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RESEARCH ARTICLE

Phytochemical, proximate analysis and antioxidant activity of the rhizome of *Alpinia nigra* (Gaertn.) B.L. Burtt (Zingiberaceae) in Tamulpur district, Assam

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Abstract

The present research examines the chemical composition, proximate characteristics and antioxidant analysis of *Alpinia nigra*'s rhizome extract. Results of GC-MS analysis revealed a total of 22 volatile compounds of which Tris (tert-butyldimethylsilyloxy) arsane (2.89%), 2-ethylthiolane, S, S-dioxide (3.47%), Ambrial (6.68%) and Silicic acid, diethyl bis (trimethylsilyl) ester (4.59%) were the major compounds. The rhizome was found to have variable quantities of proximate compositions such as moisture content (55.5%), ash content (4.72%), crude fat (52%), crude fibre (41.25%), crude protein (28.95 µg/g), carbohydrate (46.27%) and dry matter (33.4%). The crude extraction of the rhizome was done by Soxhlet apparatus. The phytochemical screening showed the presence of alkaloid, tannin, terpenoid, glycoside, steroid, carbohydrate and a considerable amount of phenol and flavonoid. Phenolic and flavonoid content in the extracts was determined by the Folin-Ciocalteu and aluminium chloride assays as gallic acid equivalent and quercetin equivalent, respectively. To evaluate the antioxidant activity of rhizome extract, phosphomolybdenum assay, ferric-reducing antioxidant power (FRAP), and 1,1-diphenyl-2-picryl-hydrazil (DPPH) were used. The findings indicate that the rhizome extract is rich in phenol and flavonoid content and would serve as a promising source of natural antioxidants in the food, cosmetics, and pharmaceutical industries.

Keywords

Alpinia nigra; antioxidant; nutrition; phytochemicals; rhizome

Introduction

Medicinal plants represent the most ancient form of medication, used for thousands of years in traditional medicine in many countries around the world. The empirical knowledge about their beneficial effects was transmitted over the centuries within human communities (1). About 80 % of people around the globe still rely primarily on traditional medicines for their primary healthcare, demonstrating the vital role played by the plant-based traditional healing system in healthcare (2). Nutrition and medicines obtained from plant resources have been extensively used by evaluating their health benefits because of its richness in antioxidant properties and other chemical constituents. Plants naturally synthesize chemical compounds known as secondary metabolites, which are distributed throughout in various parts of the plant. These secondary metabolites play a crucial role in the plant's de-

fence mechanisms and serve as a means to protect the plant from oxidative damage caused by free radicals (3). The ability of free radicals to induce oxidative stress plays an important role in human pathophysiological diseases (4, 5). Phytochemicals are able to neutralize the free radicals in the human body and hence alleviates many chronic diseases (6). In pursuit of compounds that promote natural defence, special attention is focused on secondary metabolites, generated by plants with known nutritional or therapeutic effects. Polyphenols, which include flavonoids, isoflavones, anthocyanins and catechins, are believed to possess the strongest antioxidant capabilities. Since humans cannot produce these substances, consuming them through food is crucial for maintaining good health, particularly in fighting against free radicals (7).

A recent study shows that 1403 wild species of plants under 184 families are consumed throughout India (8). Amongst these, *Alpinia nigra* (Zingiberaceae) is one of the wild herbaceous plants which have been used for therapeutic and dietary purposes. In one of the most common herbal treatments, *A. nigra* is used in Thai traditional medicine to treat stomach ailments and gastrointestinal disorders, as it has antibacterial and antifungal properties (9). It is found mostly in Yunnan and Hainan Province of China, Thailand, Bhutan, Sri Lanka and India (10). North-east India is one of the richest and most diversified regions for Zingiberaceae, where 88 different species of plants under 19 genera were documented (11). *A. nigra* occurs in marshy slope between low hillocks in different parts of Assam and Tripura. Different tribal communities of north-east India have been using *A. nigra* as a part of their diet with rice. Shoot and rhizome parts of the plant are also well known for its use in medicinal and culinary item. Indigenous people of Tripura drink the aqueous juice of *A. nigra* shoots to treat intestinal parasite infections. Although the plant is a beloved vegetable in the diet, the native people of Tripura in some areas also utilise it as a food flavouring agent. Similarly, in Assam, the aqueous extract of *A. nigra*'s shoot and rhizome has been used to treat conditions like gastric ulcers, irregular menstruation, bone weakness and jaundice (12). Leaf extract from *A. nigra* exhibits cytotoxic, antimicrobial and analgesic properties (13, 14). Experiments conducted using rhizome extract validated it as antibacterial and cytotoxic drugs (15). But there is no scientific justification on the use of rhizome of *A. nigra* as nutritional and antioxidant source. Despite this, this medicinal plant still has a bright future because more research is still needed to fully understand the phytochemical makeup and potential health benefits of many species. Therefore, this study has been devoted to work on phytochemical, proximate and antioxidant analysis of the rhizome of *A. nigra*.

Materials and Methods

Collection of plant material

The specimen, *Alpinia nigra* was collected from a village, Kekerikuchi under Tamulpur district, Assam (Fig. 1) and authenticated in Department of Botany, Bodoland University, Kokrajhar, Assam.

Preparation of rhizome

The rhizomes of *A. nigra* were separated and washed with running water for 3–5 times and further washed with distilled water. The washed specimens were cut into slices and shade dried on blotting paper at room temperature for 3–4 days (Fig. 2).

Preparation of rhizome extract

The dried rhizomes were grinded using an electronic grinder. About 10 g powdered rhizome of *A. nigra* was extracted with Soxhlet apparatus for 7–8 h using methanol and hexane as solvents separately. Filtrate obtained was concentrated in rotary evaporator and crude extracts were kept at −4 °C for further analysis. The extracts were dissolved in 5 % dimethyl sulfoxide (DMSO) at the concentration of 10 mg/mL and subjected to analysis of antioxidant activity.

