Trop. J. Eng., Sci.& Techn. 2025. 4(1):113-122 Tropical Journal of Engineering, Science and Technology

Multi-solvents Screening of Phytochemicals and Free Radical Scavenging Properties of Fruits and Leaves extracts of *Murinda lucida* and *Nauclea latifolia*

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Abstract

Free radical is an unsympathetic health factor in the human system, and this occurs when the oxidative molecule developed the loss of electron from one atom owing to the oxidative process. This study was designed to determine the phytochemicals, free radical scavenging properties and antioxidants of fruits and leaves of two plants on different solvent extracts of aqueous, ethanol and methanol. Solvent of different polarity extracts were used in evaluating the free radical scavenging activity and reducing power activity of the plants' parts. There was a high presence of phenol and flavonoid in the ethanol extracts than aqueous, and Methanol extracts. There was various levels of antioxidant activity in all the extracts. Extract of Methanol also has a good extraction capacity and showed significant antioxidant activity alongside others. From the results, we concluded that Ethanol extract fruits and leaves of Murinda lucida (ML) and Nauclea latifolia (NL) plant extracts show a significant antioxidant activity than methanol and aqueous extracts.

Keywords: medicinal plants, phytochemical, antioxidant capacity, aqueous, ethanol and methanol solvents.

Évaluation multi-solvants des composés phytochimiques et des propriétés antioxydantes des extraits de fruits et de feuilles de *Murinda lucida* et *Nauclea latifolia*

Résumé

Les radicaux libres constituent un facteur nocif pour la santé humaine, se formant lorsqu'une molécule oxydative perd un électron en raison d'un processus d'oxydation. Cette étude a été conçue pour déterminer les composés phytochimiques, les propriétés antioxydantes et le pouvoir piégeur de radicaux libres des extraits de fruits et de feuilles de deux plantes obtenus avec différents solvants aqueux, éthanolique et méthanolique. Des extraits de polarités variées ont été utilisés pour évaluer l'activité antioxydante et le pouvoir réducteur des parties des plantes. Une forte teneur en phénols et flavonoïdes a été observée dans les extraits éthanoliques comparativement aux extraits aqueux et méthanoliques. Tous les extraits ont présenté différents niveaux d'activité antioxydante. L'extrait méthanolique a également démontré une bonne capacité d'extraction et une activité antioxydante significative. Les résultats ont permis de conclure que les extraits éthanoliques des fruits et feuilles de Murinda lucida (ML) et Nauclea latifolia (NL) présentent une activité antioxydante supérieure à celle des extraits méthanoliques et aqueux.

Mots-clés : Plantes médicinales, Phytochimie, Capacité antioxydante, Solvants aqueux, éthanolique et méthanolique.

٣. الفحص المتعدد بالمذيبات للمركبات النباتية الكيميائية وخصائص مكافحة الجذور الحرة لمستخلصات ثمار وأوراق نباتي موريندا لوسيدا وناوكلاء لاتفوليا

يُعد الجذر الحر عاملاً صحيًا ضارًا في جسم الإنسان، ويحدث ذلك عندما تفقد الجزيئات المؤكسدة إلكترونًا من أحد الذرات نتيجة لعملية الأكسدة. وقد صُممت هذه الدراسة لتحديد المركبات النباتية الكيميائية، وخصائص مكافحة الجذور الحرة، ومضادات الأكسدة في ثمار وأوراق نباتين باستخدام مستخلصات مختلفة المذيبات: المائي، والإيثانولي، والميثانولي. وقد استُخدمت مذيبات ذات قطبية مختلفة لاستخلاص أجزاء النبات لتقييم نشاط مكافحة الجذور الحرة ونشاط القدرة الاختزالية. وقد وُجد تركيز عالٍ من الفينولات والفلافونويدات في المستخلصات الإيثانولية مقارنةً بالمستخلصات المائية والميثانولية. كما أظهرت جميع المستخلصات مستويات متفاوتة من النشاط المضاد للأكسدة. وأظهر مستخلص الميثانول قدرة جيدة على الاستخلاص، كما بيّن نشاطًا مضادًا للأكسدة ملحوظًا إلى جانب المذيبات الأخرى. واستنادًا إلى النتائج، نستنتج أن مستخلصات الإيثانول من ثمار وأوراق نباتي موريندا لوسيدا (ML) وناوكلاء لاتفوليا (NL) تُظهر نشاطًا مضادًا للأكسدة أعلى من مستخلصات الميثانول والماء.

