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Vol. 9(21), pp. 553-560, 8 June, 2015
DOI: 10.5897/AJPP2015. 4331
Article Number: 3D568F953902
ISSN 1996-0816

http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Improved gastric lesion of ulcerogenic mice treated with bark extracts and fractions of *Newbouldia laevis*

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Received 1 April, 2015; Accepted 8 May, 2015

Newbouldia laevis **is used in Nigerian traditional medicine for the treatment of various diseases including gastric ulcer. The present study was undertaken to validate the anti-ulcer potential of *N. laevis* stem bark. The plant was extracted using ethanol and fractionated using N-hexane, ethyl acetate and water. Phytochemical evaluation of the extract and fractions of the plant was done using standard procedures. The anti-ulcer property of the crude extract and fractions of the plant was investigated against ethanol induced gastric ulcer in white albino rats using Cimetidine (100 mg/kg) as the standard control. Primary screening of the crude ethanol extract of *N. laevis* stem bark against *Salmonella typhi, Staphylococcus aureus, Streptococcus pneumoniae, Bacillus subtilis, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa* was determined using the agar well diffusion method. Also, the minimum inhibitory concentrations (MICs) of the extract on the test organisms were determined using the agar dilution method. The result showed that the crude extract and fractions of the plant significantly ($P < 0.001$) produced protection in the ethanol- induced ulceration and reduced the ulcer index when compared to the control. Preliminary phytochemical screening of the crude extract and fractions revealed the presence of steroid, glycosides, flavonoids, saponins terpenoids, and tannins while alkaloids were absent. At the concentrations analyzed (15.625 to 500 mg/ml), the inhibition zone diameters (IZDs) produced by the crude ethanol extract against test isolates ranged from 0 to 14 mm. Also, the MICs of the plant extracts on test organisms ranged from 6.25 to 25 mg/ml. The extract recorded best antibacterial activity against *K. pneumoniae* followed by *S. typhi.* In conclusion, the present study provided preliminary data that the extract and fractions of *N. laevis* possesses significant anti-ulcer activity in animal models. The gastric anti-secretory and acid neutralizing effect of the plant plus its antibacterial activity reveals the anti-ulcer potential of the plant.**

Key words: *Newbouldia laevis*, anti-ulcer, stem bark, phytochemical, polar fractions.

INTRODUCTION

An ulcer is basically an inflamed break in the skin or mucus membrane lining the alimentary tract (Ukwuani et al., 2012). Ulceration occurs when there is a disturbance of the normal equilibrium caused by either enhanced aggression or diminished mucosal resistance (Sravani et al., 2011). About 9 out of 10 peptic ulcer cases are

duodenal but, gastric ulcers are less common (Gadekar et al., 2010). The gastric mucosa is continuously exposed to potentially injurious agents such as acids, food ingredients, bacterial products (*Helicobacter pylori*) and drugs pepsin, bile acids, (Grossman, 2009). These agents have been implicated in the pathogenesis of gastric ulcer, including enhanced gastric acid and pepsin secretion, inhibition of prostaglandin synthesis and cell proliferation growth, diminished gastric blood flow and gastric motility (Grossman, 2009). Symptoms of ulcer include epigastric pain, nausea, vomiting, belching and bloating. Complications of protracted untreated cases include weight loss attributed to a reduced appetite caused by fear of pain, anemia caused by gastro-intestinal blood loss, vomiting associated with a gastric ulcer or pyloric stenosis and mucosal perforation (Hunt et al., 2006). Current management of peptic ulcer disease involves the use of an antibiotic, metronidazole and proton pump inhibitor (PPI), (triple therapy) (Malfertheiner, 2002). Due to undesirable side effects and the high cost of conventional anti-ulcer drugs, there is, therefore, the need to develop safe, effective and affordable alternatives in the symptomatic management of peptic ulcer disease (Ukwuani et al., 2012).