Preliminary phytochemical screening of A. nigra

Methanolic extracts of rhizome was used to detect the presence of different secondary metabolites using standard laboratory procedures. The different tests, Wagner's test for alkaloid, Ferric chloride test for tannin, foam test for saponin, aluminium and ammonium test for flavonoids, Salkowski's test for terpenoid and steroid, Folin-Ciocalteu test for phenol, Keller-kiliani test for glycoside, Molisch test for carbohydrate were performed (16-22).

Alkaloid

1–2 mL of the extract was warmed with 2 % sulphuric acid (H_2SO_4) for 2 min. Appearance of reddish-brown precipitation upon the addition of Wagner's reagent indicates the presence of alkaloid.

Carbohydrate

1 mL of extract was treated with 0.5 mL of Molisch reagent and 1 mL of conc. H_2SO_4 was added to it. Appearance of purple to violet colour ring at the junction indicates the presence of carbohydrates.

Flavonoid

Ammonium test

1 mL of the extract was heated with 5 mL of ethyl acetate in boiling water for 3 min. The mixture was filtered and the filtrates were shaken with 1 mL of dilute ammonium solution (1 %). The layers were allowed to separate. A yellow colouration at ammonium layer indicates the presence of flavonoid.

Aluminium test

1 mL of the extract was heated with 5 mL of ethyl acetate in boiling water for 3 min. The mixture was filtered and the filtrates were shaken with 1 mL of 1 % aluminium chloride solution. Light yellow colouration indicates the presence of flavonoid.

Glycosides

1 mL of glacial acetic acid was mixed with 2 mL of rhizome extract and 5 % $FeCl_3$ was added to it. Then conc. H_2SO_4 was added by the side wall of the test tube. Reddish brown appearance at the junction of 2 liquids and bluish green in the upper layer confirms the presence of glycosides.

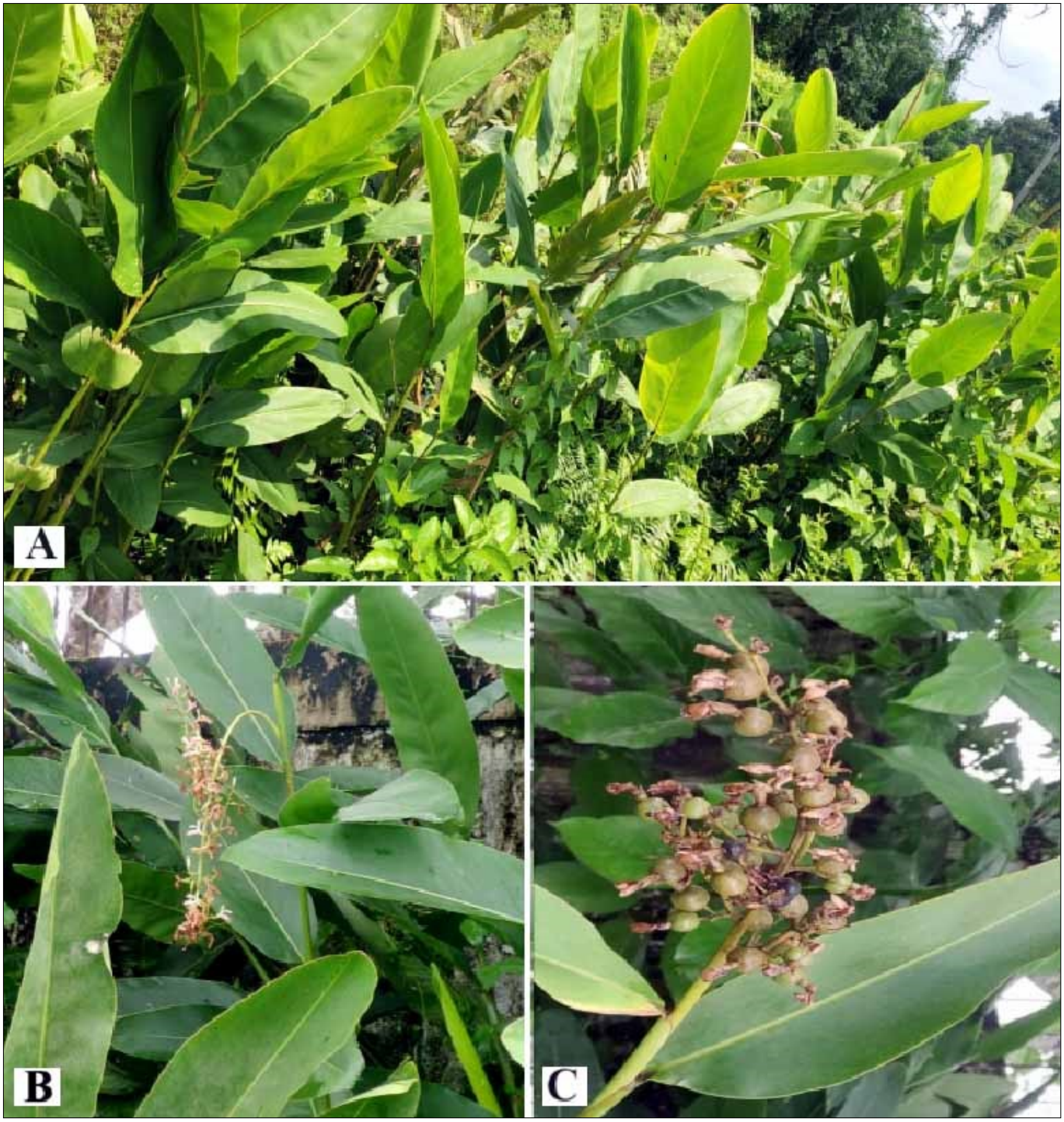

Fig. 1. *Alpinia nigra.* **A** - Habit, **B** - Inflorescence, **C** - Fruits.

