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EVALUATION OF ANTIBACTERIAL ACTIVITY AND TOXICITY EFFECTS OF NEWBOULDIA STEM BARK EXTRACT

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Abstract

Newbouldia leavis is a boundary tree. it is a medium size angiosperm which belongs to the Bignoniaceae family. It is a fast-growing evergreen shrub or small tree. This study was carried out to investigate antibacterial activity of the methanol extract of Newbouldia stem bark. The antibacterial activity of the methanol extracts was determined by the agar well diffusion method. Three standard bacterial strains: Escherichia coli, Staphylococcus aureus, and Klebsiella pneumoniae were used for the antibacterial assay. Result of screening plant extracts for antibacterial activity showed that most of the organisms were sensitive to the extract. The antibacterial test utilizing well diffusion assay showed that Gram-positive bacteria were more susceptible to the plant extract compared to Gram-negative bacteria. This study supports the use of Newbouldа leavis in traditional medicine as well as highlights the need to further explore the potentials of the plant extract as antibacterial agent.

Keywords: Evaluation, Antibacterial, Toxicity, Newbouldia leavis, Stem Bark, Extract

INTRODUCTION

The World Health Organization (WHO) has recognized the role of herbal medicines or traditional medicine in the administration of primary health care; particularly in developing countries; and has encouraged members of nations to develop national policies for the adequate; sustainable identification; proper exploitation; scientific development and appropriate use of herbal medicines adapted to the prevailing situation (Ushie et al., 2021). In Benin; some studies have been carried out on the traditional use and socio-cultural values of N. Laevis. Through the research of Dassekpo et al. in 2017 and 2020; the specific uses of N. Laevis have been documented; so, in the African culture; N. Laevis is very sought-after plant and is sometimes associated with other plants during ceremonies (marriage; coronation; for peace; fertility etc.) (Dassekpo et al., 2017). Four traditional methods of preparation of N. Laevis (boiled in infusion or decoction; macerated and pressed; cold powder often produced by manual grinding; hot powder or inflammation) and several routes of administration (oral; dermal and inhalation) were identified. Plant and all parts of N. Laevis were used as drugs. Medicinal plants are the backbone of traditional medical practice; and indigenous knowledge needed for effective herbal medicine practice differs between cultures in different communities (Dassekpo et al., 2017). The African hyssop is thus used in the treatment of several ailments such as: ear infections; bronchopneumonia; malaria; abscesses; dysentery; arthritis; conjunctivitis; in the treatment of wounds; painful pathologies: dental pain; chest pain; otalgia; neuralgia; migraine abdominal pain; in the treatment of hypertension; T2D; diarrhoea; constipation; epilepsy; convulsions; elephantiasis; joint rheumatism; gastrointestinal ulcer and buruli ulcer; sickle cell anemia dysmenorrhea; haemorrhoid disease; typhoid fever cough; snake bites; the plant is used as an oxytocic during childbirth and as a deworming agent (Benlamdini et al., 2014).

Newbouldia leavis is a boundary tree called 'Aduruku' in Hausa, 'Ogirisi' in Igbo and 'Akoko' in Yoruba languages. it is a medium size angiosperm which belongs to the Bignoniaceae family. It is a fast-growing evergreen shrub or small tree. It only reaches a height of 3 - 8 meters in the west of its range, but can attain a height of up to 20 meters in the east. It has many stem forming clumps of gnarled branches (Arbonnier, 2004). The tree, and especially the bark, is widely used in traditional medicine in Africa. Newbouldia laevis is one of such plants that its leaves are used in South-eastern Nigeria to hasten parturition and to expel the placenta after delivery. Agents that stimulate uterine contraction are classified as oxytocic's and are employed clinically for the induction and

support of labour as well as in the management of the third stage of labour (Bafor and Sanni, 2009). The leaves of the plant were reported to have hepatoprotective activity (Hassan et al., 2010). The plant has antidiabetic, uterine contractile, antihypertensive, analgesic and anti-inflammatory properties (Tanko et al., 2008).