Newbouldia laevis belongs to the family Bignoniaceae, the tree is commonly known as smooth *Newbouldia* or boundary tree. It is called 'Ogirisi in Igbo; 'Aduruku' in Hausa; Ikhimi' in Edo and 'Akoko' in Yoruba language (Hutchinson and Dalziel, 1963). It is a medium sized angiosperm which grows to a height of about 7 to 8 (up to 15 m), more, usually a shrub of 2 to 3 m, many-stemmed forming dumps of gnarled branches (Arbonnier, 2004). *N. laevis* is widely used in African folk medicine for the treatment of malaria and fever, stomach ache, coughs, sexually transmitted disease, tooth ache, breast cancer, and constipation (Iwu, 2000; Gorman et al., 2003; Eyong et al., 2005). In south eastern and part of the Midwestern Nigeria, the plant is used for the treatment of septic wounds, the young leaves are crushed in little places in water and the extract is put into the eye to cure eye inflammation and redness, it is also administered to stop vaginal bleeding in threatened abortion (Gill, 1992; Kargbo, 1982). The root and leaves are used for the treatment of round worms, elephantiasis, dysentery, migraines and convulsions (Lewis and Manony, 1977; Akunyili, 2000). About ten compounds have been isolated from the root bark of Newbouldia Leavis (Kuete et al., 2007). The phytochemical screening of the leaves and bark of congolies Newbouldia leavis revealed the absence of flavonoids, quinines, saponins, terpenes or steroids (Oliver- Bever, 1956). White alkaloids have been reported in *N. leavis* (Vollekova et al., 2001). The phytochemical screening of the crude methanol leaf extract of *N. laevis* in Nigeria revealed the presence of flavonoids, tannins, terpenes, steroidal and cardiac glycoside, but alkaloid and saponins were found to be absent (Usman and Osuji, 2007).

MATERIALS AND METHODS

Plant sample collection and preparation

Stem barks of *N. laevis* were collected from Agulu, Aniocha local government area of Anambra State, Nigeria in the month of April, 2014. The plant was identified at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria, where the specimen with the voucher specimen number (PCG/474/A/035) was deposited. The stem barks of *N. laevis* were cut into pieces and air-dried at room temperature for one month and homogenized into coarse powder using a blender.

Extraction and sequential fractionation

The coarse powdered material (400 g) was extracted using 75% ethanol in water for 24 h. Thereafter, it was filtered with muslin cloth and re-filtered using Whatman filter paper No.1. The filtrate was concentrated using rotary evaporator at 50°C, and the concentrate was then freeze-dried to give 25.57 g (6.39%) yield. Sequential fractionation of the crude extract with solvents of increasing polarity (n-hexane, and ethyl acetate) using separating funnel was carried out. The crude extract was exhaustively extracted with n-hexane, evaporated at room temperature to yield 2.01 g (0.50%). The remaining extracts which is insoluble in n-hexane was exhaustively extracted with ethyl acetate, evaporated at room temperature to yield 10.44 g (2.61%). The residue which was the aqueous fraction was evaporated at 50°C with 13.12 g (3.28%) yield.

Culture media, chemicals and reagents

Nutrient agar, Nutrient broth, Mueller Hinton agar (Oxoid Limited, England). Dimethyl sulfoxide (DMSO), MacFarland turbidity Standard (prepared from barium chloride, sulfuric acid and distilled water), Ethanol, Ethylacetate and N-hexane (Sigma Aldrich Germany). Six (6) strains of both Gram-negative and Gram-positive bacteria (*Salmonella typhi, Staphylococcus aureus, Streptococcus pneumoniae, Bacillus subtilis, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa)* were used in this study. These isolates were obtained from the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka-Nigeria.

