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Antibacterial Effect of Methanol Extract of *Newbouldia laevis* Leaves on Some Selected Resistant Pathogen on Synthesised Drugs

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Abstract

Newbouldia laevis is a tropical plant belonging to the family of Bignoniaceae. It is among the most useful plants in Africa. Historically medicinal plants have been provided a good source of inspiration for novel therapeutic drugs which has made a large contribution to health and well-being of humans. It has been used over the years to as curative agents against many infections and have been exploited in the traditional medicine with their curative potentials. Material and methods: The leaves of Newbouldia laevis was bought from a local market in Wukari, Taraba State. The leaves of Newbouldia laevis was chop into pieces, air-dried for four days and then pulverized into fine powder. About 250 g of the powdered bark extracted with 2 L of ethanol using maceration method for 72 hrs. The crude extracts of Newbouldia laevis was used in antibacterial assay. The results observed demonstrate that the Newbouldia leaves methanol crude extract possesses concentration-dependent antibacterial activity against both Gram-positive resistant bacteria (Staphylococcus aureus) and Gram-negative

(Escherichia coli, Klebsiella pneumonia) bacteria. The increasing inhibition zones with higher concentrations suggest a potential dose-response relationship.

Keywords: Antibacterial, Methanol, Extract, Newbouldia Laevis, Leaves, Resistant Pathogen, Synthesised, Drugs

INTRODUCTION

Historically medicinal plants have been provided a good source of inspiration for novel therapeutic drugs which has made a large contribution to health and well-being of humans. It has been used over the years to as curative agents against many infections and have been exploited in the traditional medicine with their curative potentials well documented. Most plants are capable of synthesizing some chemicals compounds that's are able to perform physiological action in the body. These chemicals compounds are known as phytochemicals. Plant product was used by man for health care delivery for centuries. The use of plant extract as medicine is wide spread throughout the world. While, plant disease remedies are as old as human history, it was estimated that about 80% of useful bioactive plant derived pharmaceuticals are used around the world as was discovered by University academicians, researcher from the field of traditional herbal medicine (Diba *et al.,* 2013).

Newbouldia laevis is a tropical plant belonging to the family of Bignoniaceae. It is among the most useful plants in Africa and grows up to 10 m height with a cauliflorous habit (Akerele *et al.,* 2011).

Scientific reports on the phytochemical constituents of the plant revealed the presence of alkaloids and phenylpropanoids in the root (Germann et al., 2006), flavonoids, and tannins in the leaf (Usman and Osuji, 2007). Wound infection is detrimental to wound healing which is a complex process that can be delayed by many potential factors (Edwards and Harding, 2004). A variety of disorders commonly affect the eye and vary in severity from mild but annoying allergic conjunctivitis to sight threatening infections (Anton et al., 1996). As it has been asserted that medicinal plants constitute a continuous source of new compounds with the potential to act against multi-resistant bacteria (Al-Bayati et al., 2008).

Newbouldia laevis has been reported to have medicinal value ranging from anti-inflammatory, antioxidant, antimicrobial, anti-fungi, analgestic and wound healing properties (Agbafor *et*

al., 2015). 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 (Enye *et al.,* 2015). More, alkaloids, saponins, flavonoids, glycosides, tannins and cyanides are regularly reported to be present in *N. laevis* extracts (Burkill, 1997)

According to Fifa *et al.* (2014), phytochemical analysis of *N. laevis* leaf extracts revealed the presence of flavonoids, tannins, terpenoids, steroids, cardiac glycosides, alkaloids and saponins with the excemption of chloroform extract which only shows the presence of alkanoids, 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 Fatunla *et al.* (2018), on antibacterial effect of N. laevis leaf extract on vancomycin and methicillin resistant bacterial isolates and (Anaduaka *et al.,* 2013) on some important phytochemical of *N. laevis*. It was also similar to the report of Usman and Osuji (2007), on phytochemical and in vitro antimicrobial assay of the leaf extract of *N. laevis* and on involvement of tannins and flavonoids in the in-vitro effects of *N. laevis* (Azando *et al.,* 2011).