Phenol

1–2 mL of the extract was treated with few drops of 5 % ferric chloride solution. Appearance of bluish black colour indicates the presence of phenol.

Saponin

A small amount of the extract was shaked with 5 mL of distilled water in a test tube. Appearance of foamy layer on the top indicates the presence of saponin.

Steroid

1–2 mL of the extract was treated with 2 mL of chloroform and 3 mL of conc. H_2SO_4 was added carefully by the side wall of test tube. Formation of greenish yellow fluorescent in the chloroform layer with acid confirms the presence of steroid.

Tannin

A small amount of the extract was diluted with distilled water and 2–3 drops of 5 % ferric chloride solution was added. If the solution turns transient green to black colour, it indicates the presence of tannin.

Terpenoid

1–2 mL of extract was treated with 2 mL of chloroform and 3 mL of conc. H_2SO_4 was added carefully. Formation of reddish-brown colouration in the interface indicates the presence of terpenoids.

Proximate analysis of rhizome of A. nigra

Moisture content

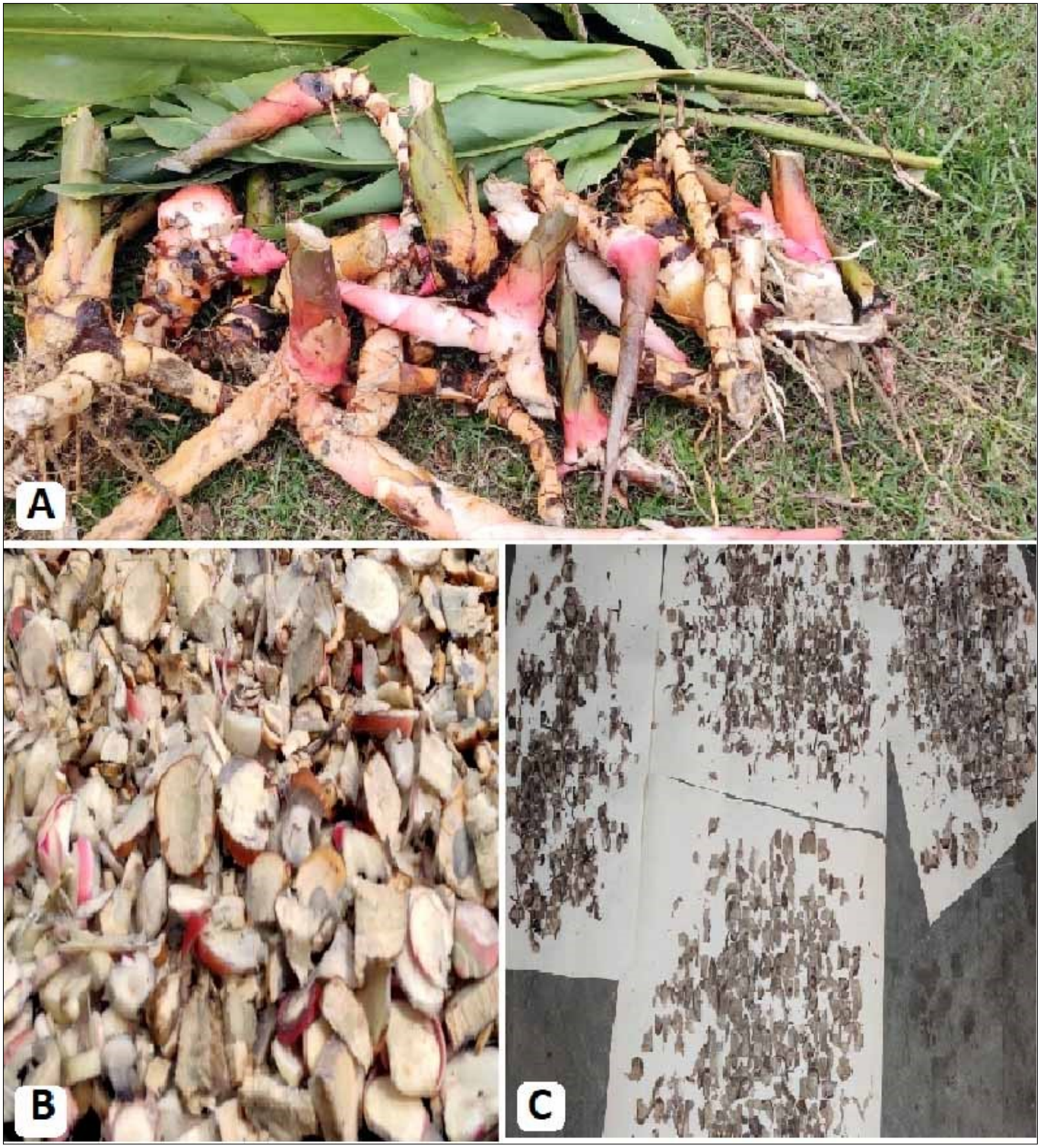

Fig. 2. *Alpinia nigra*: **A** - Rhizome, **B** - Slicing of the specimen, **C** - Drying of the specimen.

