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Research Article

Antioxidants and Antimicrobial Activities of Methanol Extract of *Newbouldia laevis* and *Crateva adansonii*

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Abstract

Plants and plant products are continuously being explored in medicine against free radicals induced oxidative stress and increasing No. of antibiotic resistant organisms. In the present study antioxidants and antimicrobial activity of methanol leaf extract of *N. laevis* and *Crateva adansonii* against some pathogenic microorganism were carried out. The antioxidants activities were conducted using DPPH radical scavenging assay. The antibacterial activity was screened against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus* and *E. coli* using agar well diffusion method at various concentrations (160, 120 and 80 mg mL^{-1}). The Minimum Inhibitory Concentration (MIC) was determined using serial dilution method, while Minimum Bacterialcidal Concentration (MBC) by plating various dilution of extract. Results revealed the presence of alkaloid, flavonoids, saponins, terpenoids, glycoside and anthraquinone in methanol leaf extract of *N. laevis*. *Newbouldia laevis* and *Crateva adansonii* extracts and the standard antioxidant (Vitamin E) promoted an inhibition of DPPH radical with increasing concentrations. The IC_{50} values of *C. adansonii*, *N. laevis* and Vitamin E were 1562.52, 155.17 and 83.65 mg mL^{-1}, respectively. The zone of inhibition demonstrated by the two extracts increase with increase concentration. The MIC of both *e*xtracts was in range of 120-160 mg mL^{-1}, while the MBC range between 120-160 mg mL^{-1}. It is concluded that *N. laevis* contains some useful phytochemicals with potential antioxidants and antibiotic reputations. Thus, it may be considered as a natural source of antimicrobials and antioxidants for therapeutic purposes.

Key words: *Newbouldia laevis*, *Crateva adansonii*, antimicrobials, antioxidants, DPPH

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

World Health Organization (WHO) recognizes traditional based medicine particularly from plant extract as an important alternative healthcare delivery system for most of the world's population[1]. Literatures have documented the potential beneficial effects of African medicinal plants and other African natural products in the traditional management of different parasitic infectious, metabolic and oxidative stress induced disease[2-4]. They are also known for considerably huge amount of novel bioactive compounds that could serve as a lead for the development of new and effective drugs for the treatments of several diseases[5-7]. African natural products are therefore noteworthy for there remarkable healing properties as revealed in the various citations above.

The recent growth in knowledge of free radicals and Reactive Oxygen Species (ROS) in biological systems is producing a medical revolution that promises a new age of health. Under a situation of oxidative stress, reactive oxygen species such as superoxide ($O_2^{\bullet-}$), hydroxyl ($OH^{\bullet}$) and peroxyl ($OOH^{\bullet}$, $ROO^{\bullet}$) radicals are generated. These reactive oxygen species play an important role in degenerative or pathological processes, such as aging, cancer, coronary heart disease, alzheimer's disease, atherosclerosis, cataracts and inflammation[8]. The major roles of antioxidants are in preventing the oxidation of other molecules by inhibiting the instigation or promulgation of oxidizing chain reactions by free radicals and they may reduce oxidative damage to the human body[9]. Many natural antioxidants compounds are abound in plants. The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics has raises concern on untreatable bacterial infections and call for urgent search for new infection fighting strategies and new effective antimicrobial and antioxidants agents.

Crateva adansonii (family: Capparidaceae) also called sacred garlic pea or temple plant (English), eegun orun or ajanaka (Yoruba-Western Nigeria), ungududu (Hausa-Northern Nigeria) and amakarode (Igbo-Eastern Nigeria). The bark is widely used for stomach troubles and held to have tonic properties. In Africa the roots figure in several treatments for infectious disease, syphilis, jaundice yellow[10,11]. *Newbouldia laevis* also referred to as seeman or boundary tree which is known as Aduruku in Hausa, Ogirisi in Igbo, Akoro in Yoruba and Dinberechianmile in Nupe. It is a shrub or small tree reaching to 7-8 m high. It is shrubby or erect with vertically ascending branches of wooded savanna and deciduous forest. Traditionally, the dried bark and young twigs of *Newbouldia laevis* are pounded up with spices given in decoction or infusion for complaints such as uterine colic, dysmenorrhoea, etc.[12]. A decoction of the bark is given to children for epilepsy and convulsions. The bark is used as a stomachic and in the form of an enema for constipation and piles; the bark is also said to cure septic wounds[13].