الكلمات المفتاحية: النباتات الطبية، المركبات النباتية الكيميائية، القدرة المضادة للأكسدة، المذيبات المائية، الإيثانول، والميثانول.

Introduction

Free radical is an unsympathetic health factor in the human systems, and this occurs when the oxidative molecule developed the loss of electron from one atom owing to the oxidative process. Free radical occurred due to environmental pollutants, chemicals, radiation, toxins, foods as well as physical stress and weakening of immune human body system (Halliwell 1994, Kühnau 1976, Younes and Siegers 1981). Oxidative stress and reaction are the critical part to formation human disorders. The formation of free radical is artificially controlled with plant derivate compounds such as polyphenol compounds, vitamin A, C, beta-carotene. *Morinda lucida* Benth. (Rubiaceae) is amid several native plants used in the local treatment of Diabetic Mellitus among Yorubas (South-West Nigeria). Several parts of the plant are attributed to diverse therapeutic benefits. Morinda lucida is a medium-sized shrub used as a medicinal plant in West Africa (especially in Nigeria) in the treatment of malaria locally and other delirious conditions, hypertension, diabetes, dysentery cerebral congestion, (Halliwell 1994) This plant different parts have been reported to possess antimicrobial (Kühnau 1976). The aqueous leaf extract of *M. lucida* has been reported to cause antidiabetic effects in streptozotocin-induced diabetic rats, while the ethanolic leaf extract of the same plant elicited antidiabetic effects in both normal and alloxan-induced diabetic rats (Irulandi and Mehalingam)

Nauclea latifolia Smith (Rubiaceae) is a multi-stemmed shrub or small tree widespread in tropical Africa and Asia. The plant is traditionally used for treatment of various diseases. The roots are used in a part of Nigeria, by some traditional medicine to treat hypertension and the bark for the treatment of wounds, coughs, and gonorrhea in Nigeria. Biological activities reported on the roots include antibacterial, antifungal, anti-influenza, and antihyperlipidemic (Oyetayo *et al*., 2021).

Influence of different extraction solvents has been reported by many researchers, and their techniques on the content of natural antioxidants in extracts (Michiels *et al*., 2012),

Methods of solvents and their efficiency are strongly dependent on plant matrix used (Zhou and Yu 2004, Spigno *et al*.,2007, Michiels *et al*., 2012). Solvents, such as methanol, ethanol, acetone, propanol and ethyl acetate have been commonly used for the extraction of phenolics from fresh product. The extracting solvents properties significantly affected the measured total phenolics content (±25% variation) and antioxidant capacity (up to 30% variation) in fruits and vegetables (Michiels *et al*., 2012). Solvent polarity is very important parameter in extraction. The higher the polarity, the better the solubility of phenolic compounds (Halliwell 1994). The highest extract yields (up to 22.8%) were obtained with polar alcohol-based solvents (Savithramma and Suhrulatha 2011). Water addition to ethanol improves extraction rate, but too high-water content brought an increased concomitant extraction of other compounds, and, then to lower phenols concentrations in the extracts (Savithramma and Suhrulatha 2011). For wheat, 50% acetone extracts contained the highest level of total phenolics, whereas ethanol is the least effective solvent for extracting phenolics from wheat bran samples (Zhou and Yu 2004). Literature data shows that extraction efficiency of solvents is strongly dependent on food matrix and the aim of current research was to determine best method for extraction of phenolic compounds from Fruits and Leaves extracts of *Murinda lucida* and *Nauclea latifolia* showing high antiradical activity.

