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Phytochemical Screening, GC-MS Analysis, and Evaluation of Antioxidants, Cytotoxicity, Analgesic, and Anti-Diarrheal Activity of the Extracts of the Leaves of *Syzygium reticulatum* Wight Walp

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Abstract

Syzygium genus is extensively used in managing different diseases such as antimicrobial, anti-diarrheal, antidiabetic, analgesic, anti-inflammatory, antioxidant, and anticancer activities. The present study aims to investigate phytochemicals by screening tests and GC-MS analysis and evaluate the antioxidants, cytotoxicity, analgesic, and anti-diarrheal activity of the extracts of the leaves of *Syzygium reticulatum*. The leaves of the *S. reticulatum* were extracted with hexane and methanol by the maceration process successively, denoted as SRH and SRM. The extract's phytochemical analyses found flavonoids, alkaloids, saponins, tannins, glycosides, and carbohydrates. The GC-MS analysis of the methanol extract identified 8 compounds. The major components were identified as Diisooctyl phthalate (96.903 %), 2-methyltetracosane (1.094 %), and others with fewer amounts. The result of the antioxidant study of the extracts as total phenolic content of SRH 8.92 ± 0.62 and SRM 340.107 ± 11.11 mg/g, respectively, total flavonoids content of SRH 7.85 ± 0.71 and SRM 105.16 ± 0.93 mg/g, IC50 values of the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay of SRM was 14.67 µg/mL, SRH was 41.26 µg/mL and ascorbic acid 14.08 µg/mL; the total antioxidant capacity of SRH was 21.33 ± 2.52 mg/g, whereas SRM was 353.71 ± 4.59 mg/g. The results of these studies suggest that methanol extract has a more potent antioxidant capacity than hexane extract. The methanol extract also exhibited higher toxicity (LC50 value 49.99 µg/mL) compared to hexane extract (LC50 value 370.348 µg/mL), as revealed by the Brine shrimp nauplii cytotoxic study. An analgesic study of the methanol extract found that a lower dose (250 mg/kg) has more significant analgesic activity than the higher dose and standard drug aspirin. At 90 min, the hot plate's highest percentage of latency elongation was 110.32, while the acetic acid-induced writhing inhibition was 63.04 %. At a dose of 500 mg/kg, the methanol extract also shown good efficacy in mice suffering from castor oil and magnesium sulfate-induced diarrhea. The findings from the study of the leaves of the *S. reticulatum* exhibited that methanol extracts have significant antioxidant, cytotoxic, analgesic, and anti-diarrheal activity.

Keywords: *Syzygium reticulatum,* Phytochemicals, Antioxidant, GC-MS, Cytotoxicity, Analgesic, Antidiarrheal

Introduction

Natural products are a broad family of different chemical substances that have a wide range of biological effects that have led to a variety of uses, mainly in agricultural and veterinary medicine [1]. Natural products have a wide range of applications because of their wide range of biological effects. Natural products derived from plants, animals, and minerals, as well as their byproducts, are considered to be the most effective medications for treating a wide variety of conditions, and herbal medicine has been making use of them for a very long time [2]. Phytochemicals found in plants and animals, such as alkaloids, glycosides, flavonoids, and polyphenols, have been shown to possess therapeutic qualities [3]. These properties include the ability to act as antioxidants, antitumors, analgesics, anti-inflammatory agents, hepatoprotective agents, cytotoxic agents, and antimicrobial agents, among others.

The plants of the genus Syzygium have long been revered in tropical and semitropical regions for their curative properties. Several different species of this genus have been shown to be effective in treating