About 2 g of fresh specimen was weighed and kept in hot air oven at 105 °C temperature for a few hours until the specimen was dry. Dried specimen was weighed again to determine the moisture content (23) and calculated as follows:

$$\text{Moisture (\%)} = \frac{\text{Fresh weight–Dry weight}}{\text{Fresh weight of the sample}} \times 100 \quad \text{...(Eqn. 1)}$$

Ash content

For ash content, 2 g of the powdered sample was placed in an incubated tared crucible and weighed. Then it was placed under muffle furnace at 550 °C for 5–6 h until the sample became white colour (ash) (23) and its content determined as follows:

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100 \text{..} \ldots\ldots\ldots\ldots\ldots\ldots\text{(Eqn. 2)}$$

Determination of crude fat content

A cleaned round bottom flask was dried in oven at 105 °C for 2 h to ensure the weight of the bottle is stable. About 3 g of sample was weighed and wrap with filter paper. It was

then extracted with petroleum benzene in Soxhlet apparatus. The extract was dried on a hot plate to completely dry out the solvent; cooled and weighed again (23) and calculated as follows:

Weight of fat = A (weight of round bottom flask with fat) – B (initial weight of round bottom flask) ……..(Eqn. 3)

$$\text{Crude fat (\%)} = \frac{\text{Weight of the fat}}{\text{Weight of the sample}} \times 100 \quad \text{(Eqn. 4)}$$

Determination of crude fibre

2 g of specimen was taken in a beaker and 200 mL of mixture solution (1.25 % H_2SO_4 and petroleum ether) added to it and boiled in water bath at 60–80 °C for 30 min. The residue was washed with boiled water until they are no longer acidic and again boiled with 200 mL of petroleum ether containing 1.25 % NaOH for 30 min at 60–80 °C. After filtration it was further washed with boiled water. Then the residue was dried at 50 °C for 5 h in an oven and cooled; the weight of the specimen was recorded (24) and calculated as follows:

$$\text{Crude fibre (\%)} = \frac{\text{Weight after drying}}{\text{Weight of original sample}} \times 100 \text{..(Eqn. 5)}$$

Dry matter of the plant

It was calculated using the formula given below (14).

Dry matter = 100 – Moisture (%)..... (Eqn. 6)

Determination of carbohydrate

It was estimated by the difference method based on traditional carbohydrate determination (25).

Carbohydrate (%) = 100 – [Moisture (%) + Ash (%) + Crude protein (%) + Crude fat (%)]……..……..(Eqn. 7)

Protein estimation

Lowry's method using Bovine serum albumin (BSA) as the working standard was performed for protein estimation (26). Reagents A (2 % of Na_2CO_3 in 0.1 NaOH), B (0.5 % $CuSO_4$ in 1 % NaK tartarate solution) and C (100 mL of reagent A + 2 mL of reagent B) was prepared. To 0.2 mL of the methanolic extract (2 mg/mL) taken in 3 test tubes, the volume was made up to 1 mL with distilled water and 5 mL of reagent C was added to each test tubes and incubated for 15 min at room temperature. Further 0.5 mL of FC reagent was added to the mixture and incubated for 30 min. The absorbance was measured at 660 nm against distilled water as blank in UV-VIS spectrophotometer (UV-1900i, Shimadzu). The protein in the extract was determined as micrograms of BSA equivalent (BSAE) per gram of dry weight (µg BSAE g^{-1}DW) using standard BSA graph.

Antioxidant activity of rhizome of A. nigra

Total phenolic content (TPC)

The total phenolic contents (TPC) were determined by Folin-Ciocalteu reagent after Slinkard and Singleton, 1977 using gallic acid as a standard phenolic compound (27). A total of 50 µL of the extract was taken in a test tube and diluted with distilled water (1.8 µL). Then 1 mL of a 10 % Na_2CO_3 and 150 µL of Folin-Ciocalteu was added and thoroughly mixed. The mixture was then allowed to stand undisturbed for a duration of 10–20 min. The absorbance was measured at 765 nm in UV-VIS spectrophotometer (UV-1900i, Shimadzu) against blank consisting of all the reaction agents except the extract. The total concentration of phenolic compounds in the extract was expressed as milligrams of gallic acid equivalent (GAE) per gram of dry weight (mg GAE g^{-1}DW) using standard graph.

Total flavonoid content (TFC)

Total flavonoid content (TFC) was determined following Dowd method (28). To 50 µL of extract solution, diluted using 1.95 mL distilled water, 1 mL of 2 % aluminium trichloride ($AlCl_3$) in methanol was added. The mixture was further incubated at ambient room temperature for 10 min. Absorbance was measured at 415 nm in UV-VIS spectrophotometer (UV-1900i, Shimadzu) against blank that lack extracts. The total flavonoid content was expressed as mg of quercetin equivalent (QE) per g of dry weight (mg QE g^{-1}DW) using standard quercetin graph.

Phosphomolybdenum assay

Phosphomolybdenum assay was done to evaluate total antioxidant compounds (29). The assay is used for the reduction of Mo(VI)–Mo(V) by antioxidant compound and subsequent formation of a green phosphate Mo(V) complex at low pH. Briefly, in a 3 mL vial, a 0.1 mL aliquot solution was mixed with 1 mL of a reagent solution comprising 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate and diluted upto 3 mL with distilled water. The mixture was incubated for 90 min in water bath at 95 °C. After cooling to room temperature, the absorbance was measured at 765 nm in UV-VIS spectrophotometer (UV-1900i, Shimadzu) against blank (0.1 mL of methanol in place of extract). The results were estimated from the standard ascorbic acid graph as milligrams of ascorbic acid equivalent (AAE) per g of dry weight (mg AAEg^{-1}DW).

Ferric-reducing antioxidant power (FRAP) assay

Reducing power of the extracts was determined (30), using ascorbic acid as standard. Various concentrations of the extracts (10–50 µL) were mixed with 1 mL of phosphate buffer (0.2 M, pH 6.6) and 1 mL of 1 % potassium ferricyanide [$K_3Fe(CN)_6$]. After 30 min of incubation at 50 °C in water bath, 1 mL of 10 % trichloroacetic acid was added to the mixture to stabilise the reaction and it was centrifuged (3000 rpm) for 10 min. Then, 1 mL of supernatant was mixed with 1.5 mL distilled water and $FeCl_3$ (0.5 mL, 0.1 %) and absorbance measured at 700 nm in UV-VIS spectrophotometer (UV-1900i, Shimadzu) against blank (all the reagents without extract). The reducing power is increased when the reaction mixture shows higher absorbance.