The aim of this study was to determine, comparatively the phytochemical analysis and antioxidant activity of three different solvent extracts of fruits and leaves of Murinda lucida and Nauclea latifolia respectively.

Materials and method

Sample preparations

Leaves and fruits parts of *Morinda lucida* and *Nauclea latifolia* were collected from Ifaki-Ekiti in Ekiti State. The identification was done at the University Herbarium, Plant Science and Biotechnology Department of Ekiti State University, Nigeria. The leaves and fruits were air-dried and powdered using an electric blender and stored for further analyses.

Solvent Extraction

The powdered leaves and fruits of each plant were prepared by successive extraction method using three solvents based on their polarities (aqueous, ethanol, and methanol). About 30 g of the dry leaves sample was taken in a flask and 200 mL of each solvent was added sequentially. The flask was agitated on the mechanical shaker for 24 hrs. Thereafter, the extract was filtered using Whatman filter paper and kept for further analysis. The dried extracts recovered using rotary evaporator and stored in the refrigerator for further investigation and analysis.

Quantitative phytochemical analysis

Phytochemical Screening

Tannins test

The crude extracts in methanol taken and 1-2 drop of ferric chloride was added. Resultant blue color observed indicated the positive result (Savithramma and Suhrulatha 2011).

Terpenoid test (Salkowski test)

The crude methanol extract (5 ml) taken and 2 ml of chloroform was added followed by addition 3ml concentrated sulfuric acid on the test tube's side wall. Reddish brown color observed shows the presence of Terpenoid (Savithramma and Suhrulatha 2011).

Saponins test (Foam test)

To the crude methanolic extract of the plant about (0.5 ml) 20 ml of water was added. The mixture was shaken thoroughly for 15 min. The foam layer appeared which indicated the presence of Saponins (Savithramma and Suhrulatha 2011).

Glycoside test (Keller-Kilian test)

Acetic acid (2 mL) and sulfuric acid (2 mL) were added to the plant crude extract. The Reddish color formation showed the presence of glycoside in sample (Sheel *et al.,*2014)

Flavonoids test

Crude extract of plant was added to 3 mL ammonia solution followed by careful addition of sulfuric acid. The appearance of yellow color indicated the presences of flavonoids in sample. (Soni and Sosa 2013).

Phenol test

Into the crude extract of plant, 2-10 drops of ferric chloride solution were added. The appearance greenish color shows presence of phenol (Tamilselvi *et al*., 2012).

Steroids test (Liebermann Burchard test)

To the crude plant extract, few drops of acetic anhydride were added, followed by addition of 2 ml chloroform and 2 ml of sulfuric acid. Presence of Red colour gave positive test (Soni and Sosa 2013).

Free Radical Scavenging Properties Analyses

Total phenol content Estimation

The magnitude of total phenol was determined using the Folin–Ciocalteu reagent method of Lister and Wilson, 2001 (Lister and Wilson 2001). A standard curve using different concentrations of gallic acid were prepared in solvents and their absorbance was recorded at 760 nm. Sample of about 100 µl was dissolved in 500 µl of Folin–Ciocalteu reagent (1/10 dilution) and 1 ml of distilled water was added. The contents were mixed and incubated at room temperature for 1 minute. After 1 minute, thereafter 1.5 mL of 20% sodium carbonate solution was added. The final mixture was shaken well and incubated for 2 hrs in the dark at room temperature. The absorbance was measured at 760 nm using an ultraviolet and visible (UV-Vis) spectrophotometer for all samples. The results were expressed in mg gallic acid equivalents (GAE) per milligram of dry weight of the plant.