MATERIALS AND METHODS

Plant collection: Fresh leaf of *Newbouldia laevis* and *Crateva adansonii* were collected in the month of June, 2015 from Chanchaga Local Govt area of Niger State Nigeria. It was identified and authenticated by a botanist in the Department of Biological Science, Federal University of Technology, Minna, Niger State.

Sources of microorganisms: Pure isolate of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus* and *Escherichia coli* were obtained from Microbiology Department, Federal University of Technology, Minna, Niger State. The identity of the organism were confirmed by standard biochemical test and proper Gram staining test.

Sample preparation and extraction of plant materials: The collected fresh leaves of *Newbouldia laevis* and *Crateva adansonii* were destalked, washed with clean-water, dried at room temperature and finally grounded using a grinder mill. Extraction of plant materials was performed by weighing 200 g of the powdered plants and extracted by soxhlet extraction using 600 mL each of methanol. The resulting methanol extract was concentrated in a water bath at low temperature. The concentrated extract was finally exposed to air to complete drying. The dried extracts were stored in a refrigerator at 4°C until required.

Phytochemical analysis: Methanol extract of *Newbouldia laevis* was screened prelimnary for its phytochemical contents including flavonoids, saponins, alkaloids, tannins, cardiac glycosides, anthraquinones phlobatannins and steroids according to the methods of Sofowora[14] as described by Lawal *et al*.[15].

Assay for antibacterial activity

Preparation of inoculum: Stock cultures were maintained at 4°C on nutrient agar (HiMedia) slants. Active cultures for experiments were prepared by transferring a loopful of culture to 10 mL of nutrient broth (HiMedia) and incubated at 37°C for 24 h for bacterial proliferation[16].

Agar-well diffusion method: Agar well bioassay was employed for testing antibacterial activity of the plants extract as described by Jayaraman *et al.*[16]. Each extracts were made to a final concentration of 160 mg mL^{-1}. Twenty four old cultures of test organisms (0.05 mL) were seeded onto agar (Media) plate and uniformly spread with a spreader. Wells (5 mm) were made in the agar plate with a sterile cork borer. The plant extract was introduced into the well. Ciprofloxacin (40 µg mL^{-1}) was used as standard antibacterial antibiotics. The plates were then maintained at room temperature for 2 h allowing for diffusion of the solution. All plates were then incubated at 37°C for 24 h. Sensitivity was recorded by measuring the clean zone of growth inhibition on agar surface around the disc.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC): Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) was determined by tube dilution method for each of the test organism in triplicates. To 0.5 mL of varying concentrations of the extracts (0-200 µg mL^{-1}) 2 mL of nutrient broth was added and then a loopful of test organism previously diluted to 0.5 McFarland turbidity standard for (Bacterial isolates) was introduced to the tubes. The procedures were repeated on the test organisms using standard antibiotics ciprofloxacin.

A tube containing nutrient broth only seeded with the test organisms was served as control. Tubes containing bacterial cultures were then incubated at 37°C for 24 h for bacteria. After incubation the tubes were examined for microbial growth by observing the turbidity.

To determine the MBC for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes which did not show any growth and inoculated on sterile nutrient agar by streaking. Plates inoculated with bacteria and fungi were then incubated at 37°C for 24 h. After incubation the concentration at which no visible growth was seen was noted as MBC.

