Original Research Article

Antibacterial effects of *Newbouldia laevis* {P. BEAUV} on the bacteria isolated from the Blood of Hepatitis C virus positive individuals

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ABSTRACT

The methanol, chloroform and aqueous leaf extracts of *Newbouldia laevis* were obtained using cold extraction method. Phytochemical screening {qualitative} of the extracts was investigated and the inhibitory activity of extracts against the isolates were assayed by agar well diffusion technique. The concentrations were varied from 100 mg/ml to 400 mg/ml and zones of inhibition at every concentration were recorded. The bacterial isolates including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Streptococcus pnuemoniae* and *Salmonella* sp. The extracts revealed the presence of flavonoids, tannins, terpenes, alkanoids, phenolics, saponins, and cardiac glycosides with exception of chloroform extract that revealed the presence of alkanoids, saponins and tannins only. Antibacterial activity revealed that methanol extract had the highest potency with 23.03 ± 0.33^{e} mm, followed by aqueous extract with 21.75 ± 0.22^{d} mm zones of inhibition against *S. aureus,* and the chloroform extract had the highest activity of 16.0 ± 0.59^{d} mm zone of inhibition against *Salmonella* sp. while aqueous extract had the least zone of inhibition against *P. mirabilis* with 10.07 ± 0.67^{a} mm on isolates. All the extracts, irrespective of the extracting solvents had a minimum inhibitory concentrations {MIC} range of 6.25 – 50 mg/ml and minimum bactericidal concentrations {MBC} range of 12.5 – 100 mg/ml. Findings from this research shows that *N. laevis* has high antibacterial potency against pathogens in blood even in comparison with some conventional antibiotics used.

*Keywords: [*Methanol, Chloroform, Phytochemical, Inhibition, Antibacterial, Pathogens*}*

1. INTRODUCTION

Infectious diseases account for approximately 50 percent of all deaths in tropical countries {1}. According to World Health Organization {WHO} report, about 15 million {>25%} of 57 million annual deaths worldwide are as a result of infectious diseases {2}. Microorganisms are the commonest organisms responsible for morbidity and mortality resulting from infectious disease {Shira, 2008}. As such, bacterial and fungal diseases continue to remain a major public health problem {3}.

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As a result of abuse in the use of synthetic antimicrobial drugs, microorganisms resistant and or multi resistant to major class of antibiotics have emerged in recent years {1,4}. Hence, biologically active extracts and compounds from plant species used in herbal medicines have received huge attention in recent years {5,6}. Research by {7 and 8} on antioxidant, free radical scavenging capacity and antimicrobial activities of *Mirabilis jalapa* revealed that traditional medicine is an important source of potentially useful new compounds for the development of chemotherapeutic agents.

Medicinal plants have received huge attention both in the developed and developing nations. Their economic importance has drawn attention of various world bodies mostly; the World Health Organization {WHO} which released a special document concerning collection practices for medicinal plants {9}. In Nigeria, a large percentage of the populace depends on herbal medicines because the commercially available orthodox medicines are becoming increasingly expensive and out of reach {1,10}.

Amongst the medicinal plants commonly use in Nigeria for management/treatment of various types of ailments is *Newbouldia laevis* {also called the "Tree of life} belonging to the family *Bignoniaceae*. It is commonly grown as a live fence and may be found around groves and shrines. It is called 'Aduruku' in Hausa; 'Ogirisi' in Igbo and 'Akoko' in Yoruba. It grows to a height of about 7 - 8 {up to 15} metres, more usually a shrub of 2-3 metres, many – stemmed forming clumps of gnarled branches {11}. *Newbouldia laevis* has been reported to have medicinal value ranging from anti inflammatory, antioxidant, anti-microbial, anti-fungi, analgestic and wound healing properties {12, 13, 14, 15 16 and 17}. Specifically, the stem bark mixed with clay and red pepper has been reported to be effective against pneumonia, fever, cold, cough and for treating different illness like bone lesions {18}.