Total flavonoid content Estimation of

The flavonoids content of the extract was determined spectrophotometrically by the method of (Quettier-Deleu *et al*.,2000). This method was based on the formation of a complex, flavonoid aluminum, with the absorbance at 430 nm wavelength. The calibration curve was created using rutin as standard. About 1 ml of diluted sample was mixed separately with 1 mL of 2% aluminum methanolic chloride solution. Incubated at room temperature for 15 minutes thereafter, the absorbance of the mixture was measured at 430 nm in a spectrophotometer. The flavonoid content was expressed in mg per mg of rutin equivalent.

Free radical scavenging ability (DPPH)

The scavenging ability of extracts on DPPH free radicals were estimated according to the method of (Shimada *et al*., 1992). This method hinge on the reduction of purple DPPH to yellow-colored diphenyl picryl hydrazine. About 2 mL of various concentrations (10-100 µg/mL) of the test sample was mixed with 0.5 mL of 0.01 M DPPH in methanol. Equal amount of methanol and DPPH served as a control. The mixture was shaken vigorously and then steadily kept for 30 minutes at room temperature in the dark. The absorbance of the resulting solution was measured at 517 nm against a blank UV-Vis spectrophotometer. The experiment was performed in triplicates. The DPPH radical scavenging activity was calculated by the following equation:

% DPPH radical scavenging activity=(Ao-A1)/Ao×100%.

Where, Ao is the absorbance of the control reaction and A1 is the absorbance of the sample of the tested extracts. The percentage of free radical activity was plotted against the

corresponding antioxidant substance concentration to obtain the half maximal inhibitory concentration (IC50) value, which is defined as the amount of antioxidant substance required to scavenge the 50% of free radicals present in the assay solution. IC50 values are inversely proportional to the antioxidant potential.

Reducing power ability

The reducing power ability of methanol extract was determined by the method given by Oyaizu (1986) (Oyaizu 1986). Reaction mixtures were prepared by adding 2.5 mL of phosphate buffer (0.2 M, pH 6.6), 2.5 mL potassium ferricyanide (0.1%), and varying concentrations of extracts (10-250 µg/ml). Then, the reaction mixtures were incubated at 50°C in water bath for 30 minutes and allowed to cool at room temperature. Then, 2.5 ml of 10% TCA (trichloroacetic acid) were added to each reaction mixture and centrifuged at 2000 rpm for 10 minutes. The supernatant (2.5 mL) was separated in the test tube and added with 2.5 ml of distilled water and 0.5 mL FeCl3 (1.0%). After 10 minutes of incubation at room temperature, the absorbance was measured at 700 nm. The ascorbic acid solution was used as standard.

Statistical analysis

All the experimental results were mean ± S.D of three parallel measurements.

Results

In this study, the phytochemical constituents of the leaves and fruits *Murinda lucida* (ML) and *Nauclea latifolia* (NL) plants include alkaloid, saponin, tannin and terpenoids as revealed by the positive reactions of these molecules (Table 1). The presences of these compounds explain the ability of the leaf extract to inhibit the activities of various radicals. All the solvent analyzed were able to have presence of the molecules except the tannin in Table 1 that could only the presence of the molecules in the aqueous medium except in the leaves of the *Nauclea latifolia* (NL).

Also, the inhibition percentage of different extracts were calculated and compared with standard ascorbic acid. Higher percentage of inhibition was observed Aqueous extract than other two solvent extracts. Table 2 showed the DPPH activity and Flavonoid contents of the two plant extracts with higher extraction from aqueous solvent compared to the ethanol and methanol counterpart respectively.

Total phenolic content (TPC), Plants extracts have Phenolic composition based on the following different factors – variety, storage, climate, processing etc. TPC was determined using Folin-Ciocalteu reagent which reacts nonspecifically with phenolic compounds, which can be ultimately be reduced by numbers of non-phenolic compounds, e.g., Cu (II), vitamin C, etc. The TPC determined in different solvent extracts of ML and NL fruits and leaves shown in Table 2. The quantitative analysis of phenol using these three solvents however showed that the aqueous extract had activities in the leaves than the fruits while the ethanol extract revealed more activities in the fruit extracts. Among the tested solvent, aqueous extract exhibited higher reducing activity followed by ethanol extract and the least was methanol extracts.