Test organisms

Experimental animals

Albino Wistar rats (117 to 163 g) of either sex were obtained from the Animal House, Department of Veterinary Medicine, University of Nigerian Nsukka. All animals were maintained under standard laboratory conditions in an animal house, fed with commercial pellet

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diet and water *ad libitum*. All animals were acclimatized to the laboratory before the commencement of experiment.

Preliminary phytochemical screening

Phytochemical analysis was performed using standard procedures to identify chemical constituents as described by Trease and Evans (1989), Sofowora (1993) and Harborne (1973).

Antimicrobial assay

Preparation of stock solutions

For the primary anti-bacterial screening of the crude ethanol extract of *N. laevis,* stock solution of the plant extract was prepared by dissolving 1000 mg of the extract in 2 ml of DMSO to obtain a final concentration of 500 mg/ml. For determining the minimum inhibitory concentrations (MICs) of the crude ethanol extract of *N. laevis* against test isolates, stock solutions of the plant extracts were prepared by dissolving 2000 mg of the extract in 2 ml of DMSO to attain a final concentration of 1000 mg/ml. These were transferred to a screw capped bottle and stored at 4°C.

Primary screening of the crude ethanol extract of n. laevis for antibacterial activity

The anti-bacterial activity of the crude ethanol extract of *N. laevis* was determined by the agar well diffusion method. Dilutions of 250, 125, 62.5, 31.25, and 15.625 mg/ml were prepared from the 500 mg/ml stock solution of the plant extract in a 2-fold dilution process. Twenty (20) ml of molten Mueller Hinton Agar (MHA) was poured into sterile Petri dishes (90 mm) and allowed to set. Standardized concentrations (McFarland 0.5) of overnight cultures of test isolates were swabbed aseptically on the agar plates and holes (6 mm) were made in the agar plates using a sterile metal cork-borer. Twenty (20 µl) of the various dilutions of the plant extract and control were put in each hole under aseptic condition, kept at room temperature for 1 h to allow the agents to diffuse into the agar medium and incubated accordingly. Ciprofloxacin (5 µg/ml) was used as the positive control, while DMSO was used as the negative control. The MHA plates were then incubated at 37°C for 24 h. The inhibition zones diameters (IZDs) were measured and recorded. The size of the cork borer (6mm) was deducted from the values recorded for the IZDs to get the actual diameter. This procedure was conducted in duplicate and the mean IZDs calculated and recorded.

Determination of the minimum inhibitory concentrations (mics) of the crude ethanol extract of N. laevis on test isolates

Minimum inhibitory concentrations (MICs) of the crude ethanol extract of *N. laevis*, which is defined as the lowest concentration of the antimicrobial agent that inhibits the bacterial growth, was determined against the test isolates using the agar dilution method. The stock solution of the extract (1000 mg/ml) was further diluted in a 2-fold serial dilution to obtain the following concentrations: 500, 250, 125, 62.5, and 31.25 mg/ml. Agar plates were prepared by pouring 9 ml of molten double strength MHA into sterile Petri plates containing 1ml of the various dilutions of the extract making the final plate concentrations to become 100, 50, 25, 12.5, 6.25, and 3.125 mg/ml. The test isolates which were grown overnight in broth were adjusted to McFarland 0.5 standard and streaked onto the surface of the agar plates containing dilutions of the extract. The MHA plates were then incubated at 37°C for 24 h after which all plates were observed for growth. The minimum dilution (concentration) of the extracts completely inhibiting the growth of each organism was taken as the MIC. This procedure was conducted in duplicate.