a variety of conditions, including bacteria, diarrhea, diabetes, pain, inflammation, oxidation, and cancer [4-10]. A literature review found that the leaves of *Syzygium formusum* and *Syzygium balsameum* contain a number of phytochemical components that are biologically potent. These components include alkaloids, glycosides, flavonoids, tannins, and saponins, among others. In addition, these chemicals have beneficial effects as antioxidants, cytotoxic agents, thrombolytic agents, anti-diarrheal agents, and antibacterial agents [6]. The investigation of *Syzygium formosum* using HPLC-PDA-ESI-MS/MS reveals a total of 28 compounds. These contents include 11 flavonoids, 13 triterpene acids, and 4 phenolic acids [10]. The use of several species of Syzygium as potential medicinal agents has been the focus of a great number of studies carried out all over the world over the course of the last ten years [4]. *S. reticulatum* is a Syzygium genus that may also give the above activities or contain medicinal value. No report of folk use of *S. reticulatum*. We were interested in commencing phytochemical screening, GC-MS analysis, and different pharmacological activities such as antioxidant, cytotoxic, analgesic, and anti-diarrheal activity.

Materials and methods

Chemicals and instruments

Ammonium molybdate, ascorbic acid, and other solvents such as ethanol, methanol, dichloromethane, etc. procured from Merck Germany. DPPH, gallic acid, quercetin, from Sigma Aldrich, etc. Solvents were purified by fractional distillation using a distillation apparatus before use. Shimadzu (UV-1800) spectrophotometers were used to measure the absorbance of different experiment solution and others instruments at the Laboratory, Department of Pharmacy, Gono Bishwabidyalay, Savar, Dhaka-1344. GC-MS apparatus Shimadzu (GC 2010 Plus MS) applied to determine the chemical constituents of the extract in Institute of National Analytical Research and Service, Bangladesh Council of Scientific and Industrial Research (BCSIR) Dr. Qudrat-I-Khuda Road, New Elephant Road, Dhanmondi, Dhaka-1205, Bangladesh.

Plant material & extraction

The green leaves of *S. reticulatum* were collected from the hillock of Chittagong, Bangladesh. At the Bangladesh National Herbarium, Zoo Road, Mirpur-1, Dhaka 1216, the plant was identified under the DACB number 56303. It is also found in India (Assam, Meghalaya) and Myanmar. The leaves of the plants were dried in the shade for several weeks and finally in an oven at 40 °C, then ground into a coarse powder using an electric grinder. The powdered substance (500 g) was placed in a fresh, flat-bottom glass vessel and immersed in hexane and methanol successively (2,500 mL). After being sealed for 72 h, the containers underwent periodic shaking and sonication. The extracts were then filtered on a piece of brand-new, white cotton fabric before being placed on filter paper. The filtrates were each dried separately on rotary evaporator (Bibby RE-200, Sterilin, UK). The freezer's 4 °C temperature was used to preserve the dried crude extracts. The hexane extract yielded a green viscous concentrate designed as (SRH), whereas methanol extract gave a green solid denoted (SRM).

Phytochemical screening

Following the procedures outlined by Harborne and Sazada *et al.* [11,12], the freshly obtained crude extracts were qualitatively examined to identify phytochemicals such as alkaloids, flavonoids, glycosides, saponins, polysaccharides, and tannins.

Instrument and procedure for SRM GC-MS analysis

The analytes that can endure the rigorous partitioning conditions of the gas chromatograph [13] and are volatile and thermally labile are the only ones that can be identified and quantified using the GC-MS special analysis technique. This technique shows a typical spectrum output of all the compounds from the empirical sample that can be determined. A distinct spectral pick is electronically recorded for each component. In the current study, SRM was analyzed using a GC-17A gas chromatograph coupled to an MS 2010 plus mass spectrometer utilizing the Electron Impact Ionization (EI) method. Helium was employed as the carrier gas, and its constant pressure of 49.5 kPa was used to maintain the fused silica capillary column's temperature at 40 °C. By splitting with a split ratio of 50, the sample was injected. In methanol, the material was dissolved. The following operating circumstances were applied: The column's name is SH-Rxi-5Sil, and its dimensions are as follows: Length 30 m, diameter 0.25 mm, column flow rate 1 mL/min, injector temperature 250 °C, holding time 10 min, splitting samples were injected using a split ratio of 50, carrier gas helium sample dissolved in methanol, range of linear temperature increase 10 °C per min, and m/z ratio range 50 - 800. The chemicals from the leaves of the plant *S. reticulatum* soluble in methanol could be identified and measured using the GC-MS analysis. The mass spectrum of the GC-MS

instrument was analyzed using the National Institute of Standards and Technology's database (NIST). The known component's spectra recorded in the NIST collection and the unknown molecule were compared [13,14].

