Volume 9, Issue 2, 092512 (144-153)

Trends in Phytochemical Research (TPR)

https://doi.org/10.57647/tpr.2025.0902.12

Isolation and characterization of phytoconstituents and exploration of *in vitro* and *in vivo* biological activities of *Anaxagorea luzonensis* A. Gray (Annonaceae)

Sabiha Rahman[1], Fatema-Tuz-Zohora[1], Ramisa Anjum[1], A.H.M Nazmul Hasan[1], Mohiuddin Ahmed Bhuiyan[1], Choudhury Mahmood Hasan[2], Mohammad Shahriar[1,*]

[1]*Phytochemistry Research Laboratory, Department of Pharmacy, University of Asia Pacific, Bangladesh.*
[2]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Bangladesh.*

[*]**Corresponding author:** shahriar@uap-bd.edu

Original Research

Received:
17 February 2025
Revised:
19 June 2025
Accepted:
23 June 2025
Published online:
25 June 2025

Abstract:

From the stem bark of *Anaxagorea luzonensis* A. Gray using a modified Kupchan technique, the plant's methanol, *n*-hexane, and CCl_4 extracts were subjected to phytochemical screening, isolation and pharmacological tests. Key compounds identified include alkaloids, carbohydrates, saponins, glycosides, and glucosides. Spinasterol was isolated and identified via ^{1}H-NMR and ^{13}C-NMR for the first time from this plant. The extracts exhibited significant antioxidant activity, with IC_{50} values ranging from 55.768 to 157.57 µg/mL, and the methanol extract showed the highest levels of phenols and flavonoids. Cytotoxicity was observed with IC_{50} values of 0.034 and 1.498 µg/mL for CCl_4 and *n*-hexane extracts, respectively. *In vivo* testing revealed the methanol extract's strong antipyretic effect and pain relief at 200 mg/kg, with no toxicity at high doses. These findings support *A. luzonensis* as a promising source for bioactive natural compounds.

Keywords: *Anaxagorea luzonensis* A. Gray, Annonaceae, Antioxidant, Analgesic, Antipyretic, Cytotoxic, Phytoconstituents, Spinasterol

1. Introduction

Medicinal plants have played a vital role in traditional healthcare systems due to their bioactive secondary metabolites, which exhibit significant pharmacological activities (Hounsou et al., 2024). With the increasing global interest in natural remedies, herbal medicine has gained prominence as a safer and often more effective alternative to synthetic drugs (Wang et al., 2023; Zahra et al., 2024). Plant extracts are becoming more and more popular because of their high concentration of bioactive components (Tabassum et al., 2024; Shanta et al., 2024). Traditional medicinal herbs and wild species may possess untapped potential; therefore, researchers should continue to investigate lesser-known plant sources and exploring underutilized plant parts—such as roots, stems, and leaves (Sharif et al., 2024). The pharmaceutical industry, along with the implications of modern methods, aim to achieve the highest yield of bioactive compounds that can be proposed as natural drugs. These compounds can then be further investigated in preclinical and clinical studies for a wide range of persistent diseases and pathological conditions (Singh et al., 2023). There is an unavoidable need for human beings to use different medicinal and herbal plants for a wide variety of purposes, *e.g.*, to treat different diseases, in industrial applications, cosmetics and perfumery disciplines among others (Mohammadhosseini et al., 2023). However, collecting and isolating these advantageous chemicals from natural sources continue to provide formidable obstacles for researchers (Jahan et al., 2023). The Annonaceae family, widely distributed in tropical and subtropical regions, comprises numerous species known for their therapeutic properties. This family is a significant area of focus due to the presence of various types of chemicals,

including alkaloids, non-alkaloid constituents and acetogenins. These chemicals have demonstrated a wide range of pharmacological effects and are currently undergoing clinical evaluation for the treatment of Parkinson's disease, cardiovascular disease, and viral infection (Suedee et al., 2007; Cuendet et al., 2008). One of the studies evaluated the secondary metabolites, antioxidant and antimicrobial properties of the fruit extracts of one of the members of the same family (Sathiyavani et al., 2023).

Anaxagorea luzonensis A. Gray has been traditionally used to treat ailments such as muscle pain, fever, stomach disorders, and rheumatism (Moghadamtousi et al., 2015; Gonda et al., 2000). Despite its traditional applications, limited scientific studies have explored its phytochemical composition and pharmacological potential (Tep-areenan et al., 2015; Lestari et al., 2023).

The present study aims to bridge this gap by investigating the bioactive constituents of the stem bark of *A. luzonensis* and assessing it's *in vitro* and *in vivo* biological activities. Employing a bioassay-guided approach, the study evaluates the antioxidant, cytotoxic, analgesic, and antipyretic effects of different solvent extracts. Additionally, it reports, for the first time, the isolation and structural elucidation of spinasterol from *A. luzonensis*. This research contributes to the growing body of knowledge on medicinal plants and highlights the potential of *A. luzonensis* as a source of natural bioactive compounds with therapeutic values.

2. Experimental

2.1 Reagents and chemicals

In this study, analytical grade solvents and chemicals were used. The solvents *n*-hexane, ethyl acetate, dichloromethane, carbon tetrachloride and methanol were purchased from Merk, Germany. The standards DPPH, gallic acid, ascorbic acid and others were supplied from Sigma Aldrich (Mumbai, India). All the other fine chemicals used in this study were provided by Merk, Germany.

2.2 Apparatus

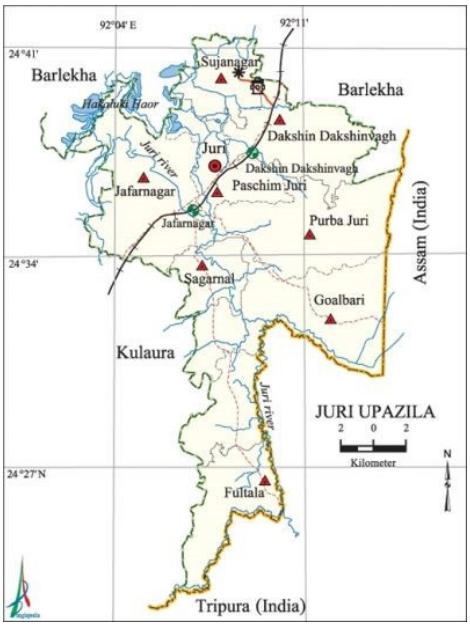
All the glassware, precoated thin-layer chromatography (TLC) aluminum sheets and other accessories were supplied from Merk, Germany. The glassware setup was used to conduct various *in vitro* tests, and plant-derived bioactive compounds were extracted, separated, and purified by using column chromatography, TLC, and preparative thin-layer chromatography (PTLC).