Scavenging DPPH radicals

The free radical scavenging activity of plant extracts was

determined by 1,1-diphenyl-2-picryl-hydrazil (DPPH) method (31, 32). Different concentrations of methanolic extract and standard in the range 10–50 µL were prepared in test tubes and added 2 mL of methanolic solution of DPPH at the concentration of 0.05 mg/mL and then the total volume in each test tube was adjusted to 3 mL by adding distilled water. The mixture was vigorously shaken and left to stand still at room temperature for a period of 30 min. Then the absorbance at 517 nm was determined using a UV-VIS spectrophotometer (UV- 1900i, Shimadzu), with methanol serving as the blank. The DPPH scavenging effect was computed using the following equation:

$$\text{DPPH scavenging effect (\%)} = (A_0 - A_1/A_0) \times 100 \ldots\ldots \text{(Eqn. 8)}$$

Where, A_0 is the absorbance of the negative control (2 mL of methanolic solution of DPPH + 1 mL of 5 % DMSO) and A_1 is the absorbance of reaction mixture or standards. Ascorbic acid was used as a standard.

GC-MS analysis

For the GC-MS analysis, the methanol extract of the sample was run in GC-MS instrument (Perkin Elmer (USA), Model Clarus 680 d amp, CalruMS, 600C MS) comprising a liquid auto-sampler. TurboMass Ver 6.1.2. software was used and the peaks were analysed by data analysis NIST-2014 software. The capillary column used was 'Elite-5MS' having dimensions-length-60 m, ID-0.25 mm and film thickness-0.25 µm. The stationary phase constituted 5 % diphenyl, 95 % dimethyl polysiloxane. In the GC-Protocol, helium gas (99.99 %) was used as a carrier gas at a flow rate of 1 mL/min. An injection volume of 2 µL was employed in splitless mode. Injector temperature was 280 °C, and ion-source temperature was 180 °C. The oven temperature was programmed at 60 °C (for 1 min), with an increase at the rate of 7 °C/min to 200 °C (hold for 3 min), then again increased at a rate of 10 °C/min to 300 °C (hold for 5 min). The total run time was ~39 min. Solvent delay was kept for 8 min. A MS protocol mass spectral measurement was taken in electron impact positive (EI+) mode at 70 eV. A solvent delay of 8 min was there for MS scan. Mass range i.e., m/z was 50-600 amu. Interpretation of the peaks obtained from GC chromatogram were done by comparing with the mass spectrum library of corresponding peaks using the database software of National Institute Standard and Technology (NIST-2014). The mass spectra of unknown components were compared with the known components of the NIST library and compounds were identified by name, molecular weight and empirical formula.

Statistical analysis

Statistical analyses were performed in excel by doing Kruskal-Wallis test in OriginPro 9.0, as the data were not normally distributed. All the values were represented as mean ± SE of three parallel measurements. Differences were considered significant at $p < 0.05$ and $p < 0.01$. The IC_{50} value (µg/mL) was calculated using the AAT Bioquest IC_{50} value calculator (AAT Bioquest, Inc., Sunnyvale, CA, USA).

Results

Qualitative phytochemical screening of A. nigra

Phytochemical investigation is of utmost significance in identifying new source of medicinally and scientifically valuable compounds. Different colours observed after performing with different tests and the inference of phytochemical screening is tabulated in Table 1.

Proximate analysis of rhizome of A. nigra

The assessment of the proximate composition of edible plant parts is necessary to determine their nutritional relevance and their byproducts are dependent on the digestion of digestible fibre in it. This will enable higher enzyme activity by increasing the food's surface area. The results of the proximate composition of rhizome of *A. nigra* are given in Table 2.

Table 1. Preliminary phytochemical screening of methanolic extract of the rhizome of *A. nigra.*

Phytochemicals	Test	Inference
Alkaloid	Wagner's test	+
Carbohydrate	Molisch test	++
Flavonoid	Ammonium test	*
	Aluminium test	*
Glycoside	Keller-Kiliani test	++
Phenol	Folin-Ciocalteu test	+
Saponin	Distilled water test	–
Steroid	Salkowski's test	++
Tannin	Ferric chloride test	+
Terpenoid	Salkowski's test	++

"**++**" = present in high concentration, "**+**" = present in low concentration, "*****" = present in very low concentration, "**–**" = absent.

Antioxidant activity of rhizome of A. nigra

Total phenolic content (TPC)

Table 2. Proximate composition of rhizome of *A. nigra.*

Proximate composition	Value
Moisture content (%)	55.50
Ash content (%)	4.72
Crude fat (%)	52.00
Crude fibre (%)	41.25
Crude protein (µg/g)	28.95
Carbohydrate (%)	46.27
Dry matter (%)	33.40

The total phenolic compounds in rhizome extracts of *A. nigra* were calculated as gallic acid equivalent (GAE) using the equation obtained from a standard gallic acid graph ($y = 0.0047x + 0.0082$, $R^2 = 0.9985$) (Fig. 3). The phenolic content was higher in the methanol extract (11.97 ± 0.38 mg GAE/g DW) than the hexane extract (3.25 ± 0.23 mg GAE/g DW).