Antioxidant activity

Total phenolic content

The colourimetric detection of phenolic and polyphenolic antioxidants in the hexane and methanol extracts uses the Folin-Ciocalteu reagent (FCR), a combination of phosphomolybdate and phosphotungstate. [15,16] It measures the quantity of the substance under test necessary to prevent the reagent from oxidizing. However, any reducing chemical in an extract causes this reagent to react. As a result, the reagent assesses a sample's overall reducing capacity rather than just the concentration of phenolic compounds. This is accomplished by tracking the reversible one- or two-electron reduction event sequences that give rise to blue species, most likely $(PMoW_{11}0_{40})^4$. The experiment was performed 3 times, with 3 test subjects. The total amount of phenolic compounds present in plant extracts were calculated as gallic acid equivalents (GAE) using the following equation:

$$C = (c \times V)/m \quad (1)$$

where C = mg/g of plant extract as GAE or the overall amount of phenolic chemicals. c is the quercetin concentration measured in mg/mL using the calibration curve. V is the extract's volume in millilitres, and m is its gram-weight.

Total content flavonoid

The total flavonoid content was determined using the aluminium chloride colourimetric technique, according to Chang *et al.* [17]. For the aluminium chloride colourimetric method, the 2 groups in which aluminium chloride creates acid-stable compounds are the C-4 keto group of flavones and flavonols and either the C-3 or C-5 hydroxyl group. Additionally, the ortho-dihydroxyl groups in the A or B ring of flavonoids interact with aluminum chloride to create complexes that are acid-labile. The complexes generated by flavonols with C-3 and C-5 hydroxyl groups, such as galangin, morin, and kaempferol, have the highest absorbance at 415 - 440 nm, according to the results of wavelength scans of the complexes of 15 standards with aluminum chloride. The quantity of flavonoid content in plant extracts was calculated using the equation below as quercetin equivalents (QE).

$$C = (c \times V)/m \quad (2)$$

C = mg/g plant extracts total flavonoid content as QE. c is the quercetin concentration measured in mg/mL using the calibration curve. V is the extract's volume in milliliters, and m is its gram-weight.

Assay for DPPH free radical scavenging

DPPH was used as a positive control to assess both extracts' antiradical efficacy. The oxidation of other substances is sparked by DPPH, a reactive free radical and an electron acceptor (oxidizing agent). Antioxidants, however, act as electron sources (reducing agents). Antioxidants oxidize in order to combat DPPH. The solid form of DPPH is a deep violet-colored crystalline powder consisting of stable free radical molecules. The transformation of the deep violet colour into pale yellow or colourless (neutralization) represents the scavenging of the DPPH free radical [19]. The calculus of the percent inhibition (PI) is as follows:

$$PI = \left(1 - \frac{Absorbance\ of\ sample}{Absorbance\ of\ Control}\right) \times 100 \quad (3)$$

Total antioxidant capacity

Antioxidants in substances at lower concentrations than those found in oxidizable substrates (proteins, lipids, carbohydrates, and DNA) greatly slow or stop oxidation [20,21]. Antioxidants' primary purpose is to shield the body against the harm that free radicals can cause [22]. The phosphomolybdenum technique of Prieto *et al.* [23] was used to assess the extract's overall antioxidant activity. The experiment is based on the Mo (VI)-Mo (V) reduction by the extract and consequent synthesis of the greenish PO_4/Mo (V) complex at an acidic medium. Each test solution required 3 test subjects, and the experiment was carried out 3 times.

The equation given below was used to calculate the total antioxidant capacity in extracts as ascorbic acid equivalents (AAE).

$$C = (c \times V)/m \quad (4)$$

where C = mg/g plant extracts as AAE total antioxidant capacity V = the volume of extracts in mL; c = the concentration of quercetin was determined from the calibration curve in mg/mL; m = the weight of plant extracts in grams.