2.3 Collection and identification of the plant

In January 2024, *A. luzonensis* stem bark was obtained from the Lathitila Reserve Forest located in Moulvibazar, Juri, Bangladesh (Fig. 1a). The plant (Fig. 1b) was recognized by Mr. Adhikary Anjon Kumar, a specialist from the Bangladesh National Herbarium, and an authentic specimen has been preserved under accession number 99071. The stem bark was thoroughly washed and cut into small pieces, then dried in the sun for 15 days and in an oven at 40 °C for an additional 2 days. Finally, the dried material was ground into a fine powder.

2.4 Extraction and isolation

Powdered *A. luzonensis* bark (1.92 kg) was soaked in methanol (4 L) for 2 weeks, filtered, and concentrated to yield a dark green residue (40 g). Using modified Kupchan partitioning procedure (Emran et al., 2015), 13 g of the crude extract was dissolved in 100 mL of methanol and partitioned with *n*-hexane until the solution became colorless, followed by partitioning with carbon tetrachloride (CCl_4). The separated CCl_4 and *n*-hexane extract fractions then air dried for a week and yielded 6.5 and 6.0 g of dried extracts, respectively. Silica column chromatography was utilized to isolate compounds from the methanolic extract



(a) Map of the Juri Upazila, Moulvibazar, Bangladesh.

(b) *Anaxagorea luzonensis* plant.

Figure 1

(16 g) of *A. luzonensis*. The column, prepared with silica gel secured by glass wool and sand, was loaded with the extract after solvent removal. Eluted fractions were monitored visually for pigmentation and under UV light for colorless compounds. TLC confirmed the purity of the compounds and facilitated their identification, while preparative TLC further purified the combined fractions by analyzing the spots under UV light or fluorescence. Using tailored solvent systems, distinct compounds were isolated: Fractions 27-29 (dichloromethane: hexane, 15:85) yielded short, needle-like white crystals and powdered crystals weighing 3.6 mg (AL1); Fractions 30-40 (dichloromethane: hexane, 15:85) produced long, needle-like white crystals weighing 2.9 mg (AL2); and Fractions 251-295 (methanol: ethyl acetate, 10:90 to 100) resulted in a thick yellow to off-white compound with a creamy or oily consistency, weighing 0.9 mg (AL3). These findings highlight the effectiveness of selective solvent systems in isolating bioactive compounds.

2.5 Phytochemical screening

The existence of various phytoconstituent groups in the plant extract, such as carbohydrates, steroids, phenolics, flavonoids, alkaloids, saponins and so on, were determined using the established protocols (Shahriar et al., 2012a; Shahriar et al., 2012b; Siddique et al., 2013; Noor et al., 2014; Shahriar et al., 2016).

2.6 *In vitro* antioxidant activity tests

2.6.1 DPPH (1,1-Diphenyl-2-picrylhydrazyl) scavenging capacity assay

DPPH, a widely used free radical for antioxidant testing, exhibits an absorbance in the range of 515 to 517 nm. Each test tube received 2 mL of the reagent solution (DPPH: 0.004%) and 200 µL of the plant extracts or standards, and was then incubated for half an hour. The solution's absorbance was measured using a spectrophotometer at 517 nm, compared to a blank (Zohora et al., 2019). The inhibitory activity was estimated as a percentage (%).

$$\text{Inhibition (\%)} = \frac{A_o - A_1}{A_o} \times 100 \quad (1)$$

Eq. 1 was used to calculate the percentage (%) inhibitory activity. Next, inhibition percentages were plotted against concentration, and the IC_{50} was determined from the graph (Fig. 4).

2.6.2 Total antioxidant content test

The phosphomolybdenum method detects antioxidants by reducing Mo(VI) to Mo(V), forming a green complex measurable at 695 nm. The test tubes contained a mixture of the sample extracts and a reagent solution. The reagent solution was prepared by combining 3.3 mL of concentrated H_2SO_4 (98%), 0.381 g of sodium phosphate and 0.494 g of ammonium molybdate and the final volume adjusted up to 100 mL with distilled water in a volumetric flask. The resulting mixture was then incubated at 95 °C for about 90 minutes and the corresponding absorbance was measured at 695 nm. A typical blank solution, consisting of 3 mL of reagent solution, was used in place of the required amount of sample solution (300 µL) of the same solvent. The results expressed as ascorbic acid equivalents (Shahriar et al., 2012b; Akhter et al., 2013; Anjum et al., 2024).

2.6.3 Determination of total phenolic content

Phenolic compounds, commonly found in food, are closely linked to the functional capacity of many bioactive chemicals (Zahra et al., 2024). The Folin-Ciocalteu reagent (FCR) method measures phenolic compounds in plant extracts using UV/Vis. spectrophotometry at 765 nm. Test tubes containing 1 mL of extracts and standard were mixed with 5 mL of FCR solution (1.9-2.1 N, diluted tenfold), followed by the addition of 4 mL of sodium carbonate solution (7.5 *w/v* %). Then, the test tubes were incubated at 20.0 °C for 30 minutes for standard solutions and one hour at 20.0 °C for the plant extracts. The solutions' absorbances were measured at 765 nm using a spectrophotometer and compared to a control. The results were expressed as gallic acid equivalents (GAE), offering a reliable assessment of bioactive phenolics calculated by using the equation $y = 0.0045x + 0.2036$, with an R^2 value of 0.9947 (Hossain et al., 2012).

2.6.4 Total flavonoid content

Flavonoids, known for their anti-inflammatory, antioxidant, anticancer, and antimicrobial effects, were measured using a colorimetric aluminum chloride method regarding quercetin as a reference. Briefly, a mixture of 5.6 mL of distilled water, 3 mL of methanol, 0.2 mL of aluminum chloride (10.0%), and 0.2 mL of potassium acetate (1.0 M) was prepared with 1 mL of the sample and the standard at different concentrations. After 30 minutes at room temperature, the reaction mixture's absorbance was measured at 415 nm using a UV/Visible spectrophotometer.

The flavonoid concentration in the plant extracts was calculated using a quercetin calibration curve ($y = 0.0104x - 0.1029$, with an R^2 value of 0.9945) (Hossain et al., 2012).