The knowledge of recent microbial resistance to antibiotics and the need to screen for new potent antimicrobial drugs from plants. This research was conducted to determine the antimicrobial effects of the aqueous, methanolic, chloroform leaf extracts of *N. laevis* on opportunistic bacteria isolated from the blood of HCV infected individuals and as well screen the plant extracts for the presence of secondary metabolites.

2. MATERIAL AND METHODS

2.1 Collection and identification of plant sample

Newbouldia laevis leaves were harvested from Ola-Ejigbo, Osun State, Nigeria around August – October 2016. The plant was authenticated by a botanist at the Biology Department of Federal Univerrsity of Technology, Akure, Ondo-State.

2.2 Preparation of crude extracts from the plant sample

The fresh leaves of *N. laevis* harvested were washed with distilled water to remove dust and other foreign particles. The leaves were then left on a clean surface to air dry at room temperature and ground into fine powder using a blender. Exactly 400 g of powdered leaf sample of *N. laevis* was each soaked in clean container containing methanol, chloroform and distilled water. The mixtures were allowed to stand for 72 hrs with intermittent stirring. This was followed by repeated filtration using sterile muslin cloth, non absorbent cotton wool and Whatman No 1 filter paper, in order to remove the marcs. The filtrates were concentrated *in vacuo* at 40^0C using a rotary evaporator to drive off organic solvent {Bibby Sterlin Ltd, England, RE. 2000} after which the aqueous part was lyophilized using a lyophilizer {Aqua Lyovac GT2, Germany}. The crude extracts obtained were stored in a tight container and kept in the refrigerator 4^0C for further use.

2.3 Determination of amount of extract in yield of solvent

After the extraction, amount of extract recovered was calculated using the following formula:

$$\frac{\text{Weight of dried extract after extraction} \quad \text{X} \quad 100}{\text{Initial weight of plant part before extraction}}$$

2.4 Phytochemical analysis

A small portion of the dried leaf extract of *N. laevis* was subjected to phytochemical tests for the screening and identification of bioactive chemical constituents using standard procedure {17, 19, 20, 21}.

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2.4.1 Test for terpenoids and steroids

Exactly 9 ml of ethanol was added to 1 g of the sample, refluxed for a few minutes and filtered. The filtrate was concentrated to 2.5 ml on a boiling water bath, and 5 ml of hot water was added. The mixture was allowed to stand for 1hour, and the waxy matter filtered off. The filtrate was extracted with 2.5 ml of chloroform using a separating funnel. One millilitre of concentrated sulphuric acid was added to 0.5 ml of the chloroform extract in a test tube to form a lower layer. A reddish-brown interface showed the presence of steroids.

Another aliquot of 0.5 ml of the chloroform extract was evaporated to dryness on a water bath and heated with 3 ml of concentrated sulphuric acid for 10 minutes on water bath. A grey colour indicated the presence of terpenoids.

2.4.2 Test for tannins

Two drops of 5% $FeCl_3$ was added to 1 cm^3 of solution of the extract. A dirty-green precipitate was observed in the extract confirming the presence of tannins.

2.4.3 Test for glycosides

Exactly ten 10 cm^3 of 50% H_2SO_4 was added to 1 cm^3 of each solution of the extract in a test tube. The mixture was heated in boiling water for 5 minutes. Ten cm^3 of Fehling's solution {5 cm^3 of each solution A and B} was added and boiled. A brick red precipitate indicated the presence of glycosides

2.4.4 Tests of saponins

Exactly 2 cm^3 of each extract in a test tube was shaken vigorously for 2 minutes. Frothing indicated the presence of saponin.

2.4.5 Test for Flavonoids

Exactly 2 cm^3 of extract solution was heated with 10 cm^3 of ethyl acetate on a water bath and cooled. The layers separated and the colour of the NH_3 layer was noted {red colouration formation}.

2.4.6 Test for phenolic compounds

Exactly 500 mg of each of the extract was dissolved in 5 ml of distilled water. Few drops of neutral 5% ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds.

2.4.7 Test for alkaloids

Exactly 2 g of each of the extract was stirred in 10 ml of 1% aqueous hydrochloric acid on steam bath for 30 minutes. The contents were then filtered and 1.0 ml of the filtrate was treated with a few drops of Meyer's reagents. A Whitish or cream coloured precipitate indicates the presence of alkaloids.