The leaves of the plant were reported to have hepatoprotective activity (Hassan et al., 2010). The plant has antidiabetic, uterine contractile, antihypertensive, analgesic and anti-inflammatory properties (Tanko et al., 2008). The bark has analgesic and stomachic properties. A decoction is used in the treatment of cough, diarrhoea and dysentery, while it is also given to children for the treatment of epilepsy and convulsions. A decoction of the bark, combined with chillies, is used in the treatment of chest pains (Bafor and Sanni, 2009). The dried bark and young twigs, pounded up with spices such as Xylopia, are given in decoction or infusion to treat such complaints as uterine colic, dysmenorrhea, etc (Bafor and Sanni, 2009).

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The trado-medical uses of N. laevis widely depend on the ethnic location of the plant and the part of the plant used. The Togolese and Nigerians use the leaves prepared as a decoction alone or in combination with other plants and administered orally for the treatment of malaria and fever (Iyamah and Idu, 2015). Also, the Tiv people of North-Central Nigeria prepare a polyherbal decoction of the leaves mixed with leaves of Crossopteryx febrifugg and Morinda lucida and taken orally to treat malaria (Yusuf et al., 2016). Inhabitants of Omo Forest Reserve in Western Nigeria boil the leaves in combination with leaves of Mangifera indica and M. lucida and taken orally to treat malaria and fever (Tor-anyiin et al., 2003). Similarly, in Southern Nigeria, the Ikwere people use the leaves, stem bark and roots for treating migraine, skin infections, fever, malaria, stomach

ache, epilepsy and conjunctivitis (Adekunle, 2008). Furthermore, the Ijebu people of Southwest Nigeria use the leaves decoction in combination with Momordica charantia, Vernonia amygdalina and Ocimum gratissimum leaves to treat measles, while the stem bark boiled with sugar cane juice is used in the treatment of dysmenorrhea by the Ibibio of Southern Nigeria (Nwauzoma and Dappa, 2013). In other reports, Ghanaians, Cameroonians and Nigerians use the bark, roots and leaves for the treatment of toothache, stomach-ache, diarrhoea, dysentery, malaria, fever, breast cancer, sexually transmitted diseases (STDs), anaemia, ulcer, arthritis, rheumatism, haemorrhoids, constipation cardiovascular diseases, diabetes, cough, elephantiasis and urinogenital tract infection (Appiah et al., 2019).

MATERIALS AND METHODS

Plant Material

Stem bark of Newbouldia laevis were collected from the uncultivated farm land of Wukari Taraba State, Nigeria. The stem bark was taxonomically identified and authenticated in the Department of Plant Science of Modibbo Adama University of Technology, Yola, Nigeria. The stem bark was washed with distilled water before being air-dried for thirty days and then ground into powder by grinding with a mortar and pestle. One hundred and fifty grams (150g) of the powdered stem bark was cold macerated in 500ml of methanol in an Erlenmeyer flask and shaken at one-hour intervals. Then was left to stand at room temperature for 48 hours and filtered using Whatman's filter paper. The extracts were then concentrated to dry using a rotary evaporator and stored frozen until needed.

Antibacterial Assay

Preparation of test samples

The crude extracts of Newbouldia laevis was used in antibacterial assay, the methanol crude extracts. The crude extracts were tested by disc diffusion method on nutrient agar medium as described by (Umaru et al., 2018). Exactly 3 mg of the crude sample was dissolved homogeneity in 3 mL of methanol giving a stock solution of 1000 µg/ mL. Different volumes from the stock solution were taken, amounted to 50, 100, 250, 500 ppm each, and dissolved in 5 mL of methanol to make final concentration respectively.

Preparation of agar plates

Preparation of agar plates was performed based on method described by (Umaru et al., 2018). Nutrient agar was prepared according to manufacturer's instruction with 14 g of dried agar dissolved in 500 mL distilled water. The agar solution was heated until boiling followed by sterilization in autoclave at 121°C. The agar solution was then poured into a sterile petri plate and allowed to cool down and forming a gel. The plate was divided into eight sections by making a line marking on the outside surface of the plate. The eight sections were for each test samples namely the 50, 100, 250, ppm samples, tetracycline 30 µg (positive control) and methanol (negative control). The plate was sealed using parafilm and kept chilled at 4°C upon bacteria inoculation.