2.7 Brine shrimp lethality assay

This investigation utilized *Artemia salina*, or brine shrimp, as the test organism. Cysts were hatched in saline water (38 g/L NaCl) for 48 hours, transforming into living nauplii. Carbon tetrachloride, methanolic and *n*-hexane extracts were prepared in various concentrations using dimethyl sulfoxide (DMSO) as the solvent. Each test tube was inoculated with ten nauplii, and the volume was adjusted with saline water. Vincristine sulfate, a highly cytotoxic alkaloid, served as the positive control, tested at concentrations of 25, 12.5, 5, 1, 0.5, 0.25, 0.125, and 0.06 µg/mL. Three premarked test tubes holding 4.9 mL of simulated sea water each received 50 µL of DMSO. Ten shrimp nauplii were added to serve as negative control groups. After a 24-hour rest period, all test tubes were counted to determine the number of living and dead nauplii using a magnifying glass against a black background. The brine shrimp lethality assay evaluates the cytotoxicity of plant extracts by exposing brine shrimp larvae (nauplii) to various extract concentrations for 24 hours and recording mortality rates. Using 2-20 mg of extract dissolved in DMSO and diluted with saltwater, nauplii survival is analyzed under black light. Mortality percentages are plotted against the logarithm of concentra-

tions to determine the median lethal concentration (LC_{50}), indicating the concentration required to kill 50% of nauplii. This simple, cost-effective assay correlates brine shrimp toxicity with potential therapeutic applications (Shohan et al., 2024).

2.8 *In vivo* pharmacological activities

2.8.1 Experimental animal

Swiss albino mice of either sex, 4-5 weeks of age, weighing between (10-24 g) were collected from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), Dhaka. Animals were kept under standard environmental conditions (temperature: 27 ± 1 °C, relative humidity: 55-65%, and a 12-hour light/12-hour dark cycle) and had unrestricted access to food and water. The animals were acclimatized to laboratory condition for one week prior to experiments (Aziz et al., 2014).

2.8.2 Evaluation of analgesic activity

The acetic acid writhing test in mice was conducted as described by Khandaker et al. (2016). Thirty-six mice were divided into six groups. Group I served as the control, while Group II served as the standard. Group III and IV received methanolic extract (ME) of 100 and 200 mg/kg and Group V and VI received the same amounts of crude CCl_4 extract (CE). In the current experiment, diclofenac was used as a typical medicine.

2.8.3 Antipyretic activity study

This study assessed the plant extract's ability to reduce fever caused by Brewer's yeast in mice (Khandaker et al., 2016). A digital thermometer was used to gauge basal rectal temperature at zero hours. Animals with an increase of at least 0.6 °C over 18 hours were chosen for the investigation. Pyrexia was produced by administering 10 mL/kg of body weight of a suspension of Brewer's yeast (15 *w/v*%) in distilled water subcutaneously. Total thirty-six mice were divided into six groups. Group I served as the control, while Group II served as the standard. Group III and IV received methanolic extract (ME) of 100 and 200 mg/kg and Group V and VI received the same amounts of crude CCl_4 extract (CE). Tween-80 (1.0%) and paracetamol were used as control and standard, respectively.

2.9 Acute toxicity test

The acute toxicity of *A. luzonensis* stem bark extracts was assessed by conventional method (Malik et al., 2007). Fifty-four mice were divided into nine groups. Group I served as the control with water. Groups II to V received oral dosages of 500 to 4000 mg/kg of crude methanolic extract, and Groups VI to IX received the same amounts of crude CCl_4 extract (Shahriar et al., 2014).

Statistical analysis

Data was expressed as mean ± standard error of mean (SEM). The results were analyzed statistically by ANOVA followed by Dunnet's test. Results with $p < 0.05$, $p < 0.01$ and $p < 0.001$ were considered statistically significant.

3. Results and discussion

3.1 Phytochemical screening

This study conducted a phytochemical screening of both polar and nonpolar extracts from the stem bark of *A. luzonensis*. The methanolic extract contained carbohydrates, glycosides, glucosides, saponins, flavonoids, and alkaloids. Similarly, the CCl_4 extract also exhibited the presence of these compounds, including carbohydrates, glycosides, glucosides, saponins, flavonoids, and alkaloids (Table 1).

3.2 Characterization of isolated compound

Compound **1** (Fig. 2) from the fractions 27-29 (AL1), obtained as white crystals, gave a quenching spot under 254 nm and a blue fluorescent spot under 356 nm UV light on a TLC plate. The ^{1}H-NMR spectrum (Fufa et al., 2018) and ^{13}C-NMR spectrum data (Fieser et al., 1949) were directly compared to those of spinasterol that had previously been extracted to identify the structure. To the best of our knowledge, this is the first report on the isolation and characterization of spinasterol from *A. luzonensis*. Spinasterol (**1**): White crystal mass (3 mg); ^{1}H-NMR (600 MHz, $CDCl_3$): 3.59 (m, H-3), 5.15 (br s, H-7), 0.55 (3H, s, H-18), 0.80 (3H, s, H-19), 1.03 (d, J = 6.6 Hz, H-21), 5.02 (dd, J = 8.4 Hz, H-22), 5.16 (dd, J = 9.3 Hz, H-23), 0.85 (d, J = 6 Hz, H-26), 0.85 (d, J = 6 Hz, H-27), 0.81 (t, J = 7.2 Hz, H-29) (Fig. 3a).

Table 1. Phytochemical screening of *A. luzonensis*.

Phytochemical tests	Methanolic extract	*n*-hexane extract	CCl_4 extract
Carbohydrate	++	+	++
Glycoside	+	+	+
Glucoside	++	++	++
Saponin	++	++	++
Tannin	-	-	-
Flavonoid	++	++	+
Alkaloid test by different reagents			
Hager's reagent	++	++	++
Dragendroff's reagent	++	++	++

(+) = Present in mild quantity; (++) = Present in moderate quantity; (-) = Absent

