Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.13, No.10, 2022

Comparative Assessment of the Phytochemical Composition and Antimicrobial Activity of Ogirisi (Newbouldia Leavis) Rootbark and Rootwood Extracts

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Abstract
The study was aimed at a comparative evaluation of the phytochemical constituents and antimicrobial activity of the rootwood and rootbark of Newbouldia leavis extracts. The powdered samples were explored using two solvents (ethanol and ethylacetate) as extractants. Phytochemical assay of the crude extracts were done following standard procedures. The results of preliminary phytochemical screening of samples revealed that carbohydrates, alkaloids, flavonoids, phenolics and terpeniods were present in all extracts; saponins and glycosides in ethanol extracts and tannins in all extracts except ethylacetate. The results also show the absence of steroids and phlobatannins in all extracts. The comparative phytochemical evaluation of the ethanol extracts of both the rootwood and rootbark samples showed the presence of these bioactive compounds in varying concentrations. Ethanol proved to be a better extractant for saponins and tannins than ethylaceate and this could be attributed to the difference in their polarities. The antimicrobial activity was carried out using the agar well diffusion method against: Escherichia coli, Staphylococus aureus, Candida albicans and Salmonella typhi. The results of the anti-microbial tests revealed that the rootbark extracts showed a broader spectrum of activity than the rootwood. The antibacterial and antifungal activities of the extracts were notably inhibitory against the test pathogens at MIC values of 2.5 – 10 mg/ml as against the MIC values of 1.25 mg/ml obtained for the standard antibiotics. In conclusion, the wide range of inhibitory zone diameter recorded could be as a result of singular or synergistic action (s) of the bioactive compounds present.
Keywords: Phytochemicals, Antimicrobials, *Newbouldia leavis*
DOI: 10.7176/JNSR/13-10-01
Publication date: May 31st 2022

1.0 INTRODUCTION

Prior to the mainstreaming of allopathic medicine, traditional medical healers have continued to rely on plant resources for treating various diseases. Plants produce important chemical molecules known as 'phytochemicals' in their various parts. These chemicals such as flavonoids, saponins, alkaloids and phenolics are considered to be bioactive compounds which play significant roles in the treatment of numerous diseases (Ndukwe *et al.,* 2011).

In general, plant's phytochemicals also known as secondary metabolites are deemed to have some anti-microbial activity such as antibacterial, antifungal, anti-inflammatory, and antioxidant properties (Nemanja *et al*., 2015). Consequently, most modern drugs are developed having these natural products as their major constituents (Mzwandile *et al*., 2017).

Popularly known as 'boundary tree', *Newbouldia laevis* is a green plant of about 7-8m that belongs to the Bignoniaceae family commonly found in the tropics of Africa. In Nigeria, trado-medical practitioners use parts such as the root, leaves, stem seed and flowers in the treatment of diseases. *Newbouldia leavis* is one of several plant species commonly used in the treatment of ailments such as convulsion, headache, fever, and infertility; the roots also serves as painkillers (Ainooson *et al*., 2009). The three core tribes in Nigeria have special local names for the plant as follows: Akoko (Yoruba), Aduruku (Hausa), and Ogirisi (Igbo). Till date, there is scarce information as to a comprehensive, well-researched and detailed documentation of the medicinal uses of the extracts of core parts of the plant.

2.0 MATERIALS AND METHODS

***2.1 Chemicals and reagents*:** Only chemicals and reagents of analytical grade were used in this study. Such chemicals include sodium hydroxide, conc. sulphuric acid (H_2SO_4), sodium sulfate, methyl red, conc. hydrochloric acid (HCl), zinc metal, n-hexane, potassium bismuth (Dragendroff's reagent), distilled water, olive oil, chloroform, 10 % ammonia solution, 0.1 %. $FeCl_3$, Fehling solutions A and B, Benedict's reagent, acetic

acid, ethanol and ethyl acetate.