2.5 Source of clinical isolates

The clinical isolates used for this research work were obtained from the Microbiology Laboratory of Federal University of Technology, Akure prior to the work done on HCV positive blood samples.

2.6 Standardization of test organisms

A loopfull of test organisms was inoculated into 5.0 ml of nutrient broth and incubated at 37^0C for 24 hrs. A 0.2 ml from the 24 hrs inoculum of the organisms was dispensed into 20 ml sterile nutrient broth and incubated for 3-5 hrs to standardize the culture to 10^{-6} cfu/ml {22}.

2.7 Antibacterial assay

The pour plate method described by {23} was adopted for this test. Exactly 1 ml of nutrient broth containing the test organism was introduced into sterile Petri dish, and sterile Mueller Hinton agar medium which has cooled to about 45^0C was poured on it. The plate was gently agitated and the agar was allowed to solidify. Afterwards, wells were dug in the plates with the aid of a sterile cork borer of 6 mm in diameter. The extract was allowed to diffuse into the medium for one hour at room temperature. This was then incubated at 37^0C for 24 hrs after which the zones of inhibition were measured and recorded in millimeter. The control was set up in a similar manner with 30% DMSO and conventional antibiotic {ciprofloxacin} respectively.

2.8 Determination of minimum inhibitory concentrations {MIC}

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Prior to the results obtained from the antimicrobial assay, the MIC for the *N. laevis* leaf extracts using the susceptible organisms was carried out as described by {Ubulom *et. al.,* 2013}. Broth dilution method was employed in the experiment. From the stock solution, extract concentrations were obtained using double fold serial dilution. Standardized inoculums {$1x10^{-6}$cfu/ml} of the test bacterial species were each introduced into the test tubes containing different concentrations of the extracts. All the tubes were covered with sterile cotton wool to avoid cross contamination and incubated at 37^0C for 24 hrs. The tubes were thereafter examined for turbidity. Clear tubes were recorded as negative {no growth} and turbid tubes recorded as positive {Growth}. The least concentration of the extract with no turbidity was taken as the minimum inhibitory concentration **{**MIC} {1, 24}.

2.9 Determination of minimum bactericidal concentrations {MBC}

The minimal bactericidal concentration was determined from broth dilution test resulting from the MIC tubes as described previously {17, 25} by inoculating the content of each test tube on a sterile Mueller Hinton agar plate. The plates were then incubated at 37^0C for 24 hrs. The lowest concentration of the extract that showed no growth was noted and recorded as the minimum bactericidal concentration {MBC}.

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2.17 Determination of minimum inhibitory concentrations {MIC}

Prior to the results obtained from the antimicrobial assay, the MIC for the *N. laevis* leaf extracts using the susceptible organisms was carried out as described by {26}. Broth dilution method was employed in the experiment. From the stock solution, extract concentrations was obtained using double fold serial dilution. Standardized inoculums {$1x10^{-6}$cfu/ml} of the test bacterial species were each introduced into the test tubes containing different concentrations of the extracts. All the tubes were covered with sterile cotton wool to avoid cross contamination and incubated at 37^0C for 24 hrs. The tubes were thereafter examined for turbidity. Clear tubes were recorded as negative {no growth} and turbid tubes recorded as positive {Growth}.The least concentration of the extract with no turbidity was taken as the minimum inhibitory concentration **{**MIC} {24}.

2.18 Determination of minimum bactericidal concentrations {MBC}

The minimal bactericidal concentration was determined from broth dilution test resulting from the MIC tubes as described previously {17, 25} by inoculating the content of each test tube on a sterile Mueller Hinton agar plate. The plates were then incubated at 37^0C for 24 hrs. The lowest concentration of the extract that showed no growth was noted and recorded as the minimum bactericidal concentration {MBC}.