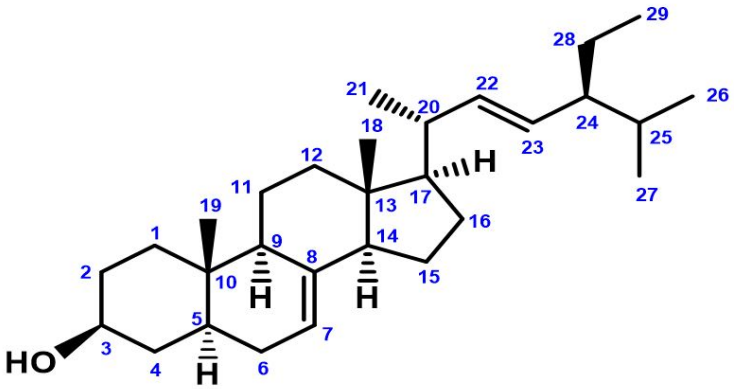
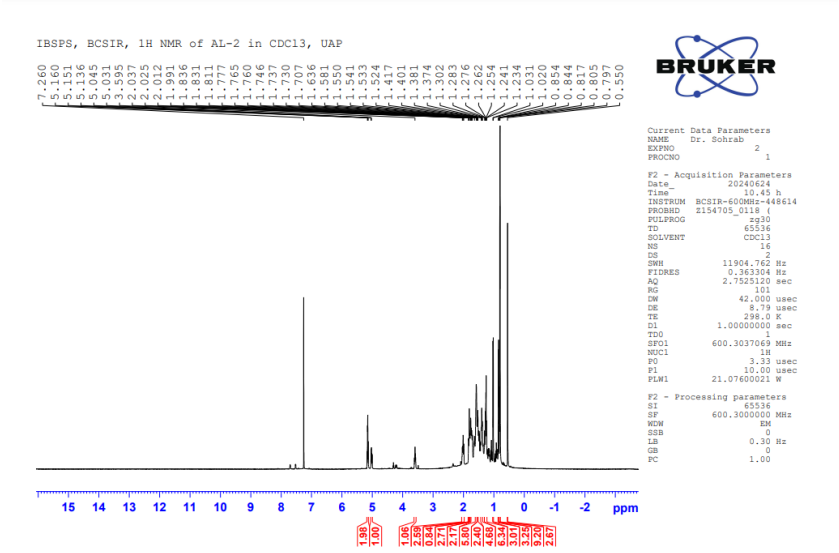
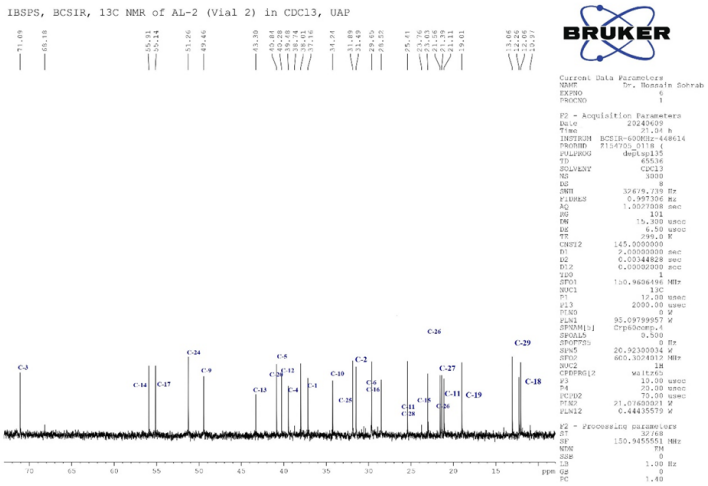


Figure 2. Chemical structure of spinasterol.

^{13}C-NMR (150 MHz, $CDCl_3$): 37.16 (C-1), 31.49 (C-2), 71.09 (C-3), 38.01(C-4), 40.28 (C-5), 29.65 (C-6), 117.48 (C-7), 139.59 (C-8), 49.46 (C-9), 34.24 (C-10), 25.41(C-11), 39.48 (C-12), 43.3 (C-13), 55.91 (C-14), 23.03 (C-15), 29.65 (C-16), 55.14 (C-17), 12.06 (C-18), 19.01 (C-19), 40.84 (C-20), 21.11(C-21), 138.19 (C-22), 129.46 (C-23), 51.26 (C-24), 31.89 (C-25), 21.56 (C-26), 21.39 (C-27), 25.41(C-28), 12.26 (C-29) (Fig. 3b).



(a) ^{1}H-NMR (600 Hz, $CDCl_3$) spectrum of AL2_$CDCl_3$.



(b) ^{13}C-NMR (600 Hz, $CDCl_3$) spectrum of AL2_$CDCl_3$.

Figure 3

Isolate no 2: Fractions 30-40 (dichloromethane: hexane, 15:85) (AL2), and isolate no 3: Fractions 251-295 (methanol: ethyle acetate, 10:90 to 100) (AL3) remained unidentified as pure compounds.

3.3 Antioxidant activity

Linear regression analysis was conducted to assess the DPPH radical scavenging activity of methanol, *n*-hexane, and CCl_4 extracts, alongside ascorbic acid serving as a standard reference (Fig. 4). The IC_{50} values were 76.89, 55.77, and 157.57 µg/mL for methanol, *n*-hexane, and CCl_4 extracts, respectively (Table 2).

3.3.1 Total antioxidant capacity

The total antioxidant capacity of various extracts was assessed using the phosphomolybdenum method and expressed as ascorbic acid equivalents (mg/g). The methanol extract exhibited the highest antioxidant capacity (30.22 ± 1.16 mg/g), followed by the *n*-hexane extract (27.48 ± 1.42 mg/g), with the CCl_4 extract showing the lowest activity (17.62 ± 0.58 mg/g) (Table 2).

3.3.2 Total phenolic content

The total phenolic content of the methanolic, *n*-hexane, and CCl_4 extracts of *A. luzonensis* stem bark was determined using the Folin-Ciocalteu reagent and expressed as gallic acid equivalents (mg/g). The methanol extract exhibited the highest phenolic content (22.99 ± 0.28 mg/g), followed by the *n*-hexane extract (19.58 ± 1.96 mg/g). The CCl_4 extract showed lower phenolic content, highlighting methanol as the most effective solvent for phenolic compound extraction (Table 2).

3.3.3 Total flavonoid content

The flavonoid content of *A. luzonensis* stem bark extracts was quantified using the aluminum chloride and expressed as quercetin equivalents (mg/g). The methanolic extract exhibited the highest flavonoid content (19.14 ± 0.16 mg/g), followed by the CCl_4 extract (17.65 ± 0.31 mg/g) and *n*-hexane extract (15.10 ± 0.36 mg/g) (Table 2).

3.4 Brine shrimp lethality assay

Vincristine sulfate (VS), the positive control in this investigation, has an LC_{50} value of 0.164 µg/mL. When VS was utilized as the negative control, the death rate significantly increased. Next, the LC_{50} values of each extract were evaluated in comparison to the negative control. The results showed that the LC_{50} values for the CCl_4, *n*-hexane, and methanolic extracts were 1.611, 1.498 and 0.034 µg/mL, respectively (Table 3).