2.2 Sample Collection: The root resources were collected from the bushes around Amankwo, Iwollo and Akma Oghe all in Ezeagu Local Government of Enugu State, Nigeria. It was authenticated by Prof. C. N. Eze of the Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology, Agbani, Enugu, Nigeria. The root bark and root wood were air-dried for twenty one days and then crushed to powdery form using mortar and pestle. The powdered sample was later stored in a polythene bag until required for analysis.

2.3 Preparation of Plant Extract: The powdered sample was exhaustively explored and extracted using ethanol and ethyl acetate as solvents through cold maceration. The root extract was concentrated through the use of a rotatory evaporator. The residue /crude extract was then dried under vacuum by standard method (Handa *et al.,* 2008.)

2.4 Phytochemical Screening: The two root extracts were subjected to preliminary phytochemical screening using standard methods as follows:
2.4.1 Test for saponins: Twenty milligram of each extract was boiled in 20 ml of distilled water in a water bath for five minutes and filtered. Then 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for froth formation. Afterwards, 3 drops of olive oil were mixed with froth, shaken vigorously and observed for emulsion development.
2.4.2 Test for terpenoids: About 5 ml (1 mg/ml) of extract was mixed with 2 ml of chloroform, followed by 3 ml of conc.H_2SO_4. A reddish brown colouration at the interface confirmed the presence of terpenoids.
2.4.3 Test for Steroids: Five drops of concentrated H_2SO_4 was added to 1 ml of the extract in a test tube. A red colouration indicates the presence of steroid.
2.4.4 Test for Flavonoids: A quantity (50 mg) of extract was suspended in 100 ml of distilled water to get the filtrate. A 5 ml of diluted ammonia solution was added to 10 ml of filtrate followed by few drops of concentrated H_2SO_4. Formation of yellow colouration confirms the presence of flavonoids.
2.4.5 Test for Tannins: A quantity (50 mg) of extract was boiled in 20 ml of distilled H_2O and filtered. A few drops of 0.1 % $FeCl_3$ was added into the filtrate and observed for colour change; brownish green or a blue-black colouration was taken as evidence for the presence of tannins.
2.4.6 Test for Alkaloids: About 0.5 g of extract was stirred with 8 ml of 2 % HCl and the mixture was warmed and filtered. The filtrate (2 ml) was treated with potassium bismuth (Dragendroff's reagent). Turbidity or precipitation with this reagent was taken as evidence for existence of alkaloids.
2.4.7 Test for Anthraquinones: A quantity (200 mg) of the extract was boiled with 6 ml of 1 % HCl and filtered. The filtrate was shaken with 5 ml of benzene, filtered and 2 ml of 10 % ammonia solution was added to the filtrate. The presence of a pink, violet or red colour in the ammonical phase indicated the presence of free hydroxyl anthroquinones.
2.4.8 Test for Cardiac Glycosides: To the 5ml of each extract, 2 ml of glacial acetic acid and few drops of ferric chloride solution was added and then concentrated sulphuric acid was also added and observed for a reddish brown coloration at the junction of two layers and the bluish green colour in the upper layer
2.4.9 Test for Carbohydrate: To 2 ml of test sample, 3 drops of Molisch reagent were added. The mixture was shaken and concentrated sulphuric acid was pour down into the inclined side of the test tube. An appearance of a purple ring at the junction of the two layers indicates the presence of carbohydrate.
2.4.10 Test for Reducing Sugars: A quantity (0.1 g) of the extract was shaken vigorously with 5 ml of distilled water and filtered. The filtrate was used for the following test:

a) **Fehling's Test:** To 1 ml portion of the filtrate were added equal volumes of the Fehling solutions 1 and 2 and boiled on a water bath for few minutes. A brick red precipitate indicates the presence of reducing sugar.
b) **Benedict's Test**: To 1 ml portion of the filtrate, 2 ml of Benedict's reagent were added. The mixture was shaken, heated on a water bath for 5 min. A rusty brown precipitate indicates the presence of reducing sugars.