3. RESULTS AND DISCUSSION

Percentage yield of extract obtained from leaves

The result of the percentage yield of extracts obtained from the leaves which revealed aqueous extract having the highest yield of 34.8g {8.7%} and chloroform extract having the lowest yield of 20.3g {5.1%}

Qualitative phytochemical constituent of *Newbouldia laevis* leaf extracts

Table 1 shows the qualitative phytochemical screening of three crude extracts {methanolic, chloroform and aqueous leaf extracts}

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Phytochemical analysis of *N. laevis* leaf extracts revealed the presence of flavonoids, tannins, terpenoids, steroids, cardiac glycosides, alkaloids and saponins with the exemption of chloroform extract which only shows the presence of alkaloids, tannins and saponins, this may be due to the poor solubility of these phytochemicals in chloroform. This signifies the inefficiency of chloroform to be used as phytochemical extraction solvent for *N. laevis* plant leaf. It conformed to the report of {27}, on antibacterial effect of *N. laevis* leaf extract on vancomycin and methicillin resistant bacterial isolates and {28} on some important phytochemical of *N. laevis*. It was also similar to the report of {17}, on phytochemical and *in vitro* antimicrobial assay of the leaf extract of *N. laevis* and {29}, on involvement of tannins and flavonoids in the in-vitro effects of *N. laevis*.

Table 1: Qualitative phytochemical constituent of *Newbouldia laevis* leaf extracts

Constituents	Methanol Extract	Chloroform Extract	Aqueous Extract
Alkaloids	Present	Present	Present
Tannins	“	“	“
Phenols	“	Absent	“
Saponins	“	Present	“
Terpenoids	“	Absent	“
Steroids	“	“	“
Cardiac Glycoside	“	“	“
Flavonoid	“	“	“

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Methanol, chloroform and aqeueous extracts exhibited range of susceptibility against the bacterial isolates at different concentrations {concentrations varied from 100mg/ml – 400mg/ml} but their activities was concentration based {i.e. higher the concentration higher the antibacterial activities} of which 400mg/ml exhibited the highest antibacterial activities. At 400mg/ml, bacteria isolated from HCV infected blood, Methanol extract has the highest activity on *Staphylococcus aureus* with zone of inhibition of 23.03 ± 0.33^{e} mm and aqueous extract had the least zone of inhibition of 10.07 ± 0.67^{a} mm against *P. mirabilis.*

Salmonellosis is a symptomatic infection caused by bacteria of the *Salmonella* type {30}. *Salmonella* species are intracellular pathogens, Typhoid fever occurs when Salmonella invades the bloodstream - the typhoidal form; or in addition spreads throughout the body, invades organs, and secretes endotoxins - the septic form. This can lead to life-threatening hypovolemic shock and septic shock {31}. The antibiotic ciprofloxacin was used as positive control which was more effective when compared with the crude extracts. It shows highest zones of inhibition on all the tested organisms, it potency may be traced to the level of purification attained {it was more purified than the crude extracts}. This confirms the effectiveness of the drug in combating or treating infections associated with the tested organisms, with highest activity against *K. pneumoniae* {28.67 ± 0.18^{e} mm}.

However, the extracts especially the methanol and aqueous extracts exhibited considerable activity against *S. pneumoniae;* a significant human pathogenic Gram positive bacterium recognized as the main cause of community acquired pneumonia and meningitis in children and the elderly {32} and of septicemia in those infected with virus. The organism also causes many types of pneumococcal infections other than pneumonia. These invasive pneumococcal diseases include bronchitis, rhinitis, acute sinusitis, otitis media, conjunctivitis, meningitis, sepsis, osteomyelitis, septic arthritis, endocarditis, peritonitis, pericarditis, cellulitis, and brain abscess {33}. The extracts also show marked activity against *K. pneumoniae, E. coli, P. aeruginosa,* organisms that mainly cause gastroenteritis, they are also part of common cause of bacteremia in Africa as reported by {34}. Earlier research by {17; 27} using methanol extract reported the same trend.

