Original Research

Prelimilary phytochemical screening and antimicrobial activities of fruit, fruit skin, stem and root of bruce plant

*Ajayi E.I.O., Adeola A.O. & Ajayi O.B.

Abstract

Introduction: The extracts of *N. latifolia* Sm were studied for proximate, mineral, antinutritional and phytochemical compositions. The root and stem, and indeed all the parts of the plant are known to be used in folklore medicine for treatment of several infections.

Materials and Methods: Cold extraction was employed using methanol and chloroform. Proximate, antinutrient, mineral analyses and phytochemical screening of plant parts were done according standard methods. Using the **d**isc diffusion method, the methanol and choloroform extracts were tested against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Shigella somnei* for antimicrobial activities.

Results: Moisture content ranged from 5.93± 0.05% to 7.83±0.04%, ash content from 2.71±0.05% to 5.42±0.04%, and crude fiber from 11.24±0.004% to 59.38±0.11% across the samples; while fat (11.53±0.11%) and protein (12.76±0.05%) were highest in the fruit. The presence of antinutrients was confirmed in the fruit, fruit skin and root: cyanide (3.35±0.003mg/kg–11.58±0.001mg/kg) and phytic acid (6.28±0.005%–7.37±0.026%). Minerals present show sodium has the highest value (908.67±0.01ppm, root) and phosphorus, the lowest (10.75±0.01ppm, fruit). The extracts contain tannins, phlobatannins and saponins. The methanol extracts of both root and stem showed higher inhibition of the test organisms than the chloroform extracts.

Conclusion: This study confirms that *N. latifolia* has a broad spectrum of antimicrobial activities depending on the method of extraction. The antimicrobial property exhibited by the plant parts as reported here may be attributed to the presence of phytochemicals, while the edibility of the fruit may be due to the moderately high nutritive values and low cyanide content.

Keywords: *Nauclea latifolia*, phytochemicals, antimicrobial, zone of inhibition

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Biochemistry Department, Faculty of Science, Ekiti State University, Ekiti State, Nigeria.
*Ajayi O.B.
E-mail: bunmi_dave@yahoo.com

Ajayi E.I.O.
Adeola A.O.

The Bruce (African peach, "igberesi"), *Nauclea latifolia* Sm belongs to the Rubiaceae family of about 150 genus and 350 species of deciduous trees (Quattrocchi, 2000). It is a multistem shrub growing in the humid tropical rainforest zone and in the savannah woodlands of West and Central Africa. It grows as high as 200m with broad leaves having deep greenish colour, while the fruit flesh is grey with the inside turning red and white when ripe (Facciola, 1998; Barwick, 2004). The syncarp, polyseeded fruits are up to 6.5 cm in length, which look like golf ball with fragrant flavor. The shrub has an open canopy and terminal spherical headlined cymes of white flowers, which are joined with calyces (Mesia et al., 2005; Mabberley, 2008).

Nauclea latifolia contributes to the economy of rural dwellers in most parts of Eastern and Southern Nigeria through the roots, stalks, fruits and leaves (Staple and Herbst, 2005). The local uses of this important shrub include wood work (trunk), farm stakes (stem), chewing stick and toothpick (stalk), poultice (flowers, leaves, bark and roots), local drink flavor base (fruit pulp). However, the most exciting uses of *N. latifolia* are medicinal including antimalarial, antinociceptive, antipyretic (Njoroge and Bussmann, 2006; Abbah *et al*., 2010), antiviral (Ukamaka et al., 2015), antidiabetic (Gidado *et al*., 2005; Ogbuehi and Ebong, 2015), anti-ulcer (Balogun *et al.*, 2016), antibacterial (Okiei et al., 2011), anti-hypertensive, anti-depressant and anti-anxiety (Taiwo et al., 2010). All the plant parts are rich in monoterpene indol alkaloids. The roots, leaves and stem bark contain strictosamine, swerosid, loganin, rhynchophylline, naucleactonine, naucleficine, nauclefidine (Deeni, 1991; Shigemori et al., 2003). Certain saponnins, quinovic acid (a five-cyclic triterpene), quinovic acid 3-O-α-L-rhamnosyl-β-D-glucopyranosyl ester and quinovic acid 3-O-β-D-glucopyranosyl-β-D-glucopyranoside are also

present in *N. latifolia* (Lamidi et al., 2005; Agomuoh et al., 2013).

