracts. The MIC was determined using the tube dilution method. Standardized suspensions of the test organism was inoculated into series of sterile tubes of nutrient broth containing different concentrations of leaf extracts and incubated at 37^0C for 24h.The MICs were read as the least concentration that inhibited the growth of the test organisms.
The MBCs were determined by first selecting tubes that showed no growth during MIC determination: A loopful from each tube was subculture onto extract free agar plates, incubated for another 24h at 37^0C. The minimum bactericidal concentration was considered as the lowest concentration that could not produce a single bacterial colony [8].

2.7. Mineral elements evaluation

The recommended methods of the Association of Official Analytical Chemists [3] earlier used and enumerated by [13] were used for the determination of the nutritional and mineral content.

3. Results and discussion

Generallytheeffectivenessofanyantimicrobialcompounddependsontheabilityofthe antimicrobial to inhibit or stop the growth of any microorganism in the body system they infect. Because of the high genetic variability of microorganisms, they seem to however develop the ability to rapidly evade the action of antimicrobials by becoming resistant to them. It becomes necessary therefore to look for newer means of eliminating microbial threat causing infections. Percentage yield of the aqueous and ethanolic extract of the powdered leaf of *Morinda lucida* shown in table 1 are higher than that of the *Nauclea latifolia* extracts. Ethanol however seem to be the better of the two solvents used in this study. This is consistent with the results of [2] who also found ethanol to be best of all the solvents used for their analysis.
The biological function of flavonoids includes protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumours. These plants also have quantity of saponin content. Some of the general characteristics of saponin include formation of foam in aqueous solutions, haemolytic activity cholesterol binding properties and bitterness [20]. Apart from saponin other metabolite constituents of the selected medicinal plants detected include the alkaloids and tannin. Alkaloids ranked the most efficient therapeutically significant plant substance. Pure isolated plant alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic, anti-plasmodic and bactericidal effects. They exhibited marked physiological activity when administered to animals. Tannin has been identified as a Phytochemical that helps in hastening the healing of wounds and inflamed mucous membrane [20]. The presence of tannin in some of the selected plants reported (Table 2) strongly supports its use in treating wounds, burns and haemorrhoids in herbal medicine.
The antimicrobial activity of *Morinda lucinda* and *Nuclea latifolia* are presented in table 3 and table 4. The ethanol crude extracts of both leaves inhibited the growth of the five (5) test organisms at varied concentration. However the aqueous extracts inhibited all the test organisms at 200mg/ml and 100mg/ml except *Staphlococcus aureus* for *Nauclea latifolia* and *Listeria monocytogenes* for *Morinda lucinda.* The results are in line with the report of [13] who reported that the susceptibility of bacteria to crude plant extracts varies according to strain and specie.
From the results, the ethanol extracts had higher zones of inhibition against all test organisms at varying concentrations as compared to the aqueous extracts. Reports by [11] and [9] have also suggested the efficacy of alcohol based extracts against bacteria more than water based extracts. The percentage of effectiveness showed higher values for the ethanol extracts of both leaves at the concentration of 200mg/ml as compared to the antibiotic control except for *Proteus mirabilis* and *Listeria monocytogenes* for *Morinda lucinda* and *Staphylococcus aureus* and *Proteus mirabilis* for *Nauclea latifolia*. The ethanol extracts at 100mg/ml and aqueous extracts at varying concentrations despite having lower values showed that they were more than half as effective as the control. There were no zones recorded at 50mg/ml for both extracts of the leaves suggesting that the dose was too small to have an effect on the test bacteria.
Minimum inhibitory concentration values for this study are presented in table 5.The MIC for ethanol extract for both leaves ranged from 25 to 50mg/ml while the aqueous extract ranged from 50 to 100mg/ml. the values obtained are reasonable to suggest the pharmacological relevance of the leaf extracts. Differences in effectiveness of the extracts could be attributed to their impure form as the crude nature of the extracts may contain substances which may not have any antibacterial activity. This observation was also reported by [12].

Table 1: Percentage Yield

Medicinal Plant	Ethanol			Aqueous		
	Leaf powder (g)	Extract (g)	Yield (%)	Leaf powder (g)	Extract (g)	Yield (%)
Morinda lucida	100	12.9	12.9	100	9.0	9.0
Nauclea latifolia	100	12.1	12.1	100	8.4	8.4

The antimicrobial effect of all these extracts is due to the phytochemical constituents present in them. *Morinda lucida* and *Nauclea, latifola* leaves are rich in phytonutrient such as flavonoids, phenolic compound tannins, saponin, terpernoids, cardiac glycosides, coumarins and alkaloids which have been reported by [5].