Table 2: Antibacterial activity of *N. laevis* leaf extract at 100, 200, and 400 mg/ml on isolates from HCV infected blood samples

Isolates	Zones of inhibition {diameter in mm}										
	100mg/ml			200mg/ml			400mg/ml			Control	
	ME	CE	AE	ME	CE	AE	ME	CE	AE	CIP	DMSO
SA	8.03 ± 0.03^{e}	7.00 ± 0.10^{e}	3.33 ± 0.07^{c}	16.00 ± 0.58^{g}	9.79 ± 0.20^{d}	9.33 ± 0.03^{e}	23.03 ± 0.33^{e}	15.33 ± 0.16^{c}	21.75 ± 0.22^{d}	28.11 ± 0.01^{d}	0.00 ± 0.00^{a}
KP	6.93 ± 0.03^{d}	4.20 ± 0.58^{d}	5.73 ± 0.17^{c}	15.00 ± 0.12^{f}	7.70 ± 0.15^{c}	10.00 ± 0.10^{f}	21.78 ± 0.18^{c}	15.70 ± 0.21^{d}	21.67 ± 0.06^{d}	28.67 ± 0.18^{e}	0.00 ± 0.00^{a}
EC	1.70 ± 0.21^{a}	3.33 ± 0.03^{c}	2.33 ± 0.08^{a}	5.80 ± 0.20^{b}	4.33 ± 0.89^{a}	7.86 ± 0.08^{c}	15.00 ± 0.10^{a}	13.33 ± 0.33^{b}	14.27 ± 0.27^{b}	27.13 ± 0.19^{c}	0.00 ± 0.00^{a}

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PM	2.00 ± 0.05^{a}	9.70 ± 0.15^{f}	2.77 ± 0.18^{b}	4.13 ± 0.89^{a}	14.73 ± 0.15^{e}	6.00 ± 0.05^{a}	19.73 ± 0.13^{b}	20.40 ± 0.00^{e}	10.07 ± 0.67^{a}	23.40 ± 0.21^{b}	0.00 ± 0.00^{a}
PA	3.40 ± 0.06^{b}	3.33 ± 0.13^{c}	3.33 ± 0.03^{c}	9.77 ± 0.15^{d}	8.01 ± 0.14^{c}	6.07 ± 0.05^{a}	15.01 ± 0.00^{a}	12.15 ± 0.02^{a}	15.00 ± 0.58	19.73 ± 0.22^{a}	0.00 ± 0.00^{a}
SP	4.53 ± 0.23^{c}	0.00 ± 0.00^{a}	3.73 ± 0.08^{d}	9.00 ± 0.57^{c}	5.66 ± 0.12^{b}	6.66 ± 0.12^{b}	22.97 ± 0.03^{e}	15.17 ± 0.13^{c}	21.73 ± 0.15^{d}	28.43 ± 0.09^{de}	0.00 ± 0.00^{a}
SS	4.17 ± 0.09^{c}	2.53 ± 0.18^{b}	3.67 ± 0.13^{bc}	9.00 ± 0.57^{c}	5.66 ± 0.12^{b}	6.66 ± 0.12^{b}	22.10 ± 0.58^{d}	16.01 ± 0.59^{d}	22.20 ± 0.15^{e}	32.30 ± 0.12^{f}	0.00 ± 0.00^{a}

Data are presented as Mean±S.E {n=3}. Values with the same superscript letter{s} along the same column are not significantly different {P<0.05}
Key: SA: Staphylococcus aureus; KP: Klebsiella pneumoniae, EC: Escherichia coli; PM: Proteus mirabilis; PA: Pseudomonas aeruginosa; SP: Streptococcus pneumoniae; SS: Salmonella sp.*; ME: Methanol extract; CE: Chloroform extract; AE: Aqueous extract, CIP: Ciprofloxacin; DMSO: Dimethylsulfoxide.*