Cytotoxicity study

Using the Brine Shrimp lethality bioassay established by Meyer *et al.* [24], the cytotoxicity of *S. reticulatum* leaves might be evaluated. The cytotoxicity of plant extracts was evaluated using the Brine Shrimp Lethality Bioassay. *In vivo*, lethality in a basic zoological creature is used in the technique. The cytotoxicity of 9KB (human nasopharyngeal cancer) and brine are highly associated ($p = 0.036$ and kappa = 0.56). LC_{50} readings from the Brine Shrimp test are typically one-tenth of the ED_{50} values for cytotoxicity. The formula was used to determine mortality.

$$\%\ Mortality = \frac{No.of\ nauplii\ (taken - alive}{No.of\ nauplii\ alive} \times 100 \quad (5)$$

In-vivo biological assay

Experimental animals

The study obtained ethical approval from the Gono Bishwabidyalay, Center for Multidisciplinary Research Ethical Committee, with registration number CMR/EC/007. Swiss Albino adult mice of either sex, weighing 25 - 35 g and aged 6 - 7 weeks, were acquired from the animal house of the pharmacy department at Jahangirnagar University. All mice were provided with commercial pellets to consume and access to unlimited water. Before the trial began, the mice were accustomed to all the procedures for a week to reduce stress. Each mouse used in this investigation was handled according to the generally acknowledged standards for managing laboratory animals [25].

Acute toxicity test

Lorke's methodology was used to determine acute toxicity tests. Three groups of 3 Swiss albino mice of either sex, weighing 25 - 35g each, were used in this experiment. Different doses of the test substance 10, 100, 500, and 1,000 mg/kg-are given to each group of animals. The animals are kept under observation for 24 h to track their behaviour and determine whether any of them will die [26].

Evaluation of analgesic activity

Hot plate method

To determine the central analgesic effectiveness of the extracts, a mouse was placed into an open-ended conical chamber with a floor made of a metallic plate heated by a thermode. The temperature of the plate was maintained at 55 ± 1 °C, which caused the behavioural components- paw licking, paw withdrawal, and jumping- that be quantified in terms of their reaction times. All of the reactions were regarded as supraspinally integrated. Each mouse was put on a hot plate with a 15-second cutoff time in order to protect the paws from damage. The amount of time required to lick the paw or jump off the hot plate was used to estimate reaction time [27]. After administering group I (distilled water at 10 mL/kg p.o.), II (aspirin 100 mg/kg p.o.), groups III & IV (SRM at 250 and 500 mg/kg b.w., respectively), the reaction durations were measured at 0 and 30, 60, 90, 120, and 150 min. The following equation is how the % increase in response time was calculated:

$$\%\ \text{elongation} = \frac{latency\ (test - control)}{latency\ control} \times 100 \quad (6)$$

Writhing test induced by acetic acid

Through the use of overnight fasting mice with free access to water, a method for detecting peripheral analgesic activity was used. All mouse groups received an injection of "Acetic acid (0.6 % v/v) (10 mL/kg, i.p.)" 1 h after the extract, vehicle, or standard administration. The number of animals writhing for 30 min after a 5-minute latency period, which involves contracting their abdominal muscles and stretching their rear limbs, was used to measure the extract's analgesic activity [28]. The extract's analgesic effectiveness was demonstrated by a reduction in the number of writhes compared to the control group, and this was measured as a percentage inhibition of writhing as follows:

$$\% \text{ Analgesia} = \left(1 - \frac{\text{mean writhing treated}}{\text{mean } writhing \text{ control}}\right) \times 100 \quad (7)$$

Antidiarrheal test

Castor oil-induced diarrhea

The approach of Jebunnessa *et al.* (2009) was slightly adjusted to examine the anti-diarrheal potential of the plant extracts [29]. After a 12-hour fast, mice of either sex were put into 4 groups of 6 mice each. Group I served as the control group and was given 10 mL/kg of DW orally. The dose of loperamide (standard) for Group II was 10 mg/kg administered orally. Methanol extracts of S. reticulatum were given orally to groups III and IV at doses of 250 and 500 mg/kg b.w., respectively. Mice were given 0.5 mL of castor oil orally to produce diarrhea an hour after dosing. For 4 h, we counted the total amount of both dry and moist stools the animals produced every hour. The percentage inhibition of faeces and the percentage inhibition of diarrhea were used to express each group's activity [30].