2.4.11 Test for Starch
Iodine Test: A quantity (0.1 g) of the extract was mixed with 2 drops of iodine solution. A blue-black colour indicates the presence of starch.
2.4.12 Test for Resin

a) ***Precipitation test:*** A quantity (0.2 g) of the powdered material was extracted with 15 ml of 96 % ethanol. The alcoholic extract was then poured into 20ml distilled water in a beaker. A formation of precipitate indicates the presence of resins.

b) ***Colour test:*** A quantity (0.12 g) of the extract was extracted with chloroform and the extract concentrated to dryness. The residue was re-dissolved in 3ml Acetone and 3 ml concentration HCL added. This mixture was heated in a water bath for 30 min. A pink colour that changes to magnets red indicates the presence of resins.

2.4.13 Test for Protein: Presence of protein in extract was carried out by taking 2 ml (1 mg/ml) of extract and mixed with 1 ml of 10 % NaOH and 1 ml of 1 % $CuSO_4$. A violet colouration confirmed the presence of a peptide bond (protein).

2.4.14 Test for Amino acids: A quantity 5 ml (1 mg/ml) of extract was mixed with 2 ml of 0.1% Ninhydrin solution and boiled for 5 min. A violet blue colouration indicates the presence of amino acids.

2.5 ANTIMICROBIAL ACTIVITY

The antimicrobial study was carried out on the extracts using Muller Hinton agar for the bacteria and potato dextrose agar for the fungi. The organisms used were: *Escherichia coli, Staphylococus aureus*, *Candida albicans* and *Salmonella typhi.*

2.5.1 Determination of Inhibitory Activity of the Extract (Sensitivity Test): The sensitivity test was carried out using agar well diffusion method. The standard inucula of the microbes (bacterial and fungal isolates) were streaked on sterilized Mueller Hinton and Potato dextrose agar plates with the aid of a sterile swab sticks. Five wells were punched on each inoculated agar plate with a sterile cork borer. The well was properly labeled according to different concentrations of the extract prepared using serial dilution which were 20, 10, 5 and 2.5 mg/ml respectively. Each well was filled up with approximately 0.2 ml of the extract. The inoculated plates with the extract were allowed to stay on the bench for about one hour and this was to enable the extract to diffuse into the agar. The plates were then incubated at 37 ^{0}C for 24 hours (plates of Mueller Hinton agar) while the plates of potato dextrose agar were incubated at room temperature for about 3-5 days. At the end of incubation periods, the plates were observed for any evidence of inhibition of the microorganism which will appear as a clear zone that was completely devoid of growth around the wells (zone of inhibition). The diameters of the zones were measured using a pair of dividers and read off from transparent ruler calibrated in millimeter and the results were recorded. Ciprofloxacin was used as a reference drug against the selected bacteria: *Staphylococus aureus*, *Escherichia coli,* and *Salmonella typhi.* While Fluconazole was used as the standard antibiotics for the fungi, *Candida albicans*.

2.5.2 Determination of Minimum Inhibitory Concentration (MIC): The tube dilution method was used for the study on the minimum inhibitory concentrations (MIC) of the extracts. The lowest concentration of the extract showing inhibition for each organism when the extract was tested during sensitivity test was serially diluted in the test tubes containing Mueller Hinton broth. The standardized organisms were inoculated into each tube containing the broth and extract. The inoculated tubes were then incubated at 37 ^{0}C for 24 hours. At the end of the incubation period, the tubes were examined for the presence or absence of growth using turbidity as a criterion. The lowest concentration in the series without visible sign of growth (turbidity) was considered to be the minimum inhibitory concentration (MIC). The result was also recorded.