In a review work by Innocent *et al.* (2022), hlkaloids were the first class of compounds isolated from the root bark of *N. Iaevis*. Pyrazole alkaloids (withasomnine (1), 4'-hydroxywithasomnine (2), 4'-methoxywithasomnine (3) newbouldine (4), 4'-hydroxynewbouldine (5), and 4methoxynewbouldine (6)) were isolated from the plant.40,43 Also, three phenylpropanoid glycosides (verbascoside (7), martynoside (8) and newbouldioside) were isolated from the roots using a combination of chromatographic and spectroscopic methods.41 Further spectroscopic analysis of newbouldioside revealed that the compound was actually three related compounds named as newbouldiosides A-C (9-11) (Dermane *et al.,* 2020).

Also, isolated from the root bark includes naphthoquinone-anthraquinone (newbouldiaquinone (12), 2-acetylfuro-1,4-naphthoquinone (13), 2-methyl-9,10-anthracenedione (14), canthic acid (15) and lapachol (16).35 Purification of the methanol root extract led to the isolation of chrysoeriol (17) a flavonoid, quinones (newbouldiaquinone (12), 2-acetylfuro-1,4naphathoquinone (13), 2-hydroxy-3-methoxy-9,10-dioxo-9,10-dihydroanthracene-1- carbaldehyde (18) and lapachol), sterol (β-sitosterol-3-O-β-D-glucopyranoside (19), triterpenes (oleanolic acid (20) and canthic acid), ceramide

(newbouldiamide (21) and one phenolic derivative (2-(4-hydroxyphenyl)-ethyltriacontanoate (22) (Kolodziej, 2021).

Recently, a German researcher isolated three new phenylethanoid glycosides identified as newbouldiosides D-F from the stem bark (Gormann *et al.,* 2003). Natural compounds belonging to different classes of phytochemical constituents isolated from the stem bark of this plant include furanonaphthoquinones like 5-hydroxy-dehydroiso-α-lapachone (26), 2-acetyl-5-hydroxynaphtho[2,3-β]furan-4,9-dione (27), 2-isopropenylnaphtho[2,3-β]furan-4,9- dione (28), 2(1'-methylethenyl)-5-hydroxynaphtho[2,3-β] furan-4,9-dione (29), 2-(1'-methylethenyl)-7hydroxynaphtho[2,3-β] furan-4,9-dione (30), β-resorcylic acid (31), atraric acid (32); benzofuran like 2-(1'methylethenyl)-6- hydroxy-2,3-dihydrobenzo[β]furan (33), 2,3-dimethoxy-1,4-benzoquinone (34) and 2-(4-hydroxyphenyl) ethyl triacontanoate (22) (Kuete *et al.,* 2007).

Withasomnine (1), 4'-hydroxywithasomnine (2), 4'-methoxywithasomnine (3), newbouldine (4), 4'-hydroxynewbouldine (5), 4-methoxynewbouldine (6), verbascoside (7), martynoside (8), newbouldiosides A-C (9-11),newbouldiaquinone (12). Source: Innocent *et al.* (2021), 2-acetylfuro-1,4-naphthoquinone (13), 2-methyl-9,10-anthracenedione (14), canthic acid (15), lapachol (16), chrysoeriol (17), 2-hydroxy-3-methoxy-9,10-dioxo-9,10-dihydroanthracene-1carbaldehyde (18), sterol (β-sitosterol-3-O-β-D-glucopyranoside (19), oleanolic acid (20), newbouldiamide (21), 2-(4-hydroxyphenyl)-ethyltriacontanoate (22). Source: Innocent *et al.* (2021)

Newbouldiosides D-F (23-25), 5-hydroxy-dehydroiso-α-lapachone (26), 2-acetyl-5hydroxynaphtho[2,3-β]furan-4,9-dione (27), 2-isopropenylnaphtho[2,3-β]furan-4,9-dione (28), 2(1'-methylethenyl)-5-hydroxynaphtho[2,3-β]furan-4,9-dione (29), 2-(1'-methylethenyl)-7hydroxy-naphtho[2,3-β]furan-4,9-dione (30), β-resorcylic acid (31), atraric acid (32), 2-(1'methylethenyl)-6-hydroxy-2,3-dihydrobenzo[β]furan (33), 2,3-dimethoxy-1,4-benzoquinone (34).

The plant was reported to be;

Hepatoprotective activity.