Total flavonoid content (TFC)

TFC were determined as quercetin equivalent using the equation obtained from the standard quercetin graph ($y = 0.0121x + 0.0059$, $R^2 = 0.9984$). Hexane extract had higher flavonoid content compared to methanol extract

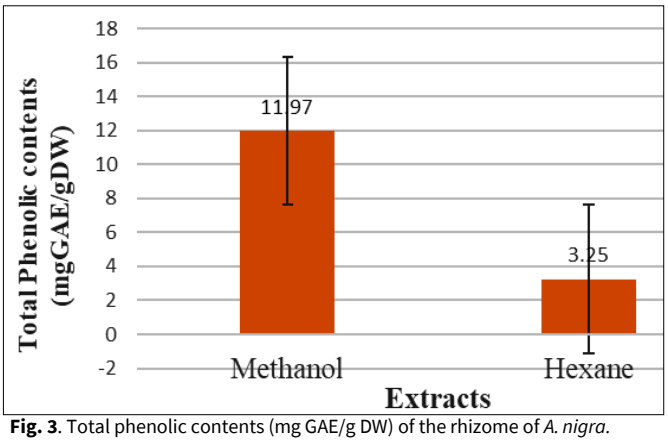

Fig. 3. Total phenolic contents (mg GAE/g DW) of the rhizome of *A. nigra.*

(Fig. 4).

Phosphomolybdenum assay

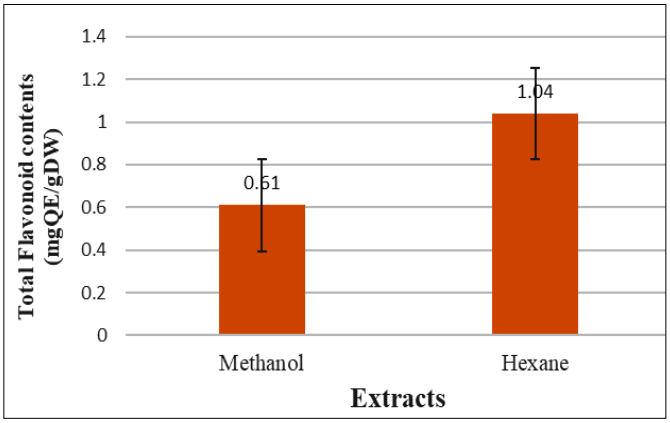

Fig. 4. Total flavonoid content (mg QE/g DW) of the rhizome of *A. nigra.*

The total antioxidant compounds were expressed as ascorbic acid equivalent using standard ascorbic acid graph (y = 0.0031x - 0.011, R^2 = 0.9981). The antioxidant compounds were found to be higher in the methanolic extract (4.23 ± 0.00 mg AAE g^{-1}DW) than the hexane extract (2.72 ± 0.67 mg AAE g^{-1}DW) (Fig. 5).

Ferric-reducing antioxidant power (FRAP) assay

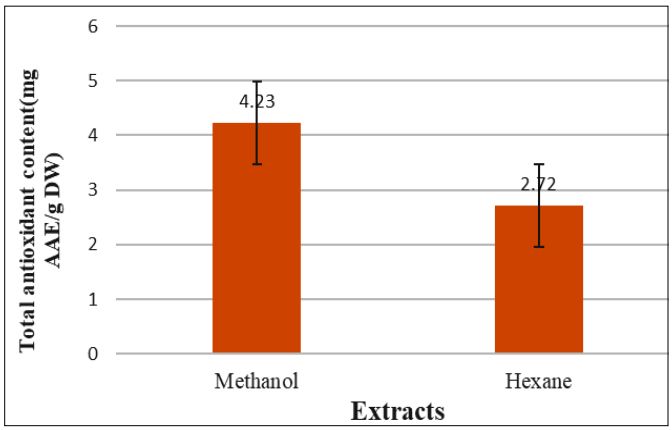

Fig. 5. Total antioxidant compounds by phosphomolybdenum method (mg AAE/g DW) of the rhizome of *A. nigra.*

The FRAP activity was expressed as ascorbic acid equivalent using the equation obtained from the standard ascorbic acid graph (y = 0.0119x – 0.053, R^2 = 0.9978). Methanol extract showed higher reducing power than hexane extract (Fig. 6).

Scavenging DPPH radicals

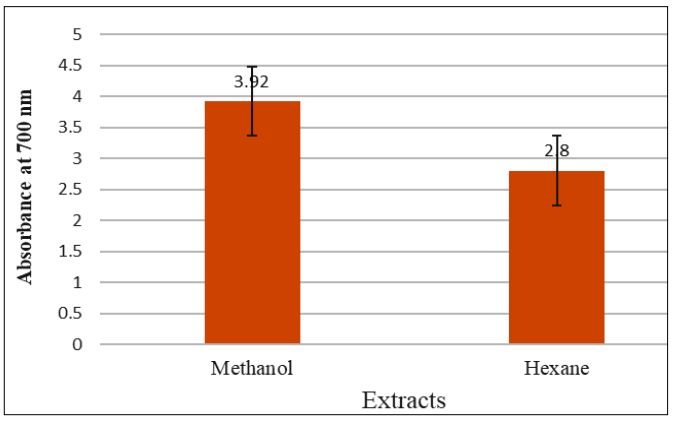

Fig. 6. Ferric-reducing antioxidant power (FRAP) (mg AAE/g DW) of the rhizome of *A. nigra.*

The percentage of scavenging DPPH radicals of the extracts are shown in Table 3. From the result it is clear that the % of free radical scavenging properties of both the methanol and ethanol extract increased linearly with the increase in concentration and was lower than the standard. However, the inhibitory effect was at equilibrium with the standard at high concentration (40 µg/mL) for methanol extract. At $p < 0.05$ and $p < 0.01$, there was a statistically significant difference among the concentration and extracts (Table 4 and 5).

GC-MS analysis

From phytochemical analysis, methanolic extracts showed better result so it was considered for further GC-MS analysis. Fig. 7 represents the chromatogram of GC-MS analysis of the extracts. Results of GC-MS analysis of the extracts revealed 22 compounds (Table 6).