The study objective was to determine the phytochemical and antimicrobial properties of the stem and root antimicrobial properties of the stem and root of *N. latifolia* with a view of finding justification for the medicinal uses of this important shrub.

Materials and methods

Preparation of Samples

N. latifolia fruits, fruit skin, stem and root were collected from the premises of the Ekiti State University, Ado-Ekiti, Nigeria. The fruits were peeled, and the four samples were air-dried under shade for three weeks. They were then pounded in wooden mortal prior grinding in warring blender to fine powder. A portion of the powdered samples were used for proximate, antinutrient, mineral analyses and phytochemical screening. The stem and root samples were soaked in methanol and chloroform for 24 hours. Following the cold extraction, the samples were sieved through Whatmann filter paper No 1 and the solvents were removed under pressure using rotary evaporator.

Proximate Analysis

The proximate composition of the fruit, fruit skin, stem and root of *N. latifolia* was determined by the official method of the Association of Official Analytical Chemicals (AOAC, 1990) as follows: Moisture (section 926.08 and 925.09), Protein (section 955.04C and 979.09), Fat (section 922.06 and 954.02), Ash (section 923.03) and Crude Fibre (section 962.09). Carbohydrate was calculated by difference.

Analysis of Anti-nutrient Content

Determination of cyanogenic glycoside: Cyanogenic glycoside was determined using alkaline picrate (Onwuka and Olopade, 2005). 5 g powdered sample was weighed into 50 cm^3 distilled water, stirred and left to stand at room temperature, overnight after which it was filtered (Inuwa *et al*., 2011). Standard concentrations of KCN solution containing between 0.1 and 1.0 mg/ml cyanide were prepared. To 1 ml of the sample filtrate and standard cyanide solution, 4 ml of alkaline picrate solution (1 g of picrate and 5 g of Na2CO3 in 200 cm3 distilled water) was added in 10 test tubes and incubated in water bath for 15 minutes at 37°C. After color development, the absorbance was read at 490 nm against blank (1 ml distilled water and 4 ml alkaline picrate solution). The cyanide content was extrapolated from the cyanide standard curve.

$$\text{Cyanogenic glycoside (mg/kg)} = \frac{\text{C (mg)} \times 100}{\text{Weight of sample}}$$

where C (mg) = Concentration of cyanide content read off the graph

Determination of Phytic acid: Phytic acid was determined by the procedure of Lucas and Markakas (1975) 2.0 g of the sample was weighed into a 250 ml conical flask. Briefly, 10 g powdered sample was soaked in 100 ml of 2% concentrated HCl for 3 hours and then filtered through Whatmann No. 1 filter paper. 50 ml of the filtrate was diluted with 10 ml distilled water to achieve optimum acidity. 10 ml of 0.3% ammonium thiocyanate solution was added and the mixture was titrated with standard $FeCl_2$ solution (0.00195 g Iron/ml). The end point yellow colouration observed persisted for 5 minutes. The percentage phytic acid was calculated as follows:

% Phytic acid = y × 1.19 × 100

where y = titre value × 0.00195 g

Analysis of Mineral Content: 5g of the powdered samples were dry-ashed in Surgifield furnace, SM1008 at 550°C for 24h. The resulting ash was cooled in a dessicator and weighed. The ash was then dissolved in 2ml concentrated HCl, adding a 3 – 5 drops of concentrated HNO_3. The solution was placed in rotary evaporator flask and evaporated to dryness under pressure. Deionized water was added to the content and poured into 100ml volumetric flask and then made to mark. Standard dilutions of mineral elements were made for mineral analysis. Calcium, magnesium and iron contents were determined using S-series atomic absorption spectrophotometer (AOAC, 1990). Phosphorus was quantified spectrophotometrically as follows: 5 drops of concentrated HNO_3 were added to 10ml sample solution in a 100 ml volumetric flask, and made up to mark. The optical density was then read at 490 nm.

Phytochemical Screening: The phytochemical constituents of the fruit, stem and root of *N. latifolia* were identified following the methods of Harbone (1973), Trease and Evans (1989) and Sofowora (1993).