3.0 RESULTS AND DISCUSSION

Table 1: Result of phytochemical evaluation of the rootwood and rootbark of Newbouldia leavis extracts

S/N	PHYTOCHEMICALS	EXTRACTS			
		ROOTWOOD		ROOTBARK	
		Ethanol	Ethylacetate	Ethanol	Ethylacetate
1	Carbohydrate	++	++	+	+
2	Protein (Amino acid)	+	–	+	–
3	Flavonoids	++	++	++	+
4	Alkaloids	+	+	++	+
5	Saponins	+	–	+	–
6	Tannins	++	+	++	+
7	Glycosides	++	+	++	+
8	Steroids	–	–	–	–
9	Terpenoids	++	+	++	+
10	Phenolics	++	+	++	–
11	Phlobatannins	–	–	–	–

KEY: + Present in trace concentration, ++ Present in moderately high concentration, +++ Present in very high concentration; – absent (not detected).

4.0 DISCUSSIONS

4.1 Phytochemical Composition: The result of preliminary phytochemical analysis of rootwood and rootbark *Newbouldia leavis* (Table 1) show that carbohydrates, alkaloids, flavonoids, terpeniods were present in all extracts and phenolics (except in ethylacetate extract of the rootbark); Tannins and glycosides were present in all extracts while saponins were present only in the ethanol extracts. The results also show the absence of steroids and phlobatannins in all extracts.

Phytochemicals are biomolecules responsible for the medicinal activity of plant resources. However, the quality of these molecules is dependent on the medium and method of extraction. The presence of saponins and phenolics contributed to the elevated antimicrobial activity of the ethanol extracts of both samples. A result which aligns with the findings on previous investigations (Mbatchou and Dawda, 2012),

that attributed the antibacterial activity of various parts of plants to the presence of saponins, alkaloids, and phenolics among other secondary metabolites.

The presence of these bioactive compounds in the sample(s) is responsible for the various pharmacological potency. According to Okwu and Okwu. (2004), molecules like flavonoids and tannins possess antioxidant properties (prevents cell damage by oxidation): inhibits growth of cancerous cells /tumors and also reduces the possibility of heart disease; alkaloids have antimicrobial activities and anti-malarial activity (Okoli *et al*., 2015).

Comparatively, the result of carbohydrate assay (Molisch's test) showed the presence of carbohydrates in both the ethanol and ethylacetate rootwood extracts to be in ++ (moderate high) concentrations. While the rootbark presented with a + (trace) concentration for the both solvent extracts. For protein analysis (Ninhydrin Test), amino acid was detected in trace (+) amounts in both ethanol and ethylaceate rootbark extracts and also in the ethanol extract of the rootwood while completely absent (not detected) in the ethylacetate extract of the same sample. In addition, the ethanol extracts of both samples showed high concentration of tannins while in trace amount only in the ethylacetate extract of the rootwood and not detected in the ethylacetate extract of the rootbark.

Furthermore, the ethanol extracts of both crude samples (rootwood and rootbark) showed trace concentrations of saponins while completely absent in the ethylacetate extracts of both. In this study, ethanol proved to be a better extractant for saponins and tannins than ethylaceate and this could be as a result of the difference in their polarities; ethanol is more polar than etheylacetate, and saponins and tannins are polar molecules (Eloff, 2004; Wagner and Bladt, 2001). Okoli *et al*. (2007) reported saponins and tannins used in herbal concoctions to have haemostatic activity (the ability to stop bleeding from cuts and wounds by precipitating protein to form vascular plugs). Thus, the presence of these key phytochemical components in both solvent (ethanol and ethylacetate) extracts of the rootwood and rootbark of *Newbouldia leavis* is responsible for their considered medicinal and antimicrobial activity.

Tables 2: Result of Antimicrobial activities of the plant extracts: IZD (mm) and MIC (mg/ml)