Table 1: Phytochemical Constituents of Fruits and Leaves extracts of *Murinda lucida* (ML) and *Nauclea latifolia* (NL) in different solvents

SCREENING	SAMPLES	SOLVENTS EXTRACTION		
		AQUEOUS	METHANOL	ETHANOL

Tannin	ML LEAF	-	+	+
	ML FRUIT	-	-	-
	NL LEAF	+	+	+
	NL FRUIT	-	-	-
Terpenoids	ML LEAF	+	-	+
	ML FRUIT	-	+	-
	NL LEAF	+	-	+
	NL FRUIT	++	+ +	+
Alkaloids	ML LEAF	+	+	+
	ML FRUIT	+	+	+
	NL LEAF	+	+	+
	NL FRUIT	++	+ +	+
Saponin	ML LEAF	++	+	++
	ML FRUIT	+	+	+
	NL LEAF	++	+	+
	NL FRUIT	+	+	-

Table 2: Free Radical Scavenging Properties of Fruits and Leaves extracts of *Murinda lucida* (ML) and *Nauclea latifolia* (NL) in different solvents

ANALYSIS	SAMPLES	SOLVENTS EXTRACTION		
		AQUEOUS	METHANOL	ETHANOL
DPPH scavenging properties	ML LEAF	0.21±0.01	0.24±0.01	0.10±0.01
	ML FRUIT	0.34±0.01	0.21±0.01	0.08±0.01
	NL LEAF	0.28±0.01	0.13±0.01	0.07±0.01
	NL FRUIT	0.34±0.01	0.12±0.01	0.11±0.01
Flavonoid Contents	ML LEAF	2.74±0.01	2.64±0.01	1.68±0.01
	ML FRUIT	2.65±0.01	2.28±0.01	1.31±0.01
	NL LEAF	2.73±0.01	2.50±0.01	1.95±0.01
	NL FRUIT	2.58±0.01	2.28±0.01	1.57±0.01
Total Phenol Contents	ML LEAF	3.06±0.01	2.67±0.01	2.60±0.01
	ML FRUIT	1.38±0.01	2.04±0.01	2.72±0.01
	NL LEAF	3.08±0.01	2.13±0.01	2.78±0.01
	NL FRUIT	1.31±0.01	0.11±0.01	2.64±0.01
Iron Chelating Properties	ML LEAF	2.46±0.01	2.59±0.01	2.15±0.01
	ML FRUIT	1.72±0.01	1.73±0.01	2.09±0.01
	NL LEAF	2.39±0.01	1.69±0.01	1.95±0.01
	NL FRUIT	2.36±0.01	1.89±0.01	1.59±0.01
Hydrogen Peroxide	ML LEAF	1.38±0.01	1.63±0.01	1.80±0.01
	ML FRUIT	1.23±0.01	1.53±0.01	1.67±0.01

	NL LEAF	2.08±0.01	2.28±0.01	2.12±0.01
	NL FRUIT	1.82±0.01	1.75±0.01	1.55±0.01
ABTS concentration	ML LEAF	0.37±0.01	0.37±0.01	0.60±0.01
	ML FRUIT	0.25±0.01	0.50±0.01	1.39±0.01
	NL LEAF	0.41±0.01	0.19±0.01	1.50±0.01
	NL FRUIT	0.28±0.01	1.05±0.01	1.33±0.01
Nitric Acid Concentration	ML LEAF	0.67±0.01	1.75±0.01	1.87±0.01
	ML FRUIT	3.08±0.01	0.93±0.01	2.97±0.01
	NL LEAF	0.82±0.01	1.75±0.01	1.09±0.01
	NL FRUIT	1.36±0.01	0.71±0.01	0.44±0.01