Table 3. Percentage scavenging rate of DPPH free radicals by the rhizome of *A. nigra.*

Concentration (µg/mL)	DPPH free radical scavenging rate (%±SE)		
	Methanol	Hexane	Ascorbic acid
10	19.30±2.03	13.63±0.51	77.14±1.16
20	40.10±0.82	23.68±1.10	81.48±1.30
30	59.42±1.12	42.57±1.22	83.66±1.18
40	85.32±0.77	64.37±0.96	85.18±1.35
50	88.58±1.05	73.78±1.16	88.44±1.06
IC50 (µg/mL)	28.82	32.71	93.61

Table 4. Kruskal-Wallis Anova of DPPH between the extracts and ascorbic acid in each concentration. (NS = not significant).

Concentration	χ2	df	p-value	Significance
10	7.20	2	0.02	*
20	7.20	2	0.02	*
30	4.36	2	0.11	NS
40	5.60	2	0.06	NS
50	5.96	2	0.05	*

Table 5. Kruskal-Wallis Anova of DPPH between the concentration in each extract.

Extracts	χ2	df	p-value	Significance
Methanol	13.5	4	0.009	**
Hexane	13.5	4	0.009	**
Ascorbic acid	12.7	4	0.01	*

Discussion

Phytochemical research is essential when looking for novel compounds with medicinal and scientific significance. The data obtained from the study demonstrated that the rhizome of *Alpinia nigra* has chemicals of medicinal importance. The rhizome contained alkaloid, carbohydrate, flavonoid, glycoside, phenol, steroid, tannin and terpenoid. The information regarding the identification and standardisation of secondary metabolites is validated by the current study. It necessitates additional research on notable sources of distinctive bioactive compounds for its therapeutic and pharmacological applications.

Proximate composition of rhizome extract showed moisture, ash, fat, fibre, protein, carbohydrate and dry matter. Since there was a fair amount of moisture content, the chances of developing microorganisms are high which shortens the sample's shelf life. Ash content was found to be low (4.72 %). It is a diagnostic purity index and represents physiological and non-physiological ash. Its determination is crucial as it helps to understand the chemical composition and potential use of the sample. The adequate carbohydrate content present in the sample signifies it as a good source of energy and facilitates digestion and absorption of other nutrients. Significant proportion

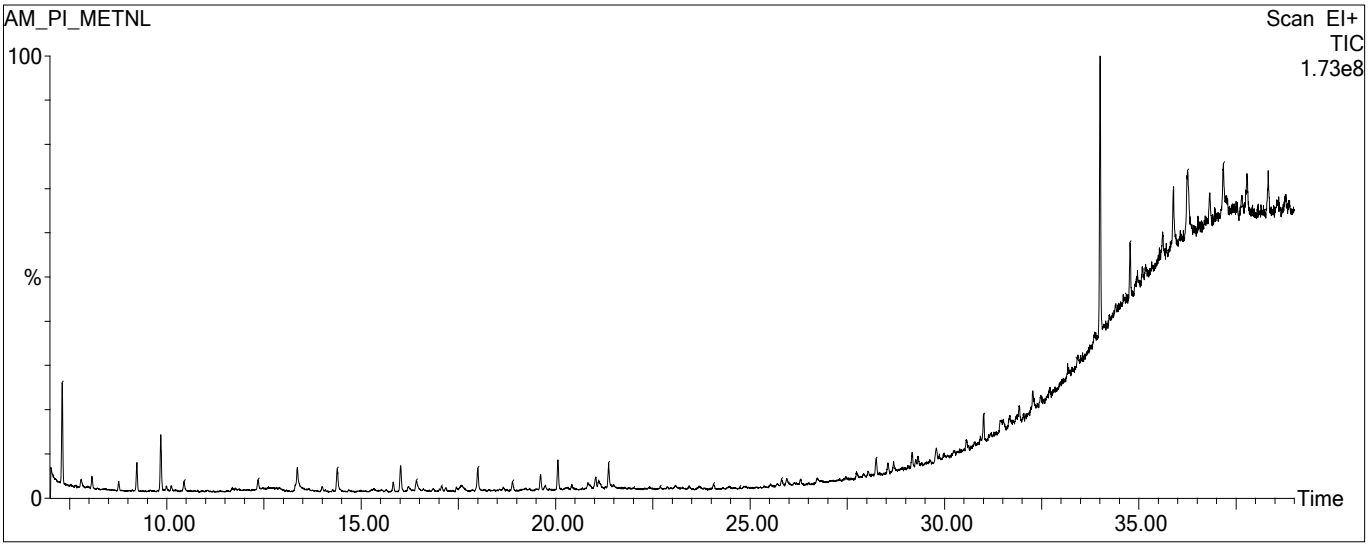

Fig. 7. GC-MS chromatogram of methanol extract of the rhizome of *A. nigra.*

Table 6. Compounds detected and identified in the methanol extract of the rhizome of *A. nigra* by GC-MS.