Test for tannins: 0.5g powdered sample was boiled in 20ml distilled water and filtered. 3 – 5 drops of 0.1% ferric chloride were added to the filtrate. Brownish-green or blue-black colouration signified the presence of tannins.

Test for alkaloids: 0.5g powdered sample was defatted with 5% ethyl ether for 15minutes. The defatted sample was extracted with 5ml aqueous HCl for 20minutes in boiling water bath. The resulting mixture was centrifuged for 10 minutes at 3000×g. 1ml each of two portions of the clarified filtrate was treated with 3 –5 drops of Meyer's and Dragendroff's reagents, separately. Turbidity signifies presence of alkaloids.

Test for saponins: 2.0g powdered sample was boiled in 20ml distilled water and filtered. Additional 5ml distilled water was added to 10ml of the filtrate and shaken vigorously for a stable froth. The persistent froth was then mixed with 3 drops of olive oil and shaken vigorously. Formation of emulsion signified the presence of saponins.

Test for phlobatannins: 0.5g powdered sample was boiled in 20ml distilled water and filtered. The filtrate was then boiled with 1% aqueous HCl. Deposition of red precipitate signified the presence of phlobatannins.

Test for anthraquinone: 0.5g powdered sample was mixed with 10ml benzene and filtered. 0.5ml of 10% ammonia solution was added to the filtrate and shaken. A violet colouration in the midlayer phase signifies the presence of anthraquinones.

Antimicrobial Activity: Using the **disc diffusion method, t**he antimicrobial activities of methanol and choloroform extracts of stem and root of Bruce were ascertained by culturing *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Shigella somnei* against several circular sterile paper discs which were each infused with 20 % (w/v) stock solution of each extract prepared in 95 % dimethyl sulfoxide (DMSO) (Nostro et al., 2000; Muiruri and Mwangi, 2016) in nutrient agar plates. The DMSO was used as negative control. The plates were then inverted and placed in an incubator at 37°C for 18h. Finally, the zone of inhibition around each disc was measured in millimeters using a graduated ruler. Zone of inhibition was indicated by clear area around the discs which corresponded to no bacterial growth

Statistical Analysis: The results were reported as Mean±SD and data were analyzed using ANOVA (SPSS version 20.0) with significance at $p<0.05$.

Results

Table 1 shows the results obtained for the fruit, fruit skin, stem and root of *N. latifolia*. The dried fruit sample contained 7.70±0.01g moisture, 3.17±0.06g ash, 11.54±0.11g fat, 12.76±0.05 protein, 22.38±0.04g crude fiber and 42.45±0.05g carbohydrate (NFE) per 100g sample. The dried fruit skin contained 7.30±0.01g moisture, 5.43±0.04g ash, 7.17±0.06g fat, 8.31±0.01 protein, 11.24±0.04g crude fiber and 60.55±1.04g carbohydrate (NFE) per 100g sample. The dried stem contained 7.83±0.05g moisture, 2.71±0.05g ash, 10.86±0.03g fat, 12.94±0.01 protein, 59.38±0.11g crude fiber and 6.28±0.15g carbohydrate (NFE) per 100g sample. The dried root contained 5.93±0.05g moisture, 3.55±0.10g ash, 7.71±0.05g fat, 8.37±0.01 protein, 40.30±0.18g crude fiber and 34.14±1.15g carbohydrate (NFE) per 100g sample.

Table 1. Proximate analysis of fruit, fruit skin, stem and root of Bruce (nauclea latifolia)

Parameters	Composition (g/100g) Fruit	Fruit Skin	Stem	Root
Moisture	7.70±0.01	7.30±0.01	7.83±0.05	5.93±0.05 ^{c,e,f}
Ash	3.17±0.06	5.43±0.04 ^{a}	2.71±0.05 ^{d}	3.55±0.10 ^{f}
Fat	11.54±0.11	7.17±0.06 ^{a}	10.86±0.03 ^{b,d}	7.71±0 .05 ^{c,f}
Protein	12.76±0.05	8.31±0.01 ^{a}	12.94±0.01 ^{d}	8.37±0.01 ^{c,f}
Crude fiber	22.38±0.04	11.24±0.04 ^{a}	59.38±0.11 ^{b,d}	40.30±0.18 ^{c,e,f}
Carbohydrate (NFE)	42.45±0.05	60.55±1.04 ^{a}	6.28±0.15 ^{b,d}	34.14±1.15 ^{c,e,f}