Antiulcer activity

The antiulcer activity of *N. laevis* crude and fractions were evaluated using ethanol induced ulceration model (Garg et al., 1993). The rats were fasted for 48 h but allowed free access to water *ad libitum*. They were randomly selected and divided into six groups of five rats each. Group I (control) received water 10 (ml/kg) body weight while group 2 received the standard drug (Cimetidine 100 (mg/kg p.o.). Groups 3, 4, 5 and 6 received 100 mg/kg of the crude extract, aqueous fraction, ethyl acetate fraction, and n-hexane fraction of *N. laevis* respectively. Thirty minutes later, ulceration was induced by gastric instillation of 1 (ml) of 99% absolute ethanol and 1 h after ethanol administration, rats were sacrificed by cervical displacement and the stomach was removed, opened along the greater curvature to examine any ulcerative lesions (elongated black-red lines parallel to the long axis of the stomach). The number, length and severity of ulcers were noted and scored on an arbitrary 0 to 3 point scale (Kodati et al., 2010). The scores were as follows:

0 = Normal colored stomach.
0.5 = Red coloration.
1 = Spot ulcers.
1.5 = Hemorrhagic streak.
2 = Ulcers.
3 = Perforation.

Mean ulcer score for each animal was expressed as ulcer index. The percentage of ulcer inhibition was determined as follows:

$$\% \text{ Inhibition of Ulcer Index} = \frac{\text{Mean ulcer index (control group)} - \text{Mean ulcer index (test group)}}{\text{Mean ulcer index (control group)}} \times 100$$

High performance liquid chromatography

Analytical HPLC was used to identify the distribution of the constituents (as peaks) from the two most active fractions as well as to evaluate the purity of the fractions. The solvent gradient used started with methanol: Nanopure water (10:90) to 100% methanol in 60 min. All peaks were detected by UV-VIS Photodiode array detector.

Statistical analysis

The data were expressed as mean ± SEM while ulcer inhibition was expressed as a percentage. The significance of the difference among the groups was assessed using one way analysis of variance Student t- tests. P values less than 0.01 were considered significance.

Table 1. Phytochemical analysis of extract/fractions of *N. laevis* Stem Bark

Extract/Fraction	Alkaloids	Saponins	Flavonoids	Tannins	Steroids	Terpenoids	Glycosides
Ethanol Extract	-	++	+	++	++	++	+++
Aqueous Fraction	-	+++	+	++	-	-	+++
Ethyl Acetate Fraction	-	+++	+	+	+	-	+++
N-Hexane Fraction	-	-	-	-	++	++	-

+ = slightly present, ++ = moderately present, +++ = highly present, - = Not present.

Table 2. Effect of *N. laevis* extract and fractions on ethanol induced gastric ulceration in white albino rats

Group	Treatments	Dose (mg/kg)	Ulcer Index	% Ulcer inhibition
1	Ethanol (control)	1 ml	4.10 ± 0.40	-
2	Cimetidine (standard drug)	100	0.90 ± 0.24**	78.04
3	Crude extract	100	0.90 ± 0.29**	78.04
4	Aqueous fraction	100	0.80 ± 0.33**	80.48
5	Ethyl acetate fraction	100	1.10 ± 0.24**	73.17
6	n- hexane fraction	100	1.20 ± 0.20**	70.73

Values are expressed as mean ± SEM. ** $p < 0.01$ significantly different when compared to the control.

RESULTS AND DISCUSSION

Preliminary qualitative phytochemical analysis of crude ethanol extract and fractions of *Newbouldia laevis* stem bark revealed the presence of tannins, saponins, terpenoids, glycosides, flavonoids and steroids while alkaloids where absent (Table 1). Active constituents such as flavonoids, tannins and terpenoids have been reported to possess antiulcer property (Sravani et al., 2011). Tannins are known to precipitate protein and 'tar' the outer most layer of the gastric mucosa rendering it less permeable and more resistant to chemical and mechanical injury or irritant (Asuzu and Onu, 1990). Flavonoids are polyphenolic compounds with known antioxidant properties in addition to strengthening the mucosal defense system through stimulation of gastric mucus secretion (Martin et al., 1994). The pathogenesis of ulcer remains unclear but its cause is known to be aggravated by an imbalance between the factors that maintain the mucosal integrity (i.e. mucus, bicarbonate and prostaglandin) and the aggressive factors (i.e. acids, pepsin and *Helicobacter pylori*) (Venkateswarlu et al., 2011). Ethanol induced damage to gastric mucosa is caused by the direct toxic effect of ethanol through reduction in mucus production, gastric mucosal blood flow, bicarbonate secretion, prostaglandin and the release of histamine, influx of calcium, generation of free radicals and leukotriene production (Glavin and Szabo, 1992).