The basic qualitative measures of the *in vitro* activity of antimicrobials are the minimum inhibitory concentrations {MIC} or minimum bacterial concentration {MBC} {35}. The minimum inhibitory concentration {MIC} is the least concentration of an antimicrobial that shows no growth of microbial isolates in broth or agar. In this study, the MIC was 6.25 mg/ml, 12.5 mg/ml and 25 mg/ml concentration against *S. aureus* for methanol, water and chloroform extracts respectively. Minimum inhibitory concentration of 25 mg/ml concentration of all extracts inhibited the growth of all the isolates except for chloroform extract against *E. coli*, *S. pneumoniae and P. aeruginosa* with MIC of 50 mg/ml concentration, while both methanol and water extracts inhibited *Salmonella* sp. at lower MIC concentration of 12.5mg/ml. Minimum inhibitory concentrations are used by diagnostic laboratories to establish resistance or to determine *in vitro* activity of new antimicrobials {36}.
On the other hand, the minimum bactericidal concentration {MBC} is the least concentration of a plant extract that will completely kill a particular microorganism and showed no growth on media. Methanol and aqueous extracts showed the lowest MBC value of 12.5 and 25 mg/ml concentration respectively against *S. aureus.* At 25 mg/ml concentration, methanol extract was effective in killing all bacterial isolates except *E. coli* which was killed at 50 mg/ml. With the concentration of 50 mg/ml all extract was able to exacted 'cidal' effects on the most of the isolates; except for the aqueous extract against *K. pneumoniae, E. coli*, *S. pneumoniae* and for Chloroform against *S. pneumoniae* which MBC of 100mg/ml concentration was observed.

In an earlier work on *N. laevis* extract {17}, found the MIC of methanol extract against test isolates to be lower than that obtained in this present study. This high MIC could be due to nature of the isolates, geographical location, age of plant at harvest, season of harvest and method of extraction, all of which affect the yield of active constituents of medicinal plants {37; 21}.

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Table 3: Minimum inhibitory concentrations {MIC} for methanol, chloroform and aqueous extracts of *N. laevis*

Bacterial isolates	Methanol extract MIC {mg/ml}	Chloroform extract MIC {mg/ml}	Aqueous extract MIC {mg/ml}
Staphylococcus aureus	6.25	25	12.5
Klebsiella pneumoniae	25	25	25
Salmonella sp.	12.5	25	12.5
Pseudomonas aeruginosa	12.5	25	50
Escherichia coli	25	50	25
Streptococcus pneumoniae	25	50	50
Proteus mirabilis	12.5	25	25

Table 4: Minimum bactericidal concentrations {MBC} for methanol, chloroform and aqueous extracts of *N. laevis*

Bacterial isolates	Methanol extract MIC {mg/ml}	Chloroform extract MIC {mg/ml}	Aqueous extract MIC {mg/ml}
Staphylococcus aureus	12.5	50	25
Klebsiella pneumoniae	50	100	100
Salmonella sp.	25	50	50
Pseudomonas aeruginosa	25	100	50
Escherichia coli	50	100	100

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Streptococcus pneumoniae	50	100	100
Proteus mirabilis	25	50	50

4. CONCLUSION

The phytochemical investigation of *N. laevis* leaf extracts revealed the presence of constituents which could be the basis for their medicinal potency against multiple antibiotics resistant organisms isolated from both HCV infected blood. The leaf extracts of *N. laevis* showed broad spectrum activity and compared favourably with the standard antibiotics used as positive control.

REFERENCES

1. Abodunrin, T. F., Oladumoye, M. K., Borishade, F. T., Akande, E. B., & Afolami, O. I. {2020}. Antibacteria Assesement of Aframomum melegueta [roscoe] k. Schum [alligator pepper] Fruit on Bacteria Pathogen Isolated from the Urinary Tract. Journal of Advances in Microbiology, 20{2}, 1-8. https://doi.org/10.9734/jamb/2020/v20i230212
2. Pinner, R.W., Teutsch, S.M., Simonsen, L., Klug, L.A., Graber, J.M., and Clerk, M.J. *et al.* {1996} Trends in Infectious Diseases Mortality in the United States. Journal of American Medical Associaton, Vol. 275, No. 3.
3. Kenrad, E.N. [2014]. *Epidermiology of Infectious disease: General principles*, Jones and Bartlett
4. Nino, J., Navaez, D.M., Mosquera, O.M. and Correa, Y.M. {2006}. Antioxidant activity of plant extracts from Colombia flora. *Brazilian J MIcrobiol* 37 {4}: 566-570.
5. Essawi, T. and Srour, M. {2000}. Screening of some Palestinian medicinal plants for antibacterial activity. *J Ethanopharmacol* 46: 343-349.
6. Orhue P.O., Edomwande E.C., Igbinosa E., Momoh A.R.M. and Asekomhe O. {2014}. *Inter. J. Herbs Pharmaco. Res.*, 3{1}: 24 – 29
7. Oladunmoye M.K. {2006}. Immodulatory effects of ethanolic extract of *Tridax procumbens* on Swiss Albino Rats Orogasrtrically Dosed with *Pseudomonas aeriginosa* {NCIB950}. *International Journal of Tropical Medicine* 1 {4}: 152-155.
8. Oladunmoye M.K. {2012}. Antioxidant, free radical scavenging capacity and antimicrobial activities of *Mirabilis jalapa*. *Journal of Medicinal plants Research* 6 {15}: 2099-2913.
9. WHO [2003]. “WHO guidelines on Good Agricultural and collection practices [GACP] for Medicinal plants”. World Health Organization, Geneva.