Preparation of bacteria broth

Several selected bacteria were used to evaluate the antibacterial activities of the crude extracts of Newbouldia laevis as obtained from the stock culture provided by Microbiology Laboratory, Federal University Wukari. The nutrient broth was prepared according to manufacturer's instruction, with 2.6 g of the dried broth dissolved in 200 mL distilled water followed by sterilization in autoclave at 121°C. The bacterial was sub-cultured in a 10 mL of broth, each in universal glass bottle for 16 hours inside an incubator equipped with shaker at 37°C (Malesh and Satish, 2008). After 16 hours of incubation, turbidity (optical density/OD) of the bacterial broth was measured by using UV mini spectrophotometer (model 1240 of Shimadzu brand), comparable to that of nutrient broth standard tube for further use. Measurement was performed at wavelength 575 nm and the bacterial broth was ready to be used when its turbidity was between OD 0.6 to 0.9. Nutrient broth was used to adjust the turbidity until the desired value was obtained.

Plate inoculation

Inoculation of the bacteria was carried out in a biohazard cabinet and the procedure was based on method described by (Umaru et al., 2018). Approximately 1 mL of the ready bacterial broth were transferred into mini centrifuge tubes. A sterile cotton swap was dipped into the mini centrifuge tube containing bacteria broth and streaked over entire of the agar plate surface, performed in 4 different directions. The agar plate was then left for 5-10 minutes before applying the test samples. The disc used was 6 mm diameter. A volume of 10 µL of the test samples of concentration 25, 50, 100, 250, ppm was each pupated onto the discs and placed onto the agar plate by using sterile forceps and gently

pressed to ensure contact. Next to be placed on the agar plate was the disc pupated with methanol as negative control, followed by 30 μg of tetracycline as standard antibacterial agent (positive control). The plates were left at room temperature for 10 minutes to allow the diffusion of the test samples and the standards into the agar. Each crude extract was tested in triplicate for each bacterium used. The plate samples were then incubated at 37°C for 24 hours before the inhibition zone around every sample disc being examined. The inhibition zone was measured in diameter to indicate the presence of antibacterial activity for each sample, as compared to the positive control.

Acute Toxicity Studies

Breeding of Animals (Albino Rats)

40 male albino rats (weighing between 150-190g.) are used for toxicological experiment. The rats are purchased from the National Veterinary Research Institute, Vom, Plateau State. The rats are maintained under standard laboratory conditions and are allowed free access to standard diet and water ad libitum. They are allowed to acclimatize for 24 hours.

The LD_{50} was to be carried out using the method of Medinat et al., (2018) with slight modification for oral routes in rats. The method consists of two phases using 24 rats. In the first phase 3 groups of 3 rats each were administered the extracts in doses of 100, 200, and 400 mg/kg body weight orally and were observed for signs of toxicity and death for 24hours. In the second phase, 3 groups each containing 1 rat were administered new doses of the extract: 100, 200 and 400 mg/kg body weight orally, they were also being observed for any sign of toxicity and death for 24 hours.

Statistical Analysis

All data were expressed as mean ± SEM and where applicable, the data were analysed statistically by Student's t-test using Graph Pad Instat software, version 2.05a. $P < 0.05$ was taken as indicative of significant difference.

RESULTS

Table 1: Effect of Newbouldia laevis stem-bark methanol crude extract (µg/mL) on Gram positive and Gram-negative bacteria in millimeter (mm)

Conc. (µg/mL)	Organism	Tetracycline (30 µg/mL)	Methanol
100µg/mL	*Escherichia coli*	21.22± 0.02	12.12± 1.13
	Staphylococcus aureus	21.43± 0.06	14.14± 0.16
	Klebsiella pneumonia	21.33± 0.03	13.12± 0.14
200µg/mL	*Escherichia coli*	21.41± 0.07	18.13±0.12
	Staphylococcus aureus	21.35± 0.05	20.11±0.11
	Klebsiella pneumoniae	21.52± 0.09	18.10± 0.5*
300µg/mL	*Escherichia coli*	21.31 ± 0.08	17.14± 0.11
	Staphylococcus aureus	21.33 ± 0.03	17.15 ± 0.13
	Klebsiella pneumoniae	21.37± 0.01	19.16± 0.12
400µg/mL	*Escherichia coli*	21.19± 0.12	14.13± 0.13
	Staphylococcus aureus	21.23± 0.08	18.14± 0.11
	Klebsiella pneumoniae	21.18± 0.09	20.15± 1.07

Result is Mean ± SD. N = 3

*= significant activity was observed when compared to the control ($p<0.05$). Concentration of standard is 30 µg/mL of tetracycline, Conc= Concentration

Table 2: Concentration of selected kidney function parameters of male albino rats.