DPPH radical scavenging activity: The DPPH (1,1-diphenyl-2-picrythydrazyl) scavenging activity was measure by the spectrometric method the principle of this assay is that the free radicals DPPH possesses a characteristic absorption at 517 nm which decrease significantly on expose to antioxidant by receiving hydrogen atom or electron a lower absorbance at 517 nm indicate a higher antioxidant capacity of the extract in this study. The DPPH solution in methanol was prepared and 3 mL of this solution was mixed with 100 µL of methanol solution of *Newbouldia laevis* and *Crateva adansonii.* The sample were incubated for 20 min at 37°C in water bath and then decrease in absorbance at 517 nm was measured (AE) A blank sample containing 100 µL of methanol in the DPPH solution was prepared and its absorbance was (AB) measured. The radical scavenging activities was calculated using the formula:

$$\text{Inhibition (\%)} = \frac{AB - AE}{AE} \times 100$$

Where:
AB = Absorbance of blank sample
AE = Absorbance of *N. laevis*

RESULTS

Phytochemical composition: The results of qualitative phytochemical composition of methanol leaf extract of *N. laevis* are showed in Table 1. The results revealed the presence of alkaloid, flavonoids, saponins, terpenoids, glycoside and anthraquinone, while steroids were absent.

Antioxidant assays

Scavenging activity on DPPH radical of *N. laevis* and *C. adansonii*: The results of DPPH radical scavenging activity of methanol leaf extract of *N. laevis*, *C. adansonii* and the standard antioxidant (Vitamin E) are presented in Table 2 and Fig. 1. The extracts and the standard antioxidant (Vitamin E) promoted an inhibition of DPPH radical with increasing concentrations. Although, the percentage inhibition of the DPPH radical by Vitamin E was higher than the extracts. DPPH radical scavenging activity of methanol leaf extract of *N. laevis* (21.45-62.90% at 10-250 mg mL^{-1}) was higher than *C. adansonii* extract which show a very low (1.22-8.68%) activity at 10-250 µg mL^{-1}. The IC_{50} (concentration that inhibits 50% of the DPPH radical) values of *C. adansonii*, *N. laevis* and Vitamin E were 1562.52, 155.17 and 83.65 mg mL^{-1}, respectively.

Table 1: Phytochemical composition of methanol, leaf extract of *Newbouldia laevis*

Phytochemicals	Inference
Alkaloids	+
Flavoinoids	+
Saponins	+
Steroids	-
Tannins	+
Terpenoids	+
Anthraquinone	+
Glycoside	+

-: Absent and +: Present

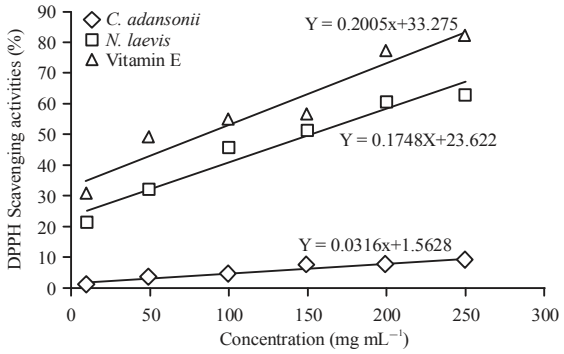

Fig. 1: DPPH radical scavenging activity of methanol leaf extract of *N. laevis* and *C. adansonii*

Table 2: DPPH radical scavenging activity of methanol leaf extract of *Newbouldia laevis* and *Crateva adansonii*

	Inhibition (%)		
Concentration (mg mL^{-1})	*Newbouldia laevis*	*Crateva adansonii*	Vitamin E
10	21.45	1.22	31.10
50	32.24	3.50	49.20
100	45.67	4.40	55.20
150	51.55	7.60	56.66
200	60.78	8.00	77.70
250	62.90	8.68	82.22

Determination of IC_{50}

For vitamin E: From the equation:

$$Y = 0.200x+33.27$$

$$At\ Y = 50$$

$$IC_{50}\ (X) = 83.65\ mg\ ML^{-1}$$

For *N. laevis*: From the equation:

$$Y = 0.174x + 23.62$$

$$At\ Y = 50$$

$$IC_{50}\ (X) = 155.17\ mg\ mL^{-1}$$

For *C. adansonii*: From the equation:

$$Y = 0.031x + 1.562$$

$$At\ Y = 50$$

$$IC_{50}\ (X) = 1562.52\ mg\ mL^{-1}$$

Zone of inhibition: The Zone of inhibition of methanol leaf extract of *N. laevis* and *C. adansonii* against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus* and *Escherichia coli* are presented in Table 2. The zone of inhibition demonstrated by the extracts increase with increase concentration. *Newbouldia laevis* extract shows significant inhibitory activities against all the microganism tested at concentration of 120 and 160, 80 mg mL^{-1}. However, at concentration of 80 mg kg^{-1} the extract show no inhibitory activities against *S. typhi*, *K. pneumonia* and *E. coli* but show significant inhibitory activities against *S. aureus* (6±0.42 mm) and *P. aeruginosa* (5±0.56 mm). *Crateva adansonii* extract shows no inhibitory activities against all the microganism tested at concentration of 80 mg mL^{-1}. However, at concentration of 120 and 160 mg mL^{-1} a significant zone of inhibitions were observed for *P. aeruginosa* (12±0.23 and 18±1.00 mm) and *S. aureus* (16±0.10 and 22±0.56 mm). However, *S. typhi* and *E. coli* were sensitive to the extract only at 160 mg mL^{-1} with inhibition zone of 16±0.65 and 17±0.20 mm, respectively (Table 3).

Minimal inhibitory concentration and minimal bacterialcidal concentration: The MIC of methanol leaf extract *N. laevis* extract against all the microoganiism tested was 120 mg mL^{-1} except for *P. aeruginosa* (160 mg mL^{-1}). Similarly, the MBC were 120 mg mL^{-1} for all the organism. For methanol leaf extract of *C. adansonii* the MIC of the extract were *K. pneumonia* (120 mg mL^{-1}), *P. aeruginosa* (160 mg mL^{-1}), *S. typhi* (120 mg mL^{-1}), *Staphylococcus aureus* (160 mg mL^{-1}) and *E. coli* (120 mg mL^{-1}), while the MBC were 160 mg mL^{-1} for all t he organism except for *E. coli* (MBC = 120 mg mL^{-1}), respectively as in Table 4.

DISCUSSION

Phytochemical constituents are secondary plant metabolites that occur in various part of plants. They have played a diverse roles in plants which include provision of vigour to plant; attraction of insect for pollination and feeding defence against predators and provision of colour[15]. However, this phytochemicals elicit varied biochemical and pharmacological actions when ingested by animals[17]. This study revealed the presence of various medically important phytochemicals in methanol, leaf extract of *N. laevis.* Phytochemical constituents demonstrated in the plants extracts were flavonoids, phenols, glycoside saponins, alkaloids, anthraquinone and tannins. The presence of important phytochemical is an indication that methanol leaf extract of *N. laevis* if properly screened could yield a drug of clinical importance. The preliminary phytochemical studies

Table 3: Zone of inhibition of methanol leaf extract of *Newbouldia laevis* and *Crateva adansonii* against some pathogenic microorganisms

	Newbouldia laevis (mg mL^{-1})			*Crateva adansonii* (mg mL^{-1})			Ciprofloxacin (mg mL^{-1})
Concentration (mg mL^{-1})	80	120	160	80	120	160	40
Zone of inhibition (mm)							
Pseudomonas aeruginosa	5±0.56	15±0.56	17±0.32	-	12±0.23	18±1.00	28±0.34
Klebsiella pneumoniae	-	12±0.50	17±0.10	-	-	-	26±0.20
Salmonella typhi	-	12±0.11	17±0.35	-	-	16±0.65	26±0.56
Staphylococcus aureus	6±0.42	9±0.05	16±0.50	-	16±0.10	22±0.56	24±0.30
Escherichia coli	-	16±0.15	19±0.32	-	-	17±0.20	26±1.09

Table 4: MICs and MBC (mg mL^{-1}) of methanol leaf extract of *Newbouldia laevis* and *Crateva adansonii* against some pathogenic organism

	Newbouldia laevis		*Crateva adansonii*	
	(mg mL^{-1})		(mg mL^{-1})	
Species	MIC	MBC	MIC	MBC
Pseudomonas aeruginosa	160	120	160	160
Klebsiella pneumoniae	120	120	120	160
Salmonella typhi	120	120	120	160
Staphylococcus aureus	120	120	160	160
Escherichia coli	120	120	120	120

also revealed glycoside was absent in *N. laevis* extract. This is in line with the study of Lawal *et al.*[15], which also found that not all phytochemicals are present in all plants and those that are presence varies with the extractant (extraction solvent) and concentration of the extract used. To confer antibacterial activity of the plant, flavonoids has been reported to singly responsible for antimicrobial activity associated with some ethnomedicinal plant. Similarly, tannins and phenolics compounds are known for there inhibitory role to bacteria growth[18].