In an in vivo study, aqueous-ethanol extract of N. laevis leaves was reported to exert good protection against a hepatotoxin, CCl4 that liver microsomal enzymes convert to its radical metabolites that alkylates cellular macromolecules and provoke peroxidation of polyunsaturated fatty acids in the membrane of the hepatocytes, causing liver damage

(Hassan et al., 2010). The pretreatment with extract (100, 200, and 300 mg/kg) prevented hepatic necrosis and steatosis, lipid peroxidation, oxidative stress/ reduction in antioxidant status and dyslipidemia associated with CCl4 intoxication comparable with silymarin (100 mg/kg). Similarly, treatment with aqueous extract of N. laevis leaves (200 mg/kg) for 30 days showed attenuation of the histological characteristics associated with cadmiummediated testicular and hepatic toxicities, both in the preventive and curative intervention models (Enye et al., 2015) However, the molecular mechanism of the tissue protection was not clearly defined by the above studies. Also, the study designs of the above study, except Enye et al. (2015), did not add any reference drug. The inclusion of a study group that will be pretreated with a known hepatoprotective drug that has antioxidant and renoprotective properties such as silymarin will have made these studies complete (Innocent et al., 2021).

Anti-ulcer activity

According to Innocent et al. (2021), aqueous-ethanol extract of N. laevis bark and its n-hexane, ethyl acetate, and aqueous fractions were examined against ethanol-induced acute stomach ulceration. It was reported that pretreatment of rats with the extract and its solvent fractions (100 mg/kg) significantly inhibited ulceration by 78, 80, 73, and 70%, respectively by extract, aqueous fraction, ethyl acetate fraction and n-hexane fraction relative to 78% inhibition by cimetidine (100 mg/kg), a positive control that act by antagonizing H2 receptors (Itez et al., 2020). This observation infers that the plant is a potential source of anti-ulcer and gastroprotective compounds; hence, further investigations are needed to exploit, isolate, and characterize these compounds.

Antidiabetic activity

In Mexico, N. laevis is well-known in folkloric for its use in controlling hyperglycemia associated with diabetes (Andrede-Cetto et al., 2005). This inspired Bosha et al. (2019) to examine the effect of methanol extract of the leaves on experimental diabetic rats induced by intraperitoneal injection of 150 mg/ kg alloxan monohydrate. It was reported that after 24 h of administration, the extract (250 mg/kg) suppressed fasting blood glucose level by 60.2% compared to 51.5% by glibenclamide (2 mg/kg).

To investigate the possible mechanism of hypoglycemic properties of the plant´s leaves, Kolawole et al. (Kolawole et al., 2013) observed that ethanol leaves extract significantly reduced postprandial glucose levels and inhibited pancreatic α-amylase activity in diabetic

rats at 500 mg/kg (IC50 = 58.7 µg/mL) relative to to an IC50 value of 92.3 µg/mL by acarbose (50 mg/kg).

The authors further assessed the inhibitory effects of the extract on baker's yeast and rat intestinal α-glucosidases and rat pancreatic α-amylase, in vitro. The extract was reported to significantly inhibit both baker's yeast and rat intestinal α-glucosidases with IC50 values of 2.2 µg/mL and 43.5 µg/ mL, respectively compared to 3.8 µg/mL and 62.7 µg/mL, respectively by acarbose. These results showed that the extract acts by inhibiting the two enzymes that play the key roles in increasing blood glucose level. Based on the reported antidiabetic activities of the plant leaves,

Mbagwu et al. (2020) evaluated the inhibitory effects of ethanol extract of the leaves on α-amylase activity and reported that the extract potently inhibited the enzyme with IC50 value of 102.91 mg/mL. Apigenin isolated from the methanol fraction of the leaves dichloromethanol-methanol extract exhibited antidiabetic property (Osigwe et al., 2107).