Parameters	Group 1 (Normal control)	Group 2 (negative control)	Group 3 (positive control)	Group 4 (100 mg/kg extract)	Group 5 (200mg/kg extract)	Group 6 (400 mg/kg extract)
Urea (mg/dL)	42.96 ± 3.36^{a}	70.03 ± 2.42^{e}	56.62 ± 1.49^{d}	41.15 ± 2.57^{a}	50.75 ± 4.52^{c}	46.72 ± 1.45^{b}
Creatinine mg/dL)	1.35 ± 0.18^{a,b}	1.12 ± 0.07^{a}	1.50 ± 0.03^{b}	1.09 ± 0.04^{a}	1.14 ± 0.04^{a}	1.81 ± 0.17^{c}
Sodium (mmol/L)	10.67 ± 0.33^{a}	55.32 ± 2.91^{b}	121. 11 ± 6.23^{d}	64.92 ± 1.84^{c}	119.78 ± 4.45^{d}	127.06 ± 3.90^{d}
Potassium (mmol/L)	5.36 ± 0.30^{a}	7.87 ± 0.61^{d}	4.98 ± 0.23^{a}	6.43 ± 0.52^{c}	6.31 ± 0.21^{c}	6.32 ± 0.04^{c}
Chloride (mmol/L)	87.65 ± 5.07^{a}	126.76 ± 5.72^{c,d}	100.11 ± 1.80^{b}	116.90 ± 7.87^{c}	119.54 ± 2.17^{c}	108.03 ± 6.81^{b}
CO_2(mmol/L)	48.22 ± 1.17^{a}	61.46 ± 0.82^{c}	54.82 ± 1.45^{b}	53.65 ± 1.00^{b}	52.30 ± 1.49^{b}	58.45 ± 0.51^{b,c}

Results are expressed as mean ± standard deviation of group results obtained (n=5).

Means in the same row having different superscripts are statistically significant ($p<0.05$).

The result of kidney function indices showed that urea increased significantly ($p < 0.05$) in all the treatment groups, except in group 4 which showed no level of significance compared to normal control; creatinine increased significantly ($p < 0.05$) in group 6, while the rest of the treatment groups show no significant difference compared to normal control. Sodium increased significantly ($p < 0.05$) in all the treatment groups; potassium, chloride and carbon dioxide were lowered significantly in all the treatment groups but not close to normal control except in potassium group 3 which was lowered almost to the same level with normal control.

DISCUSSION

Result of screening plant extracts for antibacterial activity as shown in Table 4 showed that most organisms were sensitive to the extract. In the present study, different concentrations of Newbouldia stem-bark methanolic extracts 100, 200, 300 and 400 µg/mL were tested against three isolated organisms and were sensitive to inhibition at different concentrations.

The activity of Newbouldia stem-bark methanolic extract against selected bacterial was significant when compared to the test control at all the concentration tested, except at concentration 100 µg/mL where all the extracts E. Coli did not show significant activity. Higher growth inhibition rate was observed at 200, 300 and 400 µg/mL though significant inhibition was observed in all the test bacteria except where significant inhibition was not observed at 400 µg/mL. The maximum inhibition of the methanol stem extracts was against the isolated organisms, at 200, 300 and 400 µg/mL concentration. The extract at concentration of 200 µg/mL has inhibition of 18.13+0.12 mm and at 300 µg/mL has inhibition of 17.14+ 0.11 on E. coli respectively, while Staphylococcus aureus at 200 µg/mL of 20.11+0.11 mm, at 300 µg/mL of 17.15 + 0.13mm and at 400 µg/mL of 18.14+ 0.11mm; Klebsiella pneumoniae at 200 µg/mL of 18.10+ 0.5mm, at 300 µg/mL of 19.16+ 0.12 and at 400 µg/mL of 20.15+ 1.07 mm when compared to the test control. Weaker inhibition was observed at 100 µg/mL of E. coli of 12.12+ 1.13mm and at 400 µg/mL of 14.13+ 0.13mm; Staphylococcus aureus and Klebsiella pneumoniae at 100 µg/mL of 14.14+ 0.16 mm and 13.12+ 0.14 mm respectively.