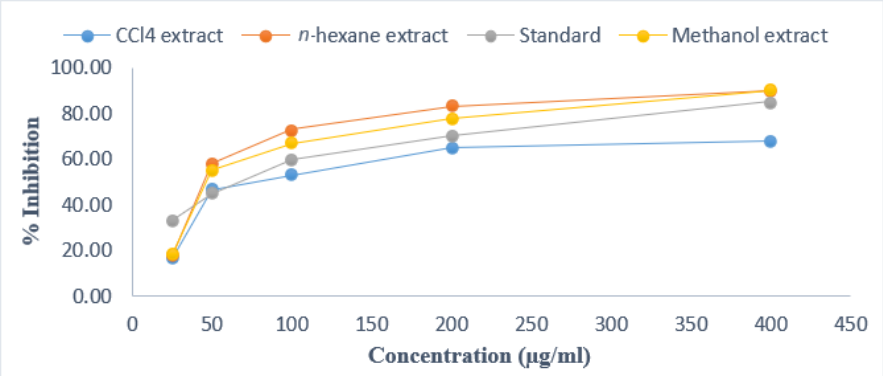


Figure 4. Comparison of DPPH scavenging activity of *A. luzonensis* stem bark extracts against standard ascorbic acid.

Table 2. Phytochemical composition and antioxidant activity of different extracts from *A. luzonensis*.

Sample	Total phenol content, mg/g GAE ± STD	Total flavonoid content, mg/g QE ± STD	Total antioxidant capacity, mg/g AAE ± STD	DPPH scavenging capacity assay, IC_{50} (µg/mL)
Methanolic extract	22.99 ± 0.28	19.14 ± 0.16	30.22 ± 1.16	76.889
n-hexane extract	19.58 ± 1.96	15.10 ± 0.36	27.48 ± 1.42	55.768
CCl_4 extract	10.15 ± 0.05	17.65 ± 0.31	17.62 ± 0.58	157.57

Table 3. LC_{50} values of *A. luzonensis* and standard.

Test samples	Regression line	R^2 values	LC_{50} (µg/mL)
Vincristine sulfate	$y = 43.584x + 42.847$	$R^2 = 0.9664$	0.164
CCl_4 extract	$y = 18.587x + 49.374$	$R^2 = 0.9477$	0.034
n-hexane extract	$y = 42.711x - 13.992$	$R^2 = 0.9643$	1.498
Methanol extract	$y = 41.92x - 17.53$	$R^2 = 0.9835$	1.611

3.5 Analgesic activity

In this study, the analgesic activity of methanolic and CCl_4 stem bark extracts of *A. luzonensis* was evaluated using the acetic acid-induced writhing assay in Swiss albino mice. Intraperitoneal administration of acetic acid elicited pain, resulting in an average of 15.17 writhing episodes in the control group. Oral administration of methanolic extract (ME) at 100 and 200 mg/kg reduced writhing by 48.36% and 64.86%, respectively, while the CCl_4 extract (CE) reduced writhing by 24.19% and 36.28% at the same doses. For comparison, diclofenac (50 mg/kg) achieved 69.23% inhibition (Fig. 5).

The methanolic extract demonstrated a dose-dependent analgesic effect, which was significant ($p < 0.05$) compared to the reference drug (Fig. 5 and Table 4). A positive correlation was observed between basal reaction time and both extract concentrations and exposure duration, underscoring the moderate analgesic potential of *A. luzonensis* extracts, particularly the methanolic extract.

3.6 Antipyretic activity

The antipyretic activity of *A. luzonensis* stem bark extracts was evaluated in Brewer's yeast-induced pyrexia in mice. Baseline body temperature was recorded before administering methanolic and CCl_4 extracts at doses of 100 and 200 mg/kg body weight. Distilled water (0.2 mL/10 g) served as the control, while paracetamol (50 mg/kg) was used as the standard. Body temperatures were monitored over three hours. Methanolic extracts exhibited significant antipyretic effects, reducing body temperature by 2.3 °F and 1.2 °F at doses of 200 and 100 mg/kg, respectively (Fig. 6). The CCl_4 extract caused reductions of 1.4 °F and 0.8 °F at the corresponding doses. Paracetamol, the standard drug, showed the highest efficacy, reducing body temperature by 3.1 °F at 50 mg/kg. These findings highlight the antipyretic potential of *A. luzonensis*, particularly its methanolic extract.

Comparing the methanol and CCl_4 extracts to the control group, statistical analysis showed that they had substantial antipyretic effects ($p < 0.05$). These results imply that *A. luzonensis* extracts may be useful as a natural antipyretic, indicating the need for more research into their processes and potential therapeutic uses.

3.7 Acute toxicity study

An acute toxicity study of *A. luzonensis* stem bark extracts was conducted in mice at oral doses of 500, 1000, 2000, and 4000 mg/kg for methanolic and CCl_4 extracts. General behavior, adverse effects, and mortality were observed over 24 hours, with a control group for comparison. The methanolic extract demonstrated no mortality across all tested doses, including 4000 mg/kg, indicating its non-toxic nature. In contrast, the CCl_4 extract exhibited toxicity, with 50% mortality at 1000 mg/kg and 100% mortality at doses of 2000 mg/kg and higher. These findings confirm the safety of the methanolic extract and highlight the acute toxicity of the CCl_4 extract at elevated doses.

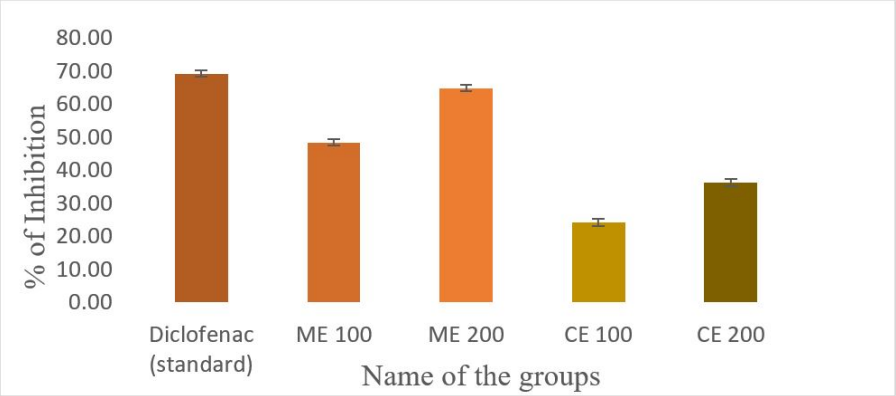


Figure 5. Comparison of writhing (%) inhibition of *A. luzonensis* different extracts (ME: Methanolic extract; CE: CCl_4 extract).