Organisim	**Extract/ Std. Antibiotics**	**Conc. (mg/ml)**					
		20	**10**	**5**	**2.5**	**1.25**	**MIC (mg/ml)**
			IZD (mm)				
BACTERIA							
S. aureus	Rootbark (Ethanol)	R	R	R	R	NA	20 ± 0.21
	Rootbark (Eth. Acet)	7	3	1	1	NA	2.5
	Rootwood (Ethanol)	R	R	R	R	NA	20
	Rootwood (Eth. Acet)	6	5	4	1	NA	2.5
	Ciprofloxacin	25	18	15	11	4	1.25
E. coli	Rootbark (Ethanol)	R	R	R	R	NA	20 ± 0.21
	Rootbark (Eth. Acet)	8	6	R	R	NA	10
	Rootwood (Ethanol)	R	R	R	R	NA	20
	Rootwood (Eth. Acet)	5	2	2	2	NA	2.5
	Ciprofloxacin	21	17	10	8	3	1.25
S. typhi.	Rootbark (Ethanol)	R	R	8	5	NA	20 ± 0.21
	Rootbark (Eth. Acet)	10	8	R	R	NA	5
	Rootwood (Ethanol)	R	R	2	2	NA	2.5
	Rootwood (Eth. Acet)	5	R	R	R	NA	20 ± 0.21
	Ciprofloxacin	27	21	14	9	5	1.25

FUNGI							
C. albicans	Rootbark (Ethanol)	3	R	R	R	NA	20
	Rootbark (Eth. Acet)	12	8	6	6	NA	2.5
	Rootwood (Ethanol)	R	R	R	R	NA	20 ± 0.21
	Rootwood (Eth. Acet)	R	R	R	R	NA	20 ± 0.21
	Fluconazole	33	26	17	11	7	1.25

KEY: R = Resistant, NA = Not applicable, IZD = Inhibition Zone Diameter, MIC = Minimum Inhibition Concentration.

4.2 Antimicrobial Analysis:

Tables 2 summarizes the results of the antimicrobial assay of the rootbark and rootwood of *Newbouldia leavis* extracts on the selected four (4) test organisms expressed in terms of diameter of zones of inhibition and Minimum Inhibitory Concentration (MIC).

The results from the antimicrobial evaluation of the separate crude extracts of both root parts showed a broad spectrum of acivity against three bacterial strains (*Staphylococus aureus*, *Escherichia coli,* and *Salmonella typhi*), and one fungal pathogen (*Candida albicans*) as tested, with the inhibition zones between 1 - 12 mm. *Specifically, S. aureus*, *E. coli,* and *S. typhi* were inhibited at a range of 1 -10 mm and *C. albicans* between 6 - 12 mm using the rootbark ethylacetate extracts only. Also, the rootwood ethylacetate extracts showed some inhibition in the zones of 1 – 6 mm. However, pathogens proved to be mostly resistant in the ethanol extracts of both samples (Table 2).

MIC investigations revealed that ethylacetae extracts of both the rootbark and rootwood samples were able to inhibit the four test organisms with corresponding values between 2.5 – 10 mg/ml. Conversely, test pathogens were mostly found to be resistant with MIC values between 20 ± 0.21 for all ethanol extracts. Thus, in terms of solvent, ethylacetate extracts proved to have more inhibitory activity on the microbes than the ethanol extracts. In all, crude extracts were largely inhibitory against the selected pathogens at MIC vaues of 2.5 – 10 mg/ml as against the MIC values of 1.25 mg/ml obtained for the standard antibiotics used. The result of this study is remarkable in that these microrganisms if not checked, would pose a lot of health risks. *E. coli* for instance is known to attack the gastrointestinal and urinary tracts of humans and animals causing them to malfunction (Nwachukwu *et al*., 2006; Nwachukwu *et al*., 2009; Prescott *et al*., 2005).

5.0 Conclusion:

The findings made in this study, is a justification for the rampant trado-medical and phytopharmaceutical applications of plant resources in treating disease caused by microbes. The preliminary phytochemical screening of samples revealed the presence of bioactive compounds with anti-microbial potency. The antibacterial and antifungal activities of the two solvent extracts of the root parts of *Newbouldia laevis* were notably inhibitory against the four test microorganisms at MIC values of 2.5 – 10 mg/ml as against the MIC values of 1.25 mg/ml obtained for the standard antibiotics. The wide range of inhibitory zone diameter recorded (Table 2) maybe as a result of singular or synergistic actions of the secondary metabolites present.

Acknowledgments:

We thank all those that have contributed to the success of this paper.

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