The antibacterial test utilizing well diffusion assay showed that Gram-positive bacteria were more susceptible to the plant extract compared to Gram-negative bacteria. This is in agreement with some published results (Mahesh and Satish, 2008; Mavri et al., 2012).

Gram-negative bacteria are usually less sensitive to antibiotics compared to Gram-positive bacteria due to the intrinsic resistance and lack of penetration to antimicrobial compounds, such as daptomycin, tetracycline (Miquel et al., 2016; Kamf, 2019). It could be due to the outer membranous make-up of the Gram-negative bacteria (Mavri et al., 2012; Baek et al., 2015).

Some researchers detected the antimicrobial activity of C. tamala against a number of organisms (Hassan et al., 2016). They reported that extract of C. tamala possessed different degrees of antimicrobial activity against all tested Gram-positive and Gram-negative bacteria similar to our result where only S. aureus was found to be effective.

Results of kidney function parameters showed treatments with standard drug and all the doses of the extract were able to counteract the elevated effect of alloxan in serum urea level. Elevated levels of serum creatinine in group 6 may confer toxic effect on the kidney. Because the kidney is in charge of filtering urea and creatinine out of the blood, urea and creatinine are frequently employ as indicators of renal function (Iseghohi and Orhue, 2017). A high concentration of these metabolites in the serum is a sign of renal impairment. The increased in sodium concentration in all the treatment groups may result to hypernatremia. Hypernatremia can be caused by a variety of conditions, such as renal illness, inadequate hydration, and water loss via diarrhoea and/or vomiting (walker et al., 1990). The lowering effects of serum potassium, chloride and carbon dioxide in all the treatment group suggests that the extract was able to facilitate the excretion of these metabolites by the kidney, this may in turn increase kidney efficiency.

CONCLUSION

This study evaluated the antibacterial activity of methanol extract Newbouldia stem bark and its toxicity on kidney. The results obtained from this study showed that methanolic extract of Newbouldia stem bark is effective as an antibacterial with little or no effects on the kidney.

Recommendations

Research on effects of Newbouldia laevis stem bark on heart function parameters should be carried out, Isolation and characterization of active ingredient responsible for lowering

blood glucose and restoration of liver and kidney indices should be carried out and mechanism and mode of actions of the individual active component should be studied.

Conflict of Interest: Author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

REFERENCES

Ushie, O. A., Longbap, B. D., Iyen, S. I., Azuaga, T.I., Ushie, O. A., Longbap, B. D., Ugwuja,, D. I. and Uba, M. (2021). Preliminary Phytochemical Screening and Proximate Analyses of Leaf Extracts of Newbouldia laevis (Boundary Tree) | *Dutse Journal of Pure and Applied Sciences*. Vol. 7(No. 3b):190-7.

Dassekpo, S.I, Achigan-Dako, E.G., Honfo, H.S., Tovissode, F., Tente, B. and Houssou, C.S. (2017). Socio-cultural and religious approaches to the uses of Newbouldia laevis and associated plants in the traditions of Benin.;14.

Benlamdini, N., Elhafian, M., Rochdi, A. and Zidane L. (2014). Floristic and ethnobotanical study of the medicinal flora of the Eastern High Atlas (Haute Moulouya). *J Appl Biosci* 78:6771.

Arbonnier, M. (2004). Trees, shrubs and lianas of west African dry zones. Germany: CIRAD Press Ltd. 243 – 248pp.

Bafor, E. and Sanni, U. (2009). Uterine contractile effects of the aqueous and ethanol leaf extracts of *Newbouldia laevis (Bignoniaceae) in vitro. Indian Journal of Pharmaceutical Science*, 71(2): 124–127.