[a, b, c, e, f indicate significant comparisons ($p<0.05$) within and across the data values following ANOVA using SPSS 20.0]

Table 2 shows the total cyanide and phytic acid contents of fruit, fruit skin and root of *N. latifolia*. The difference between total cyanide content in the samples of the same plant reflect the variations in its storage. This necessitates caution in preparation of the parts of the plant for food or medicinal purposes. The iron, magnesium, potassium, phosphorus, manganese, calcium and sodium contents in the fruit, fruit skin, stem and root of *N. latifolia* are shown in Table 3. The fruit skin contained 31.56±0.003g iron, 898.52±0.100g magnesium, 605.48±0.006g

Table 2. Anti-nutrient composition of fruit, fruit skin and root of Bruce (Nauclea latifolia)

Parameters	Fruit	Fruit Skin	Root
HCN (mg/kg)	3.36±0.003	11.58±0.001 ^{a}	9.08 ±0.002 ^{b,c}
Phytic Acid (%)	7.37±0.026	6.34±0.006 ^{a}	6.28±0.005 ^{b}

HCN = Total cyanide

[a, b, c, indicate significant comparisons ($p<0.05$) within and across the data values following ANOVA using SPSS 20.0]

potassium, 29.27±0.004g phosphorus, 12.73±0.019g manganese, 580.11±0.004g calcium and 722.19±0.00g sodium per 100g sample. The mineral content of the stem was 807.95±0.07g, 611.75±0.005g, 529.52±0.005g and 695.76±0.006g per 100g respectively for magnesium, potassium, calcium and sodium. The root contained 41.52±0.004g iron, 872.71±0.009g magnesium, 659.14±0.004g potassium, 24.40±0.013g phosphorus, 11.05±0.031g manganese, and 538.28±0.005g calcium and 908.67±0.006g sodium per 100g sample.

Table 3. Phytochemical analysis of fruit, fruit skin, stem and root of Bruce (Nauclea latifolia)

Phytochemical	Fruit	Stem	Root
Tannins	+	+	+
Alkaloids	-	-	-
Saponinis	-	+	-
Phlobatannins	+	+	+
Anthraquinones	-	-	-

"+" signifies presence of phytochemical; "-" signifies absence of phytochemical

The result of phytochemical analysis of the *N. latifolia* parts are shown in Table 4. Tannins and phlobatannins were present in the fruit, stem and root, whereas they do not contain alkaloids and anthraquinones; and only the stem contain saponins.

Table 4. Antibacterial activities of crude extracts of the stem and root of Bruce (Nauclea latifolia)

Extract (mg/ml)		Diameter of Zone of Inhibition (mm) S. aureus	Esche coli	Klebsiella pneumoniae	Shigella somnei
Chloroform extract of stem	100	12	11	14	11
	50	-	9	11	9
	25	-	7	-	-
	12.5	-	-	-	-
Methanol extract of stem	100	21	7	25	14
	50	18	-	15	11
	25	13	-	12	9
	12.5	8	-	8	8
Chloroform extract of root	100	16	9	11	21
	50	11	8	-	18
	25	-	-	-	13
	12.5	-	-	-	11
Methanol extract of root	100	21	13	21	24
	50	14	11	14	22
	25	10	8	8	20
	12.5	-	-	7	11