In the present study, *Newbouldia laevis* stem bark was found to possess a remarkable and significant ($P < 0.01$) ulcer inhibition properties in all the fraction treated groups compared to the control (Table 2). Values are expressed as mean ± SEM. ** $p < 0.01$ significantly different when compared to the control. This suggest that the observed antiulcer activity is due to the ability of *Newbouldia laevis* to antagonize these aggressive factors while enhancing the defensive mucosal factors that protect the gastric mucosa from injury (Germano et al.,1998) . Cimetidine, the standard drug produced 78.04 % inhibition of ulcer, the crude extract produced 78.04 % inhibition same as the standard drug. Aqueous fraction produced a more significant inhibition 80.48 % when compared to the standard drug, while ethyl acetate and n-hexane produced 73.17 % and 70.73 % respectively. Cimetidine belongs to the class of H2-receptors antagonists commonly used in the treatment of peptic ulcer and gastro esophageal reflux disease (Ukwuani et al., 2012).

N. laevis which has been reported to possess some antimicrobial properties (Kuete et al., 2007; Usman and Osuji, 2007; Akerele et al., 2011; Odunbaku and Amusa, 2012), showed antibacterial activity against the test organisms used in this study. The results of the antimicrobial screening of the ethanol extract of *N. laevis* stem bark on the test organisms (Figure 1) revealed that at the concentrations analyzed (15.625 to 500 mg/ml), the plant showed good antimicrobial activity against *S. typhi, S. aureus, S. pneumoniae, B. subtilis, K. pneumoniae,* and *P. aeruginosa* with inhibition zone diameters (IZDs) produced against test isolates ranging from 0 to 14 mm. Best antibacterial activity was recorded against *K. pneumoniae* followed by *S. typhi.* Also, the minimum inhibitory concentrations (MICs) of the plant extracts on the test isolates were determined and the results are shown in Table 3. At the concentrations analyzed, the MICs of the plant extracts on test

Table 3. Minimum inhibitory concentrations (MICS) of the crude ethanol extract of *N. laevis* on test organisms

Test Organisms	MICs (mg/ml)
S. typhi	25
S. aureus	12.5
S. pneumoniae	25
B. subtilis	25
K. pneumoniae	6.25
P. aeruginosa	25