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10. Lawal, I.O., Igboanugo A.B.I., Osikarbo B. R., Duyilemi O.P., Adesoga A.A., Borokini T.I. and Adeyanju B.A.; {2010}; Evaluation of plant-based non-timber forest products {ntfps} as potential bioactive drugs in South-western Nigeria. *J. Clin. Med. Res*., 3: 61-66.
11. Arbonnier, M. {2004}. *Trees, Shrubs and Lianas of West African Dry Zones.* CIRAD, Margraf Publishers, GMBH MNHN, Cote d'Ivoire, 2004; p 194.
12. Stefan, G., Jean-luc, and Wolfender Malo, {1998}. Antifungal and antibacterial nephthoquinones from *Newbouldia laevi*s roots.Ecological biochemistry. Access.sciencedirect.com/science. study in the wetlands of Udu and Ughievwan clans of Delta State, Nigeria. *Proceedings of Global Summit on Medicinal Plants.* 1: 98 – 106.
13. Aladesanmi, A. J., Nia, R. and Nahrstedt, A. {1998}. New pyrazole alkaloids from the root bark of *Newbouldia laevis, Planta Med.* 64: 90-91.
14. Chukwujeku, J.C., Staden, J.V. and Smith, P. {2005}. Antibacterial, anti-inflammatory and antimalarial activities of some Nigerian medicinal plants. *SA J Bot*. 71 {3&4}: 316–325.
15. Kuete, V., Eyong, K.O., Folefoc, G.N., Bengi, V.P., Hussain, H., Krohn, K. and Nkengfack, A.E. {2007}. Antimicrobial activity of the methanolic extract and of the chemical constituents isolated from *Newbouldia laevis. Pharmazie*, pp. 552-556.
16. Akerele, J.O., Ayinde, B.A. and Ngiagah, J. {2011}. Phytochemical and antibacterial evaluations of the stem bark of *Newbouldia leavis* against isolates from infected wounds and eyes. *Tropical Journal of Pharmaceutical Research*, 10 {2}: 211-218.
17. Usman, H. and Osuji, J.C. {2007}. Phytochemical and *in vitro* antimicrobial assay of the leaf extract of *Newbouldia laevis. African Journal of Tradit. Complement. Altern. Med.* 4{4}: 476-480.
18. Idu, M., Obaruyi, G.O. and Erhabor, J. O. {2009}. Ethnobotanical Uses of Plants Among the Binis in the Treatment of Ophthalmic and ENT {Ear, Nose and Throat} Ailments. *Ethnobotanical Leaflets* 13: 480-96.
19. Edeoga, H.O., Okwu, D.E. and Mbaebie, B.O. {2005}. Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*, 4{7}: 685- 688.
20. Sofowora, A. {1993}. *Screening Plants for Bioactive Agents*. In: Medicinal plants and Traditional Medicine in Africa. John Wiley, Chichester, 1982; pp 142-146.
21. Trease, G.E. and Evans, W.C. {2002}. Pharmacognosy. 15th Ed. Saunders Publishers, London. pp. 42 –48
22. Aiyelaagbe, O.O., {2001}. The antimicrobial activity of root of *jatropha mutifida root* root. *Fitoterpadia,* 72: 544-545.
23. Ogundare, A.O. {2007}. Antimicrobial effect of *Tithonia diversifolia* and *Jatropha gossypifolia* leaf extracts. *Trends in Applied Sciences Research* 2 {2}: 145-150.
24. Ajaiyeoba, E.O., Onocha, P.A, Nwozo, S.O. and Sama, W. {2003}. Antimicrobial and cytotoxicity evaluation of *Buchholzia coriacea* stem bark. *Fitoterapia* 74: 706-709.
25. Usman, H., A. H. Yaro and M.M. Garba. {2008}. Phytochemical and Anticonvulsant screening of the ethanolic extracts of Newbouldia laevis {Bignoniaceae} in mice. *J. Pharm. Tox.,* 3; 127 – 133.
26. Ubulom Peace M. E., Udobi Chinweizu E., Akpabio Ekaete I. and Eshiet Udeme {2013}. Antimicrobial Activities of Leaf and Stem Bark Extracts of *Blighia sapida*. *Journal of Plant Studies*. 2{2}: 1927-2388
27. Fatunla, A. O., Ogundare, O. A., Achimugu I. I. and Akindele, P. O. {2018}. Antibacterial Effect of *Newbouldia laevis* Leaf Extract on Vancomycin and Methicillin Resistant Bacterial Isolates from Federal Medical Center, Owo. *JAMB,* 1{3}: 1-11.