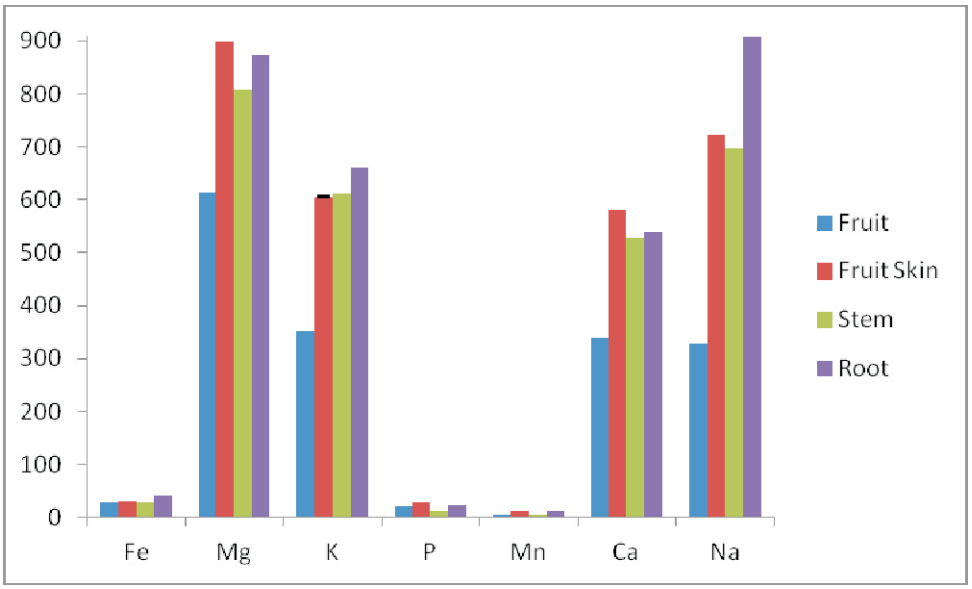
"-" signifies absence of inhibition

Crude chloroform and methanol extracts of the *N. latifolia* stem and root were screened against four important microorganisms: *Staphylococcus aureus*, *Escherichia coli, Klebsiella pneumoniae* and *Shigella somnei*. Table 5 shows that the chloroform (12mm, 11mm, 14mm and 11mm, respectively) and methanol (21mm, 7mm, 25mm and 14, respectively) extracts of the stem had zones of inhibition on the four microorganisms at 100mg/ml. The chloroform extract (50mg/ml) did not inhibit *Staphylococcus aureus*, while the methanol extract did not inhibit *Escherichia coli* at the same concentration. (16mm, 9mm, 11mm and 21mm, respectively) and methanol (21mm, 13mm, 21mm and 24mm, respectively) extracts of the root had zones of inhibition on the four microorganisms at 100mg/ml. Meanwhile, at 50mg/ml, the chloroform extract did not show inhibition against *Klebsiella pneumoniae;* whereas the methanol extract inhibited all four microorganisms (14mm, 11mm, 14mm and 22mm, respectively).

Discussion

Moisture content of fruit, fruit skin and stem of Bruce fall within the same range compared to the root which had the least moisture content. Fruit skin contained the highest ash content, while protein and fat contents in fruit and stem were higher compared to fruit skin and root. The high values of carbohydrate recorded for the fruit and fruit skin support its use as a base for soft drinks and other home-made drinks such as "zobo" (Abdelmuti, 1998). Low crude fibre content was recorded for fruit skin compared to the fruit, stem and root. This revealed that the fruit, stem and root contain high proportion of crude fibre, which is resistant to digestion by the endogenous secretions of the upper gastrointestinal tract. The crude fibre is known to interact and bind with iron *in vitro*, thus reducing the bioavailability of iron in the gut (Zhou and Erdman, 1995; Petry *et al*., 2010). Cyanide content of the root was found to be very high compared to the fruit. The low cyanide content of the fruit (3.35mg/kg) supports the consumption of the raw fruit by locals (Haque and Bradbury, 2002). Phytic acid content was also high in the fruit compared to the stem and root. Previous *in vivo* studies have shown that phytic acids also inhibit dietary iron due to the formation of di- and tetra-ferric phytates, from which iron absorption becomes poor (Torre *et al*., 1991). Phytic acid is also an important storage form of phosphorus in seeds, cereals including soybeans (Raboy, 1990). Soaking or cooking can reduce the level of cyanide and phytic acid (Enechi and Odonwodu, 2003; Soetan, 2008). Phytic acid acts to sequester phosphorus, thus becoming an important storage form of it (Sankara Rao and Deosthale, 1983). Phosphorus in turn is used in the production of energy as well as the formation of structural elements like cell membranes and skeletal muscle (Soetan *et al.*, 2010). Phytic acid also chelates other essential minerals such as zinc, magnesium, iron and calcium in the