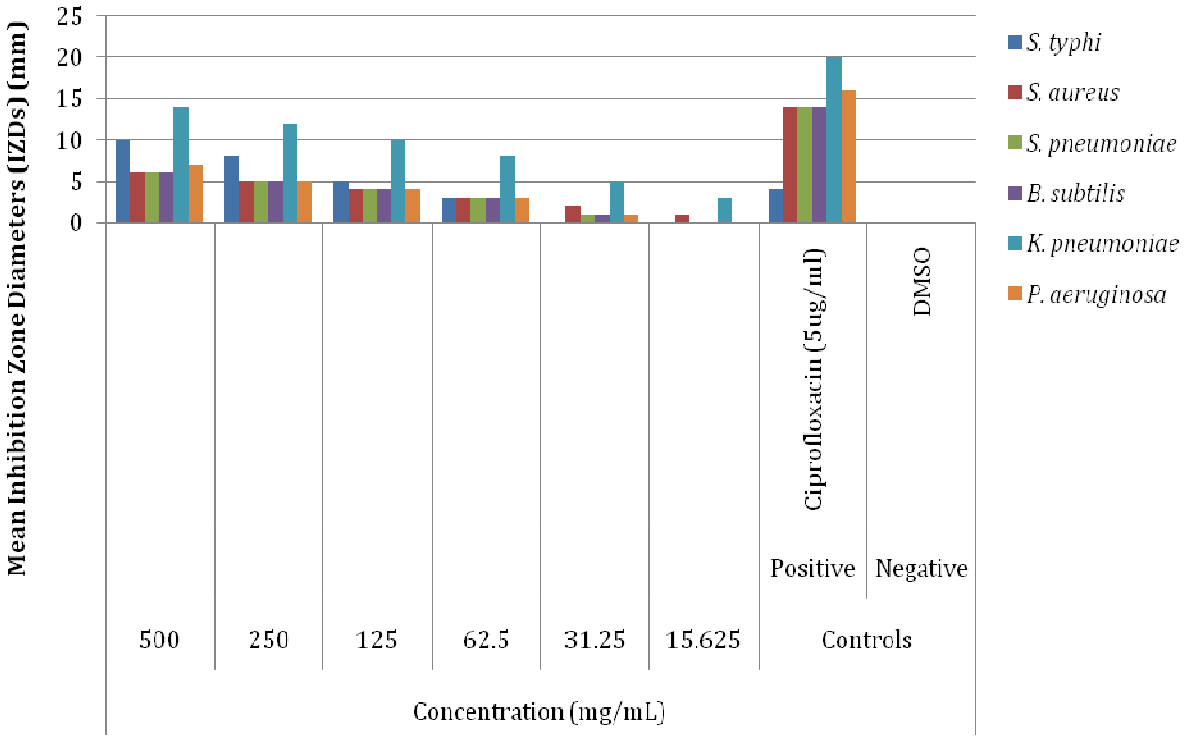

Figure 1. Mean Inhibition Zone Diameters (mm) Produced by the Crude Ethanol Extract of *N. laevis* on Test Isolates.

organisms ranged from 6.25 to 25 mg/ml. The result of the antimicrobial assay of the ethanol extract of *N. laevis* stem bark (Figure 1 and Table 3) confirms the antibacterial property of the plant. The antibacterial activity recorded by the plant may correlate with its anti-ulcer activity as several plants with antimicrobial properties have been reported to possess antiulcer activities for example, *Urtica dioica* (Gulcin et al., 2004); *Carlina acanthifolia* (Dordevic et al., 2007); *Murraya koenigii* (Harish et al., 2012)*; Allophylus serratus, Aloe vera, Glycyrrhiza glabra, Mangifera indica, Ocimum sanctum, Polyalthia longifolia, Zingiber officinalis* (Srinivas et al., 2013), etc. It can however imply that the antibacterial property of this plant may contribute to its anti-ulcer activities as the plant may play a role against *Helicobacter pylori* which is responsible for most duodenal and gastric ulcers (CDC, 1998). The antibacterial activity of the crude ethanol extract of *N. laevis* was observed at concentrations of 15.625 to 500 mg/ml. Antibacterial activity was recorded against all test isolates at concentrations of 31.25 to 500 mg/ml. At 15.625, the extract showed antibacterial activity only against *S. aureus* and *K. pneumoniae*. At the concentrations analyzed, IZDs produced by the extract against the test isolates ranged from 0-14mm with best activity recorded against *K. pneumoniae*, followed by *S. typhi.* Comparison of the HPLC chromatograms of the aqueous fraction and the ethanolic extracts showed that the main constituent of both fractions have the same chemical pattern. This suggests that these non-lipophilic constituents representing the major peaks could have been responsible for the higher anti-ulcer potentials of both extracts from more polar solvents (Figures 2 and 3). Peaks 12, 16, 20, 22 in ethanolic fraction has the same