Hassan, S. W., Salawu, K., Ladan, M. J., Hassan, L G., Umar, R. A. and Fatihu, M. Y. (2010). Hepatoprotective, Antioxidant and phytochemical properties of leaf extracts of *Newbouldia laevis. International J. of Pharm. Tech. Research* 2(1); 573-584.

Tanko, Y., Okasha, M. A., Sale, M. I. A., Mohammed, A., Yerima, M., Yaro, A. H. and Isa, A. I. (2008). Antidiabetic effect of ethanolic flower extracts of *Newbouldia laevis* (Bignoniaceae) on blood glucose levels of streptozocin-induced diabetic wister rats. *Research Journal of Medical sciences* 2 (2); 62-65.

Iyamah, P.C. and Idu, M. (2015). Ethnomedicinal survey of plants used in the treatment of malaria in Southern Nigeria. *J Ethnopharmacol.* 173:287-302.

Yusuf, L.M., Ahmed, A., Lawal, U. and Abubakar, I.U. (2016). Ethnobotanical survey of indigenous plants used in the treatment of malaria in Dutsin-Ma metropolitan area of Katsina State. *Katsina J Nat Applied Sci.* 5(1):183-89.

Tor-anyiin, T.A., Sha'ato, R. and Oluma, H.O.A. (2003). Ethnobotanical Survey of anti-malarial medicinal plants amongst the Tiv People of Nigeria. *J Herbs, Spices Med Plants*. 10(3):61-74.

Adekunle, M.F. (2008). Indigenous uses of plant leaves to treat malaria fever at Omo Forest reserve (OFR) Ogun State, Nigeria. Ethiop *J Environ Stud Manag.* 1:31-35.

Nwauzoma, A.B. and Dappa, M.S. (2013). Ethnobotanical studies of Port Harcourt Metropolis, Nigeria. ISRN Bot. 2013:829424.

Appiah, K.S., Oppong, C.P., Mardani, H.K., Omari, R.A., Kpabitey, S., Amoatey, C.A., OnwonaAgyeman, S., Oikawa, Y., Katsura, K. and Fujii, Y. (2019). Medicinal plants used in the Ejisu-Juaben Municipality, Southern Ghana: An Ethnobotanical study. *Medicines*, 6: 1.

Mahesh, B. and Satish, S. (2008). Antimicrobial activity of some important medicinal plant against plant and human pathogens. World Journal of Agricultural Science,s 4: 839-843.

Umaru, I. J., Badruddin, F. A. and Umaru, H. A. (2018). Gastroprotective Effect of *Leptadeniahastata* (Pers.) Decne and Histopathological Changes on Indomethacin-Induced Gastric Ulcer in Rats. *Int. J. Pure App. Biosci*, *6*(5): 20-27.

Mavri, A., Abramovič, H., Polak, T., Bertoncelj, J., Jamnik, P., Smole Možina, S., and Jeršek, B.(2012). Chemical properties and antioxidant and antimicrobial activities of Slovenian propolis. *Chemistry and biodiversity*, *9*(8): 1545-1558.

Miquel, S., Lagrafeuille, R., Souweine, B., and Forestier, C. (2016). Anti-biofilm activity as ahealth issue. *Frontiers in microbiology*, *7*: 592.

Kampf, G. (2019). Adaptive bacterial response to low level chlorhexidine exposure and itsimplications for hand hygiene. *Microbial Cell*, *6*(7): 307.

Baek, K. H., Sharma, A., and Bajpai, V. K. (2015). Antibacterial mode of action of Ginkgo biloba leaf essential oil: Effect on morphology and membrane permeability. *Bangladesh Journal of Pharmacology*, *10*(2): 337-50.

Iseghohi, S. O., and Orhue, N. E. (2017). Ethanolic Extract of *Dennettia tripetala* Fruits Protects the Liver and Kidney of Rats from Damage Induced by a Single Exposure to CarbonTetrachloride. *International Journal of Pharmacology, Phytochemistry and Ethnomedicine,* 68-16.

Walker, H. K., Hall, W. D., and Hurst, J. W. (1990). Clinical Methods: The History, Physical, and Laboratory Examinations. 3rd edition. Boston: Butterworths; Available from: https://www.ncbi.nlm.nih.gov/books/NBK201/