The antimicrobial activities of leaf extract of *N. laevis* and *C. adansonii* showed that the extracts had varying degree of antimicrobial activity against the test organism. It was also observed that increase in the concentration of all the extracts yielded their corresponding increase in the zones of inhibition. This linear relationship between the concentrations of extracts and zones of inhibition could be that the extracts were able to diffuse into the inoculated nutrient agar. The ability of the methanol extract of *N. laevis* to inhibit the growth *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus* and *E. coli* explains why it is used in folk medicine to treat various bacterial diseases in Nigeria and other tropical countries[19].

The extracts were found to be more active with greater zone of inhibition at concentration of 120 and 160 mg mL^{-1}, this was in accordance with the results of a study by Shahidi[20], who reported that the higher concentration of antibacterial substance showed appreciable growth inhibition of bacteria. However, despite the higher zone of inhibition demonstrated by methanol leaf extract of *N. laevis*, there zone of inhibitions were lower on all test organism compare to zone of inhibitions demonstrated by standard antibiotics drugs (ciprofloxacin) use in this study. This is an indication that the plants extract is not as effective as ciprofloxacin as an antimicrobial agents Minimum Inhibitory Concentration (MIC) is the lowest concentration of an extract that inhibit the visible growth of the test organism after 24 h incubation. In the present study MIC was measured based on turbidity or visible growth show by the organism. The MICs (mg mL^{-1}) of methanol leaf extract of *N. laevis* against all microoganism tested were 120 mg mL^{-1}, except for *K. pneumoniae* 160 mg mL^{-1}. For methanol leaf extract of *C. adansonii* the MIC of the extract were *K. pneumonia* (120 mg mL^{-1}), *P. aeruginosa* (160 mg mL^{-1}), *S. typhi* (120 mg mL^{-1}), *Staphylococcus aureus* (160 mg mL^{-1}) and *E. coli* (120 mg mL^{-1}).

According to Taiwo *et al.*[21], in the assessments of antimicrobial activities of plant extracts, MBC results obtained by plating various dilution of extract is more reliable compared to MIC results usually using turbidity as an index, consequently, In the presence study, MBC (mg mL^{-1}) of methanol leaf extract of *C. adansonii* were 160 mg mL^{-1} for all the organism except for *E. coli* (MBC = 120 mg mL^{-1}).

The DPPH radicals were widely used to investigate the scavenging activity of some natural compounds. Figure 1 shows the results of scavenging DPPH radical ability of methanol leaf extract of *N. laevis* and *C. adansonii* at various concentrations in comparison with same doses of vitamin E. *Crateva adansonii* was found to have a low antioxidant property as confirmed by DPPH radical scavenging activities. In DPPH scavenging assay the IC_{50} value of methanol leaf extract of *N. laevis* and *C. adansonii* (155.17 and 1562.52 mg mL^{-1}, respectively) was higher than IC_{50} value reported for some medicinal plant eg 55.62 µg mL^{-1} for *S. surattense*[22]. The methanol leaf extract of *N. laevis* however, showed dose-dependent DPPH radicals scavenging activity than *C. adansonii* extracts. The decrease in absorbance of DPPH caused by methanol leaf extract of *N. laevis* is due to the reaction between antioxidant molecules and radical, which results in the scavenging of the radical by hydrogen donation. Similar findings have been documented

by many authors[23,24]. The reducing power of a natural compound may serve as a significant indicator of its potential antioxidant activity[8].

CONCLUSION

This study has shown that the methanol, leaf extract of *N. laevis* and *C. adansonii* contains some useful potential antimicrobial phytochemical that are inhibitory to some pathogenic organism. *Newbouldia laevis* also had a good DDPH radical scavenging activities Thus, it may be considered as a natural source of antimicrobials and antioxidants for therapeutic purposes.

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