Figure 1. Mineral composition of fruit, fruit skin, stem and root of Bruce (Nauclea latifolia)
Fe = Iron, Mg = Magnesium, K = Potassium, P = Phosphorus, Mn = Manganese, Ca = Calcium, Na = Sodium



digestive tract and reduces their bioavailability for absorption by the body (Schlemmer et al., 2009, Gibson et al., 2010). Nevertheless, phytic acid has been known to act as an antioxidant (Coelho, et al., 2008), particularly in regards to iron, which contributes to free radical generation through the Fenton reaction thus contributing to oxidative stress in the body (Valko et al., 2005; Leopoldini et al., 2006). In this context, phytic acid's ability to tightly sequester and trap iron is beneficial (Phillippy and Graf, 2002; Valko et al., 2005). Other benefits of phytic acid include anticancer (Singh and Agarwal, 2005), nephroprotective (Grases et al., 2006), and antioxidant (Zhou and Erdman, 1995) potentials.

The mineral content of the fruit skin, stem and root was high. The various minerals present in the plant parts are major electrolytes essential for haemostatic control in the human body (Ahmed and Chandhary, 2009). Calcium, sodium, potassium and magnesium were well distributed across the plant parts tested. They are also essential for the nervous system, maintenance of correct rhythm of the heart beat, and clotting of blood (Edward et al., 1995). The involvement of these minerals and other trace elements in the maintenance of fluid volume in the body explains the use of the plant in the treatment of diarrhea and dysentery (Lamidi, 1995). Magnesium plays an important role as a cofactor to several enzymes, participates in strengthening cell membrane structure, in controlling elevated insulin levels, lowering serum cholesterol (McNair et al., 1978). The low iron content recorded in the plant parts may be due to sequestration to phytic acid. Thus, iron which is essential for erythropoiesis and oxygen transport is depleted. Other minerals present, although in low quantities in the plant parts are manganese and phosphorus, which are each essential for male fertility and bone mineralization and resorption (Heaney and Rafferty, 2001).

The few phytochemicals identified as present in the plant parts are important for various medicinal purposes. The bitter tannins and phlobatannins were found to be present in the fruit, stem and root, while saponins were found in the stem; although, Jackes (2000) reported that saponins are present in the bark, roots and leaves. These three phytochemicals have been reported to have antimicrobial properties (Sofowora, 1993).

The various concentrations (12.5 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml) of the methanol and chloroform extracts of the stem and root tested against culturing *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Shigella somnei* revealed that the extract was highly effective against all four organisms with maximum zone of inhibition of 25mm and minimum zone of inhibition of 7mm. This supports the various uses of the plant parts – for instance, the stem is used as chewing stick and protects the teeth against toothaches, dental carries and septic mouth (Asubiojo et al., 1982);

the leaves and root are used as poultice for wound treatment owing to the presence of saponins (Maobe, 2013; Ajayi *et al*., 2016) and the fruit is used to prepare drinks for treating stomach ache, dysentery and diarrhoea (Nworgu et al., 2008; Maitera et al., 2011). The large fruit is edible when ripe. It is high in vitamin C even if slightly bitter. But it is recommended that the skin should be discarded (Jackes, 2000). Staphylococcus aureus is known to cause skin disease, and it is also responsible for surgical wound infections, which can be nosocomial (Madigan et al., 2000; Ahmed, 2012). Staphylococcus aureus and Escherichia coli are known to be responsible for severe bloody diarrhea which can possibly be followed by with acute renal failure (Guentzel, 1996). In the same vein, Klebsiella spp are associated with primary cavitating pneumonia, which is typically found in patients predisposed to diabetes mellitus (Wang et al., 2005; Gadkowski and Stout, 2008). Also, Shigella spp are known to be capable of invading the mucosa, cause ulceration of the large bowel, and release toxins that precipitate defect in large bowel reabsorption, leading to dysentery and bloody diarrhea (Maurelli and Sansonetti, 1988). Thus the methanol and chloroform extracts of the stem proved more effective than those of the root extracts against the test organisms, with methanol extract of the stem showing higher inhibitory effect across the range of concentrations.

Conclusion

It can be concluded from this study that the result data supports and confirms the therapeutic importance of the fruit, fruit skin, stem and root of the Bruce plant in folklore medicine. Further isolation, purification and characterization of the bioactive compounds are needed for the possible discoveries of promising new chemical entities from this important medicinal plant.

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