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28. Anaduaka, E.G., Ogugua, V.N., Egba, S. and Apeh, V.O. {2013b}. Investigation of some important phytochemical, nutritional properties and toxicological potentials of ethanol extracts of *Newbouldia laevis* leaf and stem. *African Journal of Biotechnology*, 12{40}: 5846-5949.
29. Azando, E.V., Hounzangbe-Adote, M.S., Olounlade, P.A., Brunet, S., Fabre, N., Valentin, A. and Hoste, H. {2011}. Involvement of tannins and flavonoids in the in vitro effects of *Newbouldia laevis and Zanthoxylum zanthoxyloides* extracts on the exsheathment of third-stage infective larvae of gastrointestinal nematodes. *Vet. Parasitol.* 180{3-4}:292- 297.
30. CDC, {2015} "About Antimicrobial Resistance" . www.cdc.gov . Retrieved 2015-10-30.
31. Santos, R. L., Shuping Zhang, R. M. T., Robert A. K.. L., Gary A. and Adreas J. B. {2001}. "Animal models of *Salmonella* infections: enteritis versus typhoid fever". *Microbes and Infection*. 3: 1335–1344.
32. Van de Beek, D., De Gans, J., Tunkel, A. R. and Wijdicks, E.F.M. {2006}."Community-Acquired Bacterial Meningitis in Adults". *New England Journal of Medicine*. 354 {1}: 44–53. Retrieved 15 February 2017.
33. Siemieniuk, R. A.C., Gregson, D.B., Gill, M. J. {2011}. "The persisting burden of invasive pneumococcal disease in HIV patients: an observational cohort study". *BMC Infectious Diseases*. 11: 314.
34. Deen, J., Von Seidlein, L., Andersen, F., Elle, N., White, N. J. and Lubell, Y. {2012}. "Community-acquired bacterial bloodstream infections in developing countries in south and southeast Asia: a systematic review". *The Lancet. Infectious Diseases*. 12 {6}: 480–487.
35. Ughachukwu, P. O., Ezenyeaku, C. C. T., Ezeagwuna, D. A. and Anahalu, I. C. {2012}. Evaluation of anti-bacterial properties of ethanol extract of *Ficus exasperata* leaf, *African Journal of Biotechnology* Vol. 11{16}, pp. 3874-3876.
36. Andrews, J.M. {2002}. Determination of minimum inhibitory concentrations. *J. Antimicrob. Chemother*. 49 {6}: 1049.
37. Calixto, J.B. {2000}. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines. *Braz. J. Med. Biol. Res.* 33: 179-189.

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