Treatment of adrenaline-generated hyperglycemic rats with apigenin (25 and 50 mg/kg) dosedependently suppressed the glucose level more than glibenclamide (5 mg/kg) (Innocent et al., 2021). In addition, apigenin (50 mg/kg) returned the glucose level to normal within 2 h compared to 4 h by glibenclamide. Flavonoids such as rutin and apigenin have been demonstrated to protect rats' pancreatic β-cells from diabetogenics like streptozotocin in addition to their inhibition of hyperglycemia provoked by this pancreatic toxicant, (Rauter et al., 2010) partly through antioxidant mechanisms. Two caffeic acid glycosides, newbouldasides A and B isolated from the plant strongly inhibited α-amylase activity with IC50 values of 4.95 and 4.44 mg/ mL, respectively relative to reference drug, acarbose (IC50 = 4.05 mg/mL) (Murtala et al., 2019). This result supports the earlier reports that inhibition of α-amylase activity is one of the mechanisms through which the extract exhibits its hypoglycemic effect and further adds that newbouldiosides A and B are among the compounds in the plant leaves responsible for its antidiabetic property. Other compounds isolated from this plant with reported antidiabetic activity include 9-(4-nonyl-phenyl) non-8-enoic acid, β-sitosterol and β-sitosterol glucopyranoside (Bosha et al., 2015).

METARIALS AND METHODS

Sample Collection

The leaves of *Newbouldia laevis* was bought from a local market in Wukari, Taraba State.

Extract preparation

The leaves of Newbouldia laevis was chop into pieces, air-dried for four days and then pulverized into fine powder. About 250 g of the powdered bark extracted with 2 L of ethanol using maceration method for 72 hrs. The methanol was concentrated in a rotary evaporator after filtration. The extract obtained was kept in an air-tight container in the refrigerator till required. This gave the ethanol extract.

Fractionation of crude methanol extract of*. N. laevis* stem bark

50 ml of crude methanol extract was measured using a measuring cylinder into a separating funnel; the methanol fraction settled at the bottom of the separating funnel. The methanol fraction was collected into a beaker and was concentrated using rotary evaporator under reduced pressure and concentrates transferred into air-tight container and preserved in the refrigerator at 4ºC prior to administration.

Antibacterial

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 keep 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 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.

RESULTS AND DISCUSSION

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

Conc. (µg/mL)	Organism	Tetracycline (30 µg/mL)	Methanol
100 µg/mL	*Escherichia coli*	21.33±0.11	15.13 ± 1.17
	Staphylococcus aureus	21.13±0.12	18.19 ± 0.18
	Klebsiella pneumonia	21.14±0.13	18.25 ± 0.18
200 µg/mL	*Escherichia coli*	21.22±0.26	20.34 ± 0.19
	Staphylococcus aureus	21.19±0.15	19.56 ± 0.16
	Klebsiella pneumonia	21.23±0.11	20.44 ± 0.15
300 µg/mL	*Escherichia coli*	21.24±0.21	19.23 ± 0.16
	Staphylococcus aureus	21.31±0.23	20.19 ± 0.18
	Klebsiella pneumonia	21.33±0.18	20.45 ± 0.19
400 µg/mL	*Escherichia coli*	21.20±0.16	19.78± 0.13
	Staphylococcus aureus	21.19±0.17	20.18± 0.16
	Klebsiella pneumonia	21.18± 0.25	**20.67± 1.34**

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

The results as displayed in table 1, demonstrate that the Newbouldia leaves methanol crude extract possesses concentration-dependent antibacterial activity against both Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli, Klebsiella pneumonia) bacteria. The increasing inhibition zones with higher concentrations suggest a potential dose-response relationship. The observed activity is significant compared to the control (tetracycline), emphasizing the potential of the extract as a source of antibacterial compounds.

From the result it was observed that the extract showed higher inhibition at concentration 400µg/mL with 20.67+ 1.34mm on *Klebsiella pneumonia* when compared with the

control of 21.18+ 0.25mm. while lower inhibition on the resistant bacterial was observed at concentration of 15.13 + 1.17mm on *Escherichia coli* when compared to the control of 21.33+0.11mm.

Report by Fifa *et al.* (2014), Innocent et al. (2022), Dermane et al., (2020), Kolodziej, (2021), Kuete et al., (2007), revealed that the presence of this phytochemicals especially the tannates reduce the mucosal motility and make the intestinal mucosa more resistant. This suggests that tannins present in the aqueous leaves extract of Newbouldia laevis Leaves (Nugroho et al., 2019) mediated the antidiarrhoeal activity of the aqueous leaves extract of the plant.

CONCLUSION

These results indicate that the aqueous leaves extract of *Newbouldia laevis* has a significant dose-dependent antibacterial activity. The reduction in the resistant bacterial transit prompted by the extract provides a rationale for the usage of the Leaves in the management of resistant bacteria as it inhibits the growth of this pathogens.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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