Magnesium sulfate-induced diarrhea

Four groups of 6 mice each were fed nothing for 12 h. Group I served as the control group and was given 10 mL/kg of DW orally. The standard loperamide for Group II was 10 mg/kg administered orally. SRM were given orally to Groups III and IV at doses of 250 and 500 mg/kg b.w., respectively. Each mouse was given magnesium sulfate (2 g/kg b.w.) orally after 1 h to cause diarrhea. Each animal was housed in its cage on top of white filter paper [29,30]. The number of feces and diarrhea each group prevented was used to measure their activity. The formula for percent inhibition (PI) was as follows:

$$\text{PI} = \left(1 - \frac{mean\ defecation\ treated}{mean\ defecation\ control}\right) \times 100 \quad (8)$$

Statistical analysis

SPSS (Statistical Package for Social Sciences) version 26 and Microsoft Excel 2019 were used to analyse the results. All results are express as Mean ± SEM (Standard error of the mean). The results were deemed statistically significant at p 0.05. Statistical significance was established by utilizing a One-way Analysis of Variance (ANOVA) followed by a Dunnett post hoc test to compare changes among groups. The results were compared to the vehicle control group, with p-values less than 0.05, 0.01 and 0.001, denoting statistically significant, very significant, and significant levels of significance, respectively.

Results and discussion

Phytochemical studies

The n-hexane and methanol extracts of *S. reticulatum* have undergone preliminary phytochemical screening, and the results show the existence of several bioactive substances such as alkaloids, glycosides, saponins, etc. which are listed in **Table 1(a)**.

Table 1(a) Analysis of phytochemicals' quality.

Investigated phytochemical	SRH	SRM
Alkaloids	+	+
Tannins	−	+
Flavonoids	+	+
Saponins	−	+
Carbohydrates	+	−
Glycosides	−	+

The presence is indicated by the (+) symbol and its absence by the (−) symbol.

Result of GC-MS analysis

Spectrums of the recognized component recorded in the NIST collection and the unknown molecule were compared. **Table 2** lists the retention duration, molecular weight, and component percentage of the *S. reticulatum* sample materials. The contents in the sample were identified and quantified using the analytical GC-MS approach [14]. In total, according to our current analysis, 8 methanol-soluble chemicals were found

in the methanol extract of *S. reticulatum* leaves. The compounds' identities could be confirmed by examining the data's chemical formula, retention time, and peak area. **Table 1(b)** and **Figure S1** display the whole outcome of the GC-MS analysis.

Table 1(b) GC-MS study of methanol extracts of the leaves of *S. reticulatum.*

SI. No.	Retention time	Name of the compounds	Conc.	M.W	Molecular formula
1	23.27	Octadecane-1-sulphonyl chloride	0.175 %	353.0	$C_{18}H_{37}ClO_2S$
2	24.188	2-methyltetracosane	0.402 %	352.7	$C_{25}H_{52}$
3	25.067	zinc bis(dipentyldithiocarbamate)	0.653 %	530.2	$C_{22}H_{44}N_2S_4Zn$
4	26.250	Diisooctyl phthalate	96.903 %	390.6	$(C_8H_{17}COO)_2C_6H_4$
5	26.721	2-methyltetracosane	0.692 %	352.7	$C_{25}H_{52}$
6	27.529	Acetamide,2,2'-[(2,2-dimethyl-1,3-propanediol	0.496 %		-
7	27.963	trans-2-Tridecenal	0.246 %	196.33	$C_{13}H_{24}O$
8	28.299	L-Sorbofuranose, pentakis(trifluoroacetate) (isomer 1)	0.138 %	660.19	$C_{16}H_7F_{15}O_{11}$
9	29.342	2-bromohexadecanoic acid	0.296 %	335.32	$C_{16}H_{31}BrO_2$