Table 4. Analgesic effect of different extracts of *A. luzonensis* acetic acid induced writhing test.

Group No	Groups	Dose (mg/kg) body weight	Number of writhing	Mean± SEM
I	Control	0.2 mL/10 g of body weight	15.17	15.17 ± 1.01
II	Standard	50 mg/kg	4.67	4.67 ± 0.67
III	ME 100	100 mg/kg	7.83	7.83 ± 0.91***
IV	ME 200	200 mg/kg	5.33	5.33 ± 0.95***
V	CE 100	100 mg/kg	11.50	11.50 ± 0.43**
VI	CE 200	200 mg/kg	9.67	9.67 ± 0.49***

ME: Methanolic extract; CE: CCl_4 extract; Values are expressed as mean ± SEM ($n = 6$). Statistical significance was determined using an independent samples t-test, with significance levels indicated as follows: $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$, indicating significant differences when compared to the corresponding values of the standard group.

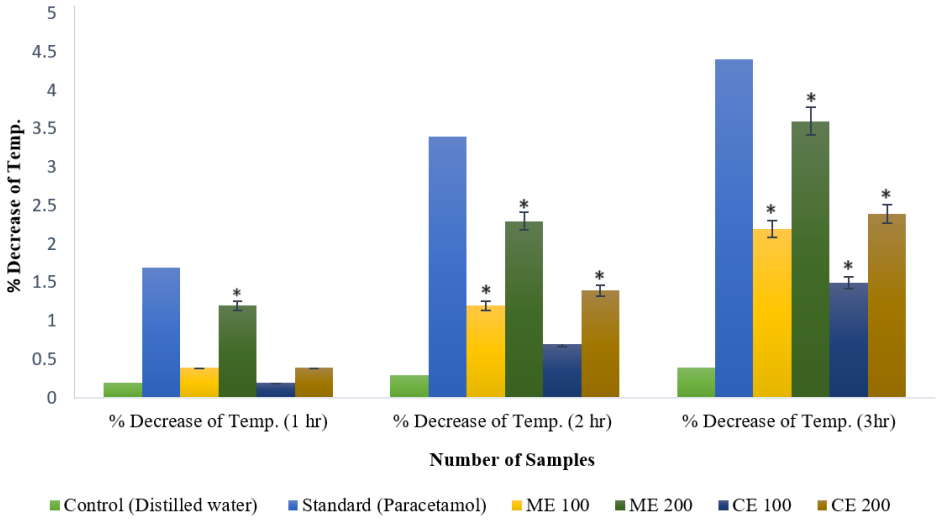


Figure 6. Comparison of the antipyretic effects of different extracts of *A. luzonensis* (ME: Methanolic extract; CE: CCl_4 extract); values are expressed as mean ± SEM ($n = 6$). Significance levels are indicated as follows: (*$p < 0.05$), (**$p < 0.01$), (***$p < 0.001$), indicating significant differences when compared to the corresponding values of the standard group, as determined by an independent sample t-test).

3.8 Comprehensive comparison

Natural antioxidants from fruits, vegetables, spices, and medicinal plants have gained significant attention for their therapeutic potential in protecting against various diseases. These biological activities are largely attributed to secondary metabolites, which play a crucial role in numerous pharmacological studies. Spinasterol, found in the methanolic extract, is a sterol compound recognized for its pharmacological properties, including anti-inflammatory, antitumor, and neuroprotective effects, which have been reported for the first time from *A. luzonensis* (Rahaman et al., 2023).

Phytochemical screening revealed the presence of alkaloids, carbohydrates, saponins, glycosides, and glucosides, aligning with previous findings. The absence of flavonoids in the CCl_4 extract underscores the therapeutic diversity of these extracts. The methanolic extract exhibited the highest total phenolic (22.99 ± 0.28 mg/g) and flavonoid content (19.14 ± 0.16 mg/g), correlating with its strong antioxidant activity (IC_{50} = 76.89 µg/mL) and total antioxidant capacity (30.22 ± 1.16 mg/g ascorbic acid). Despite lower phenolic content, the *n*-hexane extract displayed superior antioxidant activity (IC_{50} = 55.77 µg/mL), suggesting contributions from other bioactive compounds. The CCl_4 extract demonstrated moderate antioxidant activity (IC_{50} = 157.57 µg/mL), highlighting the importance of phenolic and flavonoid content in enhancing antioxidant efficacy. The results of the present study are analogous to the one conducted earlier by Yokoyama et al. (2002).

In cytotoxicity assays, the CCl_4 extract exhibited the highest toxicity, while *in vivo* studies demonstrated significant analgesic and modest antipyretic effects for the methanolic extract. At 200 mg/kg, the methanolic extract reduced body temperature by 2.3 °F and exhibited dose-dependent analgesic effects, suggesting its potential for safe therapeutic use. Conversely, the CCl_4 extract demonstrated considerable toxicity at higher doses, emphasizing the need for detailed toxicological studies.

4. Concluding remarks

This study successfully isolated and characterized spinasterol from *A. luzonensis*, marking its first reported occurrence in this species. The phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, glycosides, and carbohydrates, which contribute to the plant's pharmacological effects. Among the solvent extracts tested, the methanolic extract exhibited the highest antioxidant activity, phenolic and flavonoid content, and moderate analgesic and antipyretic effects. The cytotoxicity assessment further demonstrated the potential of *A. luzonensis* extracts in therapeutic applications. Given the observed pharmacological properties, *A. luzonensis* holds promise as a natural source for developing safe and effective herbal medicines. However, further research is necessary to elucidate its precise mechanisms of action, identify additional active constituents, and evaluate its long-term safety profile. Future studies involving molecular docking, in-depth toxicological assessments, and clinical trials could pave the way for the development of novel drugs derived from this medicinal plant.

Acknowledgment

The Department of Pharmacy at UAP is appreciated by the authors for its assistance in providing the required laboratory support, and the ICDDR, B is acknowledged for its cooperation in providing Swiss albino mice and advice on the care of laboratory animals. The BCSIR for the NMR analysis of isolated plant compounds and Bangladesh National Herbarium's taxonomist for his helpful assistance in identifying the plant are also acknowledged by the authors.