Discussion

Steroids have biological activities which are cardiotonic, insecticidal, and antimicrobial activities and these are possibly useful for development into therapeutic drugs. Tannins are recognized to be essentially important for their antiviral, antiparasitic effects, antibacterial, antibacterial, anti-inflammatory, antiulcer and antioxidant properties (Xu *et al.,* 1996, Manthey *et al*.,2001). The Saponins have been established to have antimicrobial, antifeedant, anti-inflammatory, and hemolytic effects (Francis, *et al*., 2002). Various alkaloids have been reported to have anticancer and antiviral activity. In the same vein, saponins have been reported to be cardiotonic, and flavonoids have anticancer and anti-inflammatory activity (Sheel *et al*., 2014, Shimada *et al*.,1992). The Tannins presence may be responsible for the ability of ML and NL leaves to be used in the management of diseases such as diarrhea, diabetes, and dysentery (Oyetayo *et al.,*2021)

These phytochemical studies showed that the chemical components of ML and NL fruit and seed have proven the presence of some constituents like, saponins, flavonoids, tannins, alkaloids glycosides, phenolic compounds and steroids. The presence of these compounds elucidates the capacity of the leaf extract to inhibit the activities of various radicals. From the table the results show that all the part of the plants have abundant saponin and alkaloid while the Tannin and the Terpenoid are scantly revealed especially at the fruit of each plant.

Antioxidants compounds are accountable for the protection of living organism from the damage caused by the irregular production of reactive oxygen species associated with protein damages, lipid peroxidation, and others including DNA strand breaking. They are widely believed to be an indispensable line of protection from oxidative damage, which has been indicated in a series of degenerative disorders (Stephen *et al*.,2017)

Flavonoids are a group of polyphenol compounds known properties which include free radical scavenging and oxidative enzymes inhibition of hydrolytic and inflammatory activity (Gryglewski *et al*.,1987). From this study; it is observed that quantitative analysis of phenol and flavonoid content of the extracts highly correlated to the previous results (Suja and Mohanasundari 2016). The earlier reports of P. umbellatum showed the presence of steroid, flavonoid, tannin, alkaloid, saponin, and phenol in the leaves extract (Nwauzoma and Songo 2013). The free radical scavenging activity of the plant extracts at different concentrations increases as concentrations increase.

The influence of solvent is more significant, in a conventional extraction and there are

significant differences ($p < 0.05$) especially in DPPH, ABTS and Nitric Acid while no significant difference in the others. The analyses results showed that the highest extracts seen were extracted using aqueous by cold extraction methods. Ethanol mixtures with water are commonly used for the extraction of phenols from plant materials (Yamaguchi *et al.,* 1998, Tripathi *et al*., 1996) which is majorly due to the wide range of phenols aqueous ethanol mixtures can dissolve. Furthermore, ethanolic mixtures have acceptability for human consumption models (Tripathi *et al*., 1996). Malaysia researchers reported that the maximum TPC was in 90% acetone banana pisang mas extract and 90% acetone guava extract, 70% ethanol honey pineapple extract respectively (Tripathi *et al*., 1996). The results showed that the antioxidant activity was higher for aqueous extract of both plants as followed by ethanol and methanol extracts in all the analysis except for ABTS where the reverse trend was observed. The results also showed that the antioxidant activity was higher for aqueous extract of both plants as followed by ethanol and methanol extracts in all the analysis except for ABTS where the reverse trend was observed. TPC Analysis and free radical scavenging activity of ML and NL leaves and fruits extracts showed differences depending on solvent used and extraction method.

Conclusion

This study gave insight and information for the determination of chemical composition of ML and NL using different solvents as extraction media. In the present study flavonoids, steroid, glycosides, tannins and phenolic compounds were identified from two plant part extract with different fractions. This study indicated that the three solvent extracts of ML and NL plant have potent antioxidant capacity in DPPH and ferric reducing antioxidant power assay methods. Thus, it can be concluded that good sources of natural antioxidants substances to health systems.

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