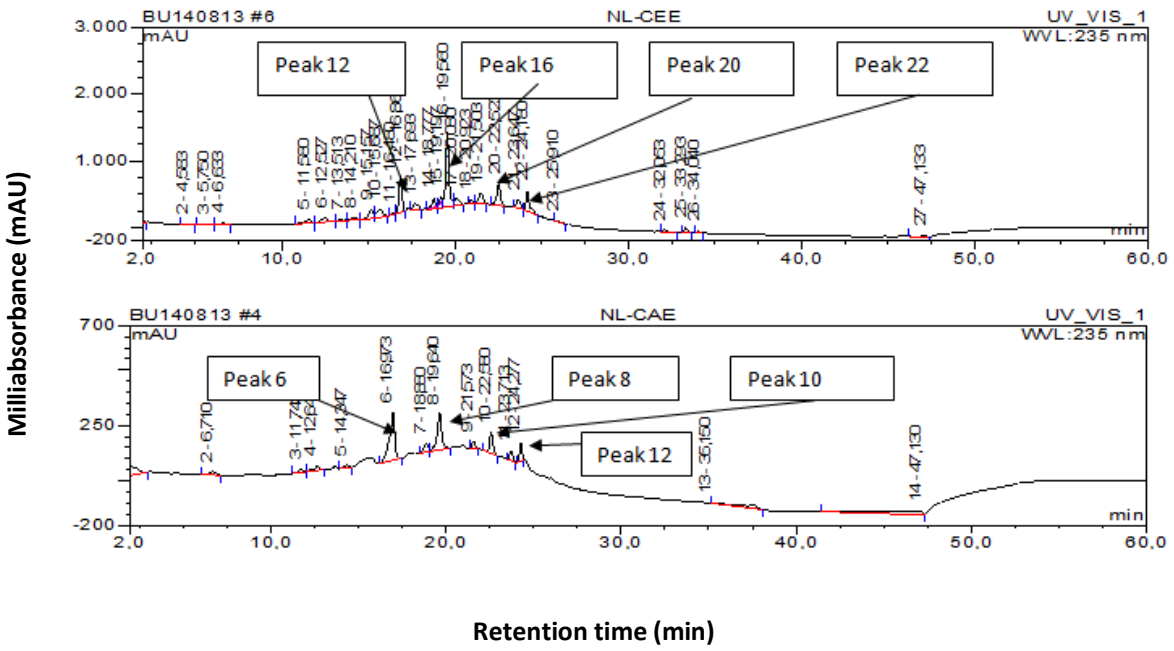

Figure 2. HPLC chromatograms of NL-CEE-Ethanolic extract and NL-CAE-Aqueous fraction.