Sl. No.	Name of the compound	RT	Peak area (%)	M.W.
1	Tris (tert-butyldimethylsilyloxy) arsane (Phenol)	7.023	2.889	468
2	2-ethylthiolane, S, S-dioxide	7.314	3.474	148
3	2-propenoic acid, 2-hydroxyethyl ester	9.850	1.483	116
4	Benzene-ethanamine, 2-fluoro-. beta.,3,4-trihydroxy-n-isopropyl-	13.361	1.359	229
5	1-(3,3,3-trifluoro-2-hydroxypropyl) piperidine	14.386	0.891	197
6	Trifluoromethyl t-butyl disulfide	16.017	0.828	190
7	N-(1-methoxycarbonyl-1-methylethyl)-4-methyl-2-aza-1,3-dioxane	20.059	0.836	203
8	Benzofuran, 2,3-dihydro-	21.364	0.847	120
9	Cis, Cis-1,6-dimethylspiro [4.5] decane	31.008	0.847	166
10	(2r,3r,4ar,5s,8as)-2-hydroxy-4a,5-dimethyl-3-(prop-1-en-2-yl) octahydro	32.269	0.950	236
11	Ambrial	33.999	6.680	234
12	Cyclotrisiloxane, hexamethyl-	34.960	0.821	222
13	4-tert-octylphenol, TMS derivative	35.085	0.706	278
14	Arsenous acid, tris (trimethylsilyl) ester	35.610	1.535	342
15	4-(4-hydroxyphenyl)-4-methyl-2-pentanone, TMS derivative	36.060	0.805	264
16	Silicic acid, diethyl bis(trimethylsilyl) ester (Phenol)	36.245	4.596	296
17	Hexestrol, 2TMS derivative	36.820	1.142	414
18	1,2-bis (trimethylsilyl) benzene	37.171	2.487	222
19	Tetrasiloxane, decamethyl-	37.251	1.234	310
20	4-tert-amylphenol, TMS derivative	37.646	1.110	236
21	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	37.776	1.771	430
22	1,2-benzisothiazol-3-amine, TBDMS derivative	38.321	1.404	264

of crude protein in *A. nigra* suggests that the rhizome can be exploited as a possible source of protein. Examining the advantages of consuming more plant protein instead of animal protein is particularly relevant in terms of lowering the risk of cancer. High crude fibre content helps the gut to absorb trace elements, avoids diverticulosis, and promotes the removal of undigested food through the intestine (20, 33, 34).

Of all the classes of plant metabolites, phenolic compounds are one of the biggest and most prevalent (35). Phenolic compounds contain hydroxyl groups, which are significantly responsible for antioxidant activity and free radical terminators. These substances are the main sources of hydrogen for free radicals to stop the initial chain reaction of lipid oxidation. The hydroxyl groups in phenolic substances can inhibit the formation of free radicals in the human body (36). They also exhibit biological attributes including prevention of ageing, reducing inflammation, preventing atherosclerosis, protecting the cardiovascular system, improving endothelial function and suppressing angiogenesis and cancerous cells (37).

Flavonoid is a very significant natural phenol with low molecular weight known to have a wide range of chemical and biological activities, including the ability to scavenge free radicals. Flavonoids have major function in how a plant interacts with its surroundings and shields it from a variety of biotic and abiotic stresses. It is understood to be a class of polyphenolic compounds with two phenyl rings connected by a propane bridge to form the distinctive 15-carbon (C_6-C_3-C_6) flavan skeleton (38). The ability to counteract free radicals depends on the number and position of the OH group, which are linked to the ability of these compounds to donate electrons (39-41). As indicated in Fig. 5 the methanol extract contained the highest concentration of antioxidant compounds, whereas hexane had the lowest antioxidant compound. The antioxidant compounds can stabilize the highly reactive free radicals (42).

The potential antioxidant activity of substances is determined by their reducing power. The reducing potential is typically associated with the presence of its reductons, generated on breaking of free radical chain by donating hydrogen atom. The reduction of ferrous ions (Fe^{2+}) to ferric ions (Fe^{3+}) is observed by the intensity of the blue-green resultant solution, which absorbs at 700 nm. The present result shows the ferric reducing activity of extracts, and is due to the presence of polyphenol compounds, which may act in a similar way to reductons by donating electrons and reacting with free radicals to convert them into more stable products and terminate chain reactions of free radicals. In this assay, there is an electron transfer mechanism present that could affect the activity of phenolic compounds present in the extracts (43).

DPPH method has been employed for the evaluation of the free radical scavenging activity of the extracts of several natural products (44). This assay is used for the preliminary test which indicates the reactivity of the test extract compounds with a stable free radical. It gives purple-coloured strong absorption maximum band at 517 nm, which on reduction by the extract compound decreases in absorbance and discolouration from purple to yellow. It has been reported that the presence of the hydroxyl functional group of phenolic compounds having redox properties attributes the antioxidant activity (45). A statistically significant difference was observed within the concentrations and between the extracts and control (Tables 4 and 5).

Rhizome extracts showed a reasonably high level of antioxidant activity that is connected to flavonoid and phenolic components and are able to neutralise free radicals including superoxide, singlet oxygen and hydroxyl radicals, which makes them considerably more effective at scavenging radicals. The discovery of this work provides strong evidence that rhizome extracts of *A. nigra* are potent radical scavengers and contain a variety of naturally occurring antioxidants and active bioactive chemicals with desired therapeutic potential.

The 4 major compounds identified in GC-MS were viz. Tris (tert-butyldimethylsilyloxy) arsane (2.89 %), 2-ethylthiolane, S, S-dioxide (3.47 %), Ambrial (6.68 %) and Silicic acid, diethyl bis(trimethylsilyl) ester (4.59 %). Tris (tert-butyldimethylsilyloxy) arsane and Silicic acid, diethyl bis(trimethylsilyl) ester are phenolic compounds with effective antimicrobial, antioxidant and anti-diabetic potential (46-48), whereas 2-ethylthiolane, S, S-dioxide is sulphur containing aromatic compounds (49) and Ambrial is a sesquiterpene which is an essential oil with strong odorant and is an important ingredient in perfumery (50).

Conclusion

The nutritional value, phytochemical screening and antioxidant activity formed the central focus of this study. The findings of the present study indicate that the rhizome of *Alpinia nigra* can be considered as good sources of nutrition. The consumption of this species as food can be nutritionally advantageous. The presence of phytoconstituents may have potential antimicrobial, anti-diabetic and antioxidant activity which are being considered as an important aspect in pharmacognostic evaluation of the medicinal plant. The outcomes are really promising, and in fact, compound analysis is required to implement them for pharmaceutical applications.

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Authors' contributions

MD carried out experimental work, PI drafted manuscript, RD designed the study. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

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