Authors contributions
Sabiha Rahman: Investigation and drafting. Fatema-Tuz-Zohora: Data curation, writing, and editing. Ramisa Anjum: Data curation, writing, and editing. A.H.M. Nazmul Hasan: Data curation, writing, and editing. Mohiuddin Ahmed Bhuiyan: Validation, writing, and editing. Choudhury Mahmood Hasan: Validation and formal analysis. Mohammad Shahriar: Supervision, visualization, conceptualization, methodology, validation, formal analysis, editing, and reviewing.

Availability of data and materials
The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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

References

Akhter, R., Haque, M.A., Bhuiyan, M.A., Shahriar, M. (2013) *In vivo* pharmacological investigation of leaf of *Polygonum hydropiper* L. *Dhaka Univ. J. Pharm. Sci.* 12(2):165–169. DOI: https://doi.org/10.3329/dujps.v12i2.17619.

Anjum, R., Hasan, A.H.M.N., Hawa, M.A.A., Rahman, M.N., Akanda, M.K.M., Wahed, T.B., Salam, F.B.A., Islam, M.R., Shahriar, M. (2024) Characterization of pharmacological properties and isolation of two bioactive compounds (ursolic acid and palmitoleic acid) from the stem bark extract of *Lannea coromandelica*. *Trends Phytochem. Res.* 8(2):106–121. DOI: https://doi.org/10.71596/tpr.2024.1126058.

Aziz, U., Akther, R., Shahriar, M., Bhuiyan, M.A. (2014) *In vivo* pharmacological investigation of *Mimosa pudica* L. *Int. J. Pharm. Pharm. Sci.* 6(2):66–69.

Cuendet, M., Oteham, C.P., Moon, R.C., Keller, W.J., Peaden, P.A., Pezzuto, J.M. (2008) Dietary administration of *Asimina triloba* (Paw Paw) extract increases tumor latency in N-methyl-N-nitrosourea treated rats. *Pharm. Biol.* 46(1-2):3–7. DOI: https://doi.org/10.1080/13880200701729497.

Emran, T.B., Rahman, M.A., Uddin, M.M.N., Rahman, M.M., Uddin, M.Z., Dash, R. (2015) Effects of organic extracts and their different fractions of five Bangladeshi plants on *in vitro* thrombolysis. *BMC Complement. Altern. Med.* 15:1–8. DOI: https://doi.org/10.1186/s12906-015-0643-2.

Fieser, L.F., Fieser, M., Chakravarti, R.N. (1949) α-Spinasterol. *J. Am. Chem. Soc.* 71(6):2226–2230. DOI: https://doi.org/10.1021/ja01174a085.

Fufa, M.F., Deressa, F., Deyou, T., Abdisa, N. (2018) Isolation and characterization of compounds from the leaves of *Melia azedarach* and stem bark of *Albizia schimperiana* and evaluation for antimicrobial activities. *Med. Chem.* 8:154–165. DOI: https://doi.org/10.4172/2161-0444.1000507.

Gonda, R., Takeda, T., Akiyama, T. (2000) Studies on the constituents of *Anaxagorea luzonensis* A. Gray. *Chem. Pharm. Bull.* 48(8):1219–1222. DOI: https://doi.org/10.1248/cpb.48.1219.

Hossain, M.I., Sharmin, F.A., Akhter, S., Bhuiyan, M.A., Shahriar, M. (2012) Investigation of cytotoxicity and *in vitro* antioxidant activity of *Asparagus racemosus* root extract. *Int. Curr. Pharm. J.* 1(9):250–257. DOI: https://doi.org/10.3329/icpj.v1i9.11615.

Hounsou, E.K., Sonibare, M.A., Elufioye, T.O. (2024) Climate change and the future of medicinal plants research. *Bioact. Compd. Health Dis.* 7(3):152–169. DOI: https://doi.org/10.31989/bchd.v7i3.1310.

Jahan, N., Shaari, K., Islam, S.N., Azam, A.Z., Ahsan, M. (2023) Chemical and biological profiling of three ferulic-acid alkyl esters isolated from *Jatropha pandurifolia* stem bark. *Biomed. Pharmacol. J.* 16(2):817–826. DOI: https://doi.org/10.13005/bpj/2664.

Khandaker, S., Das, S., Opo, F.M., Akhter, R., Shahriar, M. (2016) *In vivo* pharmacological investigations of the crude extracts of *Calamus viminalis* L. *J. Pharmacogn. Phytochem.* 5(3):263–269.

Lestari, D.A., Ningrum, L.W., Nada, M.H., Pradipta, N.N., Harsono, D.R. (2023) Flowering and fruiting phenology of *Anaxagorea luzonensis* A. Gray (Annonaceae). *Biodiversitas J. Biol. Divers.* 24(2):214–220. DOI: https://doi.org/10.13057/biodiv/d240214.

Malik, F., Singh, J., Khajuria, A., Suri, K.A., Satti, N.K., Singh, S., Kaul, M.K., Kumar, A., Bhatia, A., Qazi, G.N. (2007) A standardised root extract of *Withania somnifera* and its major constituent withanolide-A elicit humoral and cell-mediated immune responses by up-regulation of Th1-dominant polarisation in BALB/c mice. *Life Sci.* 80(16):1525–1538. DOI: https://doi.org/10.1016/j.lfs.2007.01.029.

Moghadamtousi, S.Z., Fadaeinasab, M., Nikzad, S., Mohan, G., Ali H.M., Kadir, H.A. (2015) *Annona muricata* (Annonaceae): A review of its traditional uses, isolated acetogenins and biological activities. *Int. J. Mol. Sci.* 16(7):15625–15658. DOI: https://doi.org/10.3390/ijms160715625.

Mohammadhosseini, M., Jeszka-Skowron, M. (2023) A systematic review on the ethnobotany, essential oils, bioactive compounds, and biological activities of *Tanacetum* species. *Trends Phytochem. Res.* 7(1):1–29. DOI: https://doi.org/10.30495/tpr.2023.700612.

Noor, S., Prodhan, A., Zohora, F.T., Tareq, F.S., Ahsan, M., Hasan, C.M., Islam, S.N. (2014) Phytochemical, antioxidant, antimicrobial, thrombolytic as well as cytotoxic studies on the stem bark of *Manilkara zapota* (Sapotaceae). *Asian J. Chem.* 26(18):6138–6142. DOI: https://doi.org/10.14233/ajchem.2014.16872.