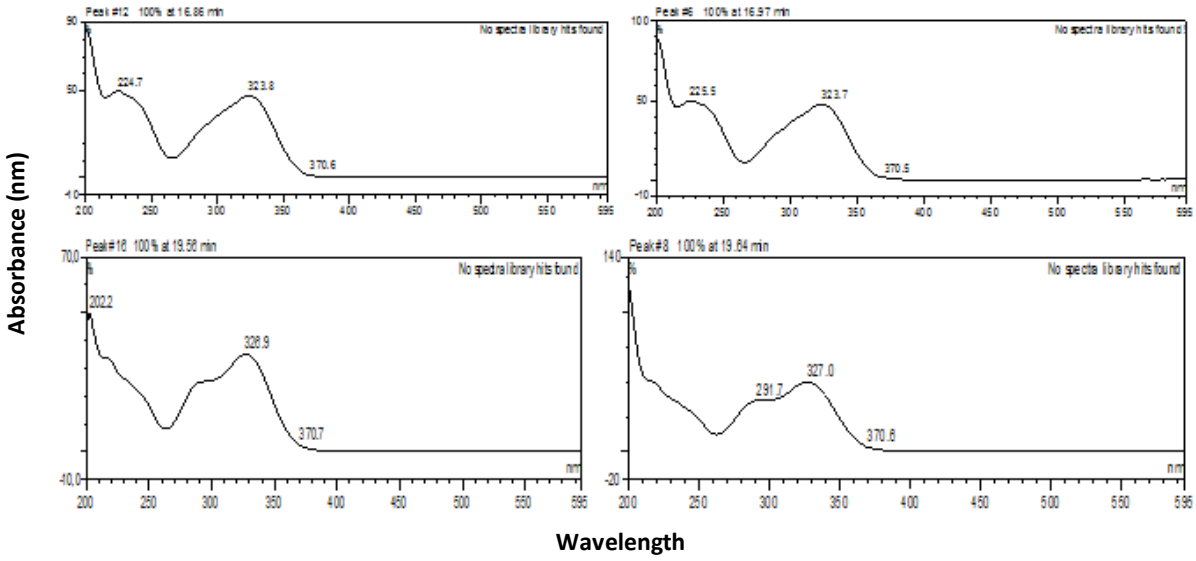

Figure 3. UV-VIS spectrum of NL-CEE-Ethanolic extract and NL-CAE-Aqueous fraction.

UV-Visible pattern as Peaks 6, 8, 10 and12 of the aqueous fraction, respectively.

Conclusion

The present study provided basic phytochemical information and preliminary data that the extract and sequential fraction of *N. laevis* possesses significant anti-ulcer activity in animal models. The gastric anti-secretory and acid neutralizing effect of the plant plus its antibacterial activity reveals the anti-ulcer potential of the plant. It is recommended that further studies are required to confirm the exact mechanism underlining the ulcer healing and protecting property of *N. laevis*.

Conflicts of Interest

The authors declare that there is no conflict of interest.

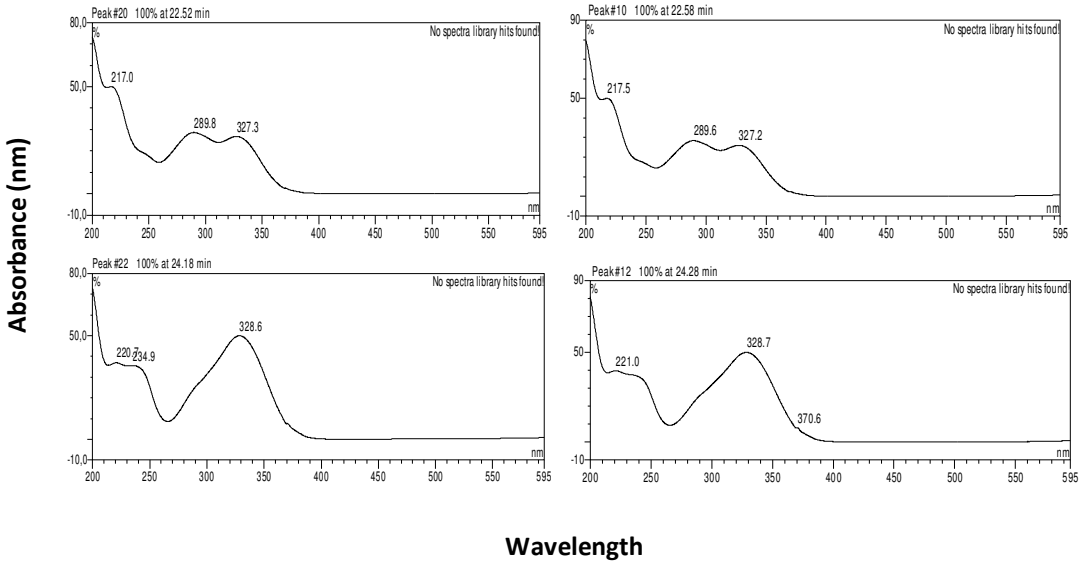

Figure 3. Cont'd.

ACKNOWLEDGEMENT

We are grateful to instutut of Pharm biology and Biotechnology; Heinrich Heine Universitat Dusseldorf Germany for providing facilities to carry out the HPLC analysis.

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