Table 2: Phytochemical Constituents of *Morinda lucida* and *Nauclea latifolia*

Phytochemicals	Ethanol		Aqueous	
	Morinda lucida	*Nauclea latifolia*	*Morinda lucida*	*Nauclea latifolia*
Cardiac Glycosides	+++	++	+	+
Tannins	++	+++	_	++
Saponins	++	+	++	++
Coumarins	+	+	+	+
Reducing Sugars	++	++	++	_
Terpenoids	++	+++	+	+
Alkaloids	+++	+++	+	++
Steriods	+	_	_	_
Flavonoids	++	+	++	++

Key: + = slightly present; ++ = moderately present; +++ = highly Present; -- = absent

Table 3: Antimicrobial Activity of *Morinda lucinda*

Isolate	Zones of inhibition in mm at different concentration (percentage of control) Ciprofloxacin (control) 10µg/ml	200mg/ml Ethanol	200mg/ml Aqueous	100mg/ml Ethanol	100mg/ml Aqueous	50mg/ml Ethanol	50mg/ml Aqueous
Staphylococcus aureus	24.0	26.6 ±0.57 (110.8)	14.0 ±1.00 (58.3)	12.4 ±2.10 (51.7)	11.0 ±1.00 (45.8)	–	–
Escherichia coli	29.0	24.0 ±2.00 (82.7)	22.0 ±2.00 (75.8)	11.0 ±1.00 (37.9)	9.0 ±1.00 (31.0)	–	–
Listeria monocytogenes	22.3	15.6 ±0.57 (69.9)	7.67 ±1.52 (34.4)	6.33 ±2.51 (28.4)	–	–	–
Pseudomonas aeruginosa	27.3	27.8 ±1.15 (101.8)	21.3 ±1.52 (78.14)	21.0 ±1.00 (76.9)	11.6 ±0.57 (42.5)	–	–
Proteus mirabilis	23.3	17.6 ±1.15 (75.5)	8.00 ±1.00 (34.3)	–	–	–	–

Table 4: Antibacterial Activity of *Nauclea latifolia* Leaf Extracts

Isolate	Zones of inhibition in mm at different concentration (percentage of control) Ciprofloxacin (control) 10µg/ml	200mg/ml Ethanol	200mg/ml Aqueous	100mg/ml Ethanol	100mg/ml Aqueous	50mg/ml Ethanol	50mg/ml Aqueous
Staphylococcus Aureus	24.0	19.0 ±1.00 (79.1)	–	13.0 ±1.73 (54.1)	–	–	–
Escherichiacoli	29.0	27.3 ±1.52 (94.2)	17.6 ±1.00 (60.91)	18.0 ±1.52 (62.1)	11.0 ±1.00 (37.9)	–	–
Listeria monocytogenes	22.3	29.0 ±1.00 (130.0)	11.0 ±1.00 (49.3)	19.3 ±0.57 (86.5)	8.36 ±1.52 (37.5)	–	–
Pseudomonas aeruginosa	27.3	28.6 ±0.57 (104.7)	20.3 ±1.52 (74.4)	18.3 ±1.15 (67.0)	9.00 ±1.00 (32.9)	–	–
Proteus mirabilis	23.3	17.6 ±0.57 (75.5)	12.0 ±2.64 (51.5)	9.6 ±2.64 (41.2)	7.00 ±0.50 (30.0)	–	–

Table 5: Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC) of *Morinda lucinda* and *Nauclea latifolia* Leaf Extracts in Mg/Ml

Isolates	*Morinda lucinda* Ethanol MIC	*Morinda lucinda* Ethanol MBC	*Morinda lucinda* Aqueous MIC	*Morinda lucinda* Aqueous MBC	*Nauclea latifolia* Ethanol MIC	*Nauclea latifolia* Ethanol MBC	*Nauclea latifolia* Aqueous MIC	*Nauclea latifolia* Aqueous MBC
Staphylococcus aureus	50	50	50	50	100	100	100	100
Escherichiacoli	50	50	50	50	50	50	100	100
Listeria monocytogenes	100	100	100	100	25	50	100	100
Pseudomonas aeruginosa	25	50	50	50	25	50	100	100
Proteus mirabilis	100	100	100	100	50	50	50	50

Table 6: Mineral Content of the Plant Extracts and Recommended Daily Allowance (RDA) in Mg/100ml

Minerals	Contents (mg/100g) *Morinda lucinda*	*Nauclea latifolia*	RDA[A] (% RDA)
Iron	53.5	36.3	400
Zinc	24.2	31.3	15
Calcium	105.9	120.3	3500
Potassium	428.3	374.6	18
Copper	2.5	2.7	90
Manganese	16.8	19.2	0.23

[A] = Recommended daily allowance [15]

The mineral analyses for the leaves of *Nauclea latifolia* and *Morinda lucida* shows the presence of micro and macro nutrients in good amounts. Potassium and calcium were the most abundant nutrients in both plants. However, the elemental concentrations in both leaves may be a reflection of the elemental composition of their parent soil.

4. Conclusion

Conclusively, it was noted that the aqueous and ethanol crude leaf extracts of *Nauclea latifolia* and *Morinda lucida* contained important nutritional and bioactive compounds with high antibacterial activity against the selected test organisms. Thus, this study reveals the therapeutic properties of the selected plants, which could be of considerable interest in the development of new drugs. Further studies however are needed to isolate and purify some of the antimicrobial agents present in the plants so as to ascertain their full potential and assist in the effective treatment of common ailments ravaging the populace.

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