Rahaman, M.M., Hossain, R., Herrera-Bravo, J., Islam, M.T., Atolani, O., Adeyemi, O.S., Owolodun, O.A., Kambizi, L., Daştan, S.D., Calina, D., Sharifi-Rad, J. (2023) Natural antioxidants from some fruits, seeds, foods, natural products, and associated health benefits: An update. *Food Sci. Nutr.* 11(4):1657–1670. DOI: https://doi.org/10.1002/fsn3.3217.

Sathiyavani, P., Vasantha, S., Gurusaravanan, P. (2023) HPLC analysis, phytochemical screening, in-vitro antioxidant and antibacterial activity of *Annona muricata* L. fruit extracts. *Trends Phytochem. Res.*7(2):117-126. DOI: https://doi.org/10.30495/tpr.2023.1984564.1335.

Shahriar, M., Akhter, S., Hossain, M.I., Haque, M.A., Bhuiyan, M.A. (2012a) Evaluation of *in vitro* antioxidant activity of bark extracts of *Terminalia arjuna*. *J. Med. Plants Res.* 6(39):5286–5298. DOI: https://doi.org/10.5897/JMPR12.580.

Shahriar, M., Hossain, M.I., Bahar, A.N.M., Akhter, S., Haque, M.A., Bhuiyan, M.A. (2012b) Preliminary phytochemical screening, *in vitro* antioxidant and cytotoxic activity of five different extracts of *Moringa oleifera* leaf. *J. Appl. Pharm. Sci.* 2(5):65–68. DOI: https://doi.org/10.7324/JAPS.2012.2510.

Shahriar, M., Islam, M.R., Hassan, M.R., Uddin, M.M.N. (2014) Biological investigations of the leaf extract of *Spondias pinnata*. *World J. Pharm. Res.* 4(3):252–256.

Shahriar, M., Khair, N., Sheikh, Z., Chowdhury, S., Kamruzzaman, M., Bakhtiar, M., Chisty, S., Narjish, S., Akhter, R., Akter, N. (2016) Phytochemical analysis, cytotoxic and *in vitro* antioxidant activity of *Erythrina variegata* bark. *Eur. J. Med. Plants* 11(3):1–5. DOI: https://doi.org/10.9734/EJMP/2016/18866.

Shanta, A.P., Zohora, F.T., Mahtarin, R., MacKerell, A.D., Ahsan, M. (2024) Isolation of phytoconstituents from an extract of *Murraya paniculata* with cytotoxicity and antioxidant activities and in silico evaluation of their potential to bind to aldose reductase (AKR1B1). *J. Biomol. Struct. Dyn.,* 1–15.

Sharif, N., Jabeen, H. (2024) Natural sources for coumarins and their derivatives with relevance to health-promoting properties: A systematic review. *Trends Phytochem. Res.* 8(3):149–162. DOI: https://doi.org/10.71596/tpr.2024.1103148.

Shohan, M.S.R., Azam, A.M.Z., Das, S., Bhuiyan, M.A., Ahsan, M. (2024) Isolation, antioxidant, cytotoxic and thrombolytic activity of *Cynometra ramiflora*. *J. Pharmacol. Pharm. Res.* 2(1):6–14. DOI: https://doi.org/10.5455/JPPR.20241023094802.

Siddique, K.I., Uddin, M.M.N., Islam, M.S., Parvin, S., Shahriar, M. (2013) Phytochemical screenings, thrombolytic activity and antimicrobial properties of the bark extracts of *Averrhoa bilimbi*. *J. Appl. Pharm. Sci.* 3(3):94–96. DOI: https://doi.org/10.7324/JAPS.2013.30318.

Singh, D., Mittal, N., Siddiqui, M.H. (2023) A review on pharmacological potentials of phenolic diterpenes carnosic acid and carnosol obtained from *Rosmarinus officinalis* L. and modern extraction methods implicated in their recovery. *Trends Phytochem. Res.* 7(3):156–169. DOI: https://doi.org/10.30495/tpr.2023.1990761.1365.

Suedee, A., Mondranondra, I.O., Kijjoa, A., Pinto, M., Nazareth, N., Nascimento, M. (2007) Constituents of *Polyalthia jucunda* and their cytotoxic effect on human cancer cell lines. *Pharm. Biol.* 45(7):575–579. DOI: https://doi.org/10.1080/13880200701501300.

Tabassum, F., Islam, S.N., Tuz-Zohora, F., Hasan, C.M., Rahman, K.M., Ahsan, M. (2024) Isolation of a new iso-quinoline alkaloid and cytotoxicity studies of pure compounds and crude extracts of *Ravenia spectabilis* Engl. *Heliyon* 10(14):e34508. DOI: https://doi.org/10.1016/j.heliyon.2024.e34508.

Tep-areenan, P., Wetchasit, P., Sawasdee, P. (2015) Extracted *Anaxagorea luzonensis* A. Gray restored impairment of endothelium-dependent vasorelaxation induced by homocysteine thiolactone in rat aortic rings. *J. Med. Assoc. Thai.* 98(Suppl. 10):S31–S37.

Wang, H., Chen, Y., Wang, L., Liu, Q., Yang, S., Wang, C. (2023) Advancing herbal medicine: Enhancing product quality and safety through robust quality-control practices. *Front. Pharmacol.* 14:1265178. DOI: https://doi.org/10.3389/fphar.2023.1265178.

Yokoyama, S., Takeda, T., Takahashi, H., Ohta, Y., Park, S.H., Nishikawa, T., Abe, M. (2002) Lipid peroxidation and the antioxidant effects of 3,5,7,4'-tetrahydroxy-2'-methoxyflavone; direct observation by atomic force microscopy. *J. Oleo Sci.* 51(1):63–67. DOI: https://doi.org/10.5650/jos.51.63.

Zahra, S.F.T., Shahriar, M., Zohora, F.T., Das, S., Akter, R., Bhuiyan, M.A. (2024) Phytoconstituent screening and analysis of antioxidant, membrane-stabilising and thrombolytic properties of leaves and rhizomes of *Schumannianthus dichotomus* (Roxb.) Gagnep. *Trop. J. Nat. Prod. Res.* 8(9):8474–8481. DOI: https://doi.org/10.26538/tjnpr/v8i9.33.

Zohora, F.T., Islam, S.N., Khan, S.A., Hasan, C.M., Ahsan, M. (2019) Antioxidant, cytotoxic, thrombolytic and antimicrobial activity of *Zanthoxylum rhetsa* root bark with two isolated quinolone alkaloids. *Pharmacol. Pharm.* 10(3):137–145.