Table 1 shows that approximately 100 % of the indicated components were present in the crude methanol extract of *Syzygium reticulatum* leaves. The major components were identified as Diisooctyl phthalate (96.903 %), 2-methyltetracosane (1.094 %), zinc bis (dipentyldithiocarbamate) (0.653 %), Acetamide,2,2'-[(2,2-dimethyl-1,3-propanediol] (0.496 %), Hexadecanoic acid, 2-bromo- (0.296 %) and others with less amount. The literature review found that some of the above compounds have antioxidant and antimicrobial properties [31-37]. Literature also found that hexadecanoic acid, 2-bromo- is used as a PPARδ agonist, inhibit fatty acid oxidation, inhibit DHHC-mediated palmitoylation, and promote glucose uptake in rat cardiac cells and the insulin-sensitive murine fibroblast line A31-IS [36,37]. That indicates that SRM may be used in several biological activity.

***In-vitro* antioxidant study**

Total phenol content

The total phenolic content of the SRH and SRM was calculated using the Folin-Ciocalteu reagent and is expressed as Gallic acid equivalents (GAE) per gram of plant extract. The total phenolic contents are calculated using the gallic acid calibration graph ($y = 0.0093x + 0.1114$, $R^2 = 0.9954$). The results are presented in **Tables 2**, **S1** and **Figure 1**.

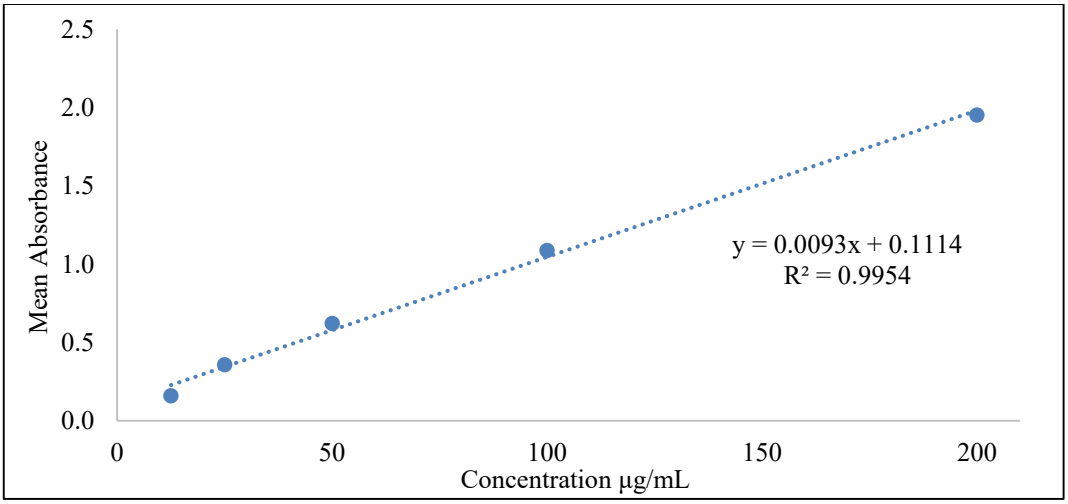

Figure 1 Galic acid calibration curve.

Table 2 Total phenolic contents of the SRH and SRM.

Sample name	Total phenolic content (mg/g, GAE) Mean ± SEM	99 % Confidence interval for mean (lower bound-upper bound)
SRH	8.92 ± 0.62	2.763 - 15.083
SRM	340.107 ± 11.11	229.837 - 450.376

The present study exhibited lower phenolic content (8.92 ± 0.62 mg/g) in the hexane extract, and methanol extract (340.107 ± 11.11 mg/g) exhibited higher phenolic content equivalent to standard GAE. Indicates SRM have good antioxidant property.

Total flavonoid content

The aluminum chloride colourimetric method, the total flavonoid concentration of the extracts was determined. Using the quercetin standard curve (y = 0.0062x + 0.0186; R^2 = 0.9996), the quantity of all flavonoids in the plant extract was measured and expressed as quercetin equivalents (QE) per gram. **Tables 3**, **S2** and **Figure 2** present the findings of the total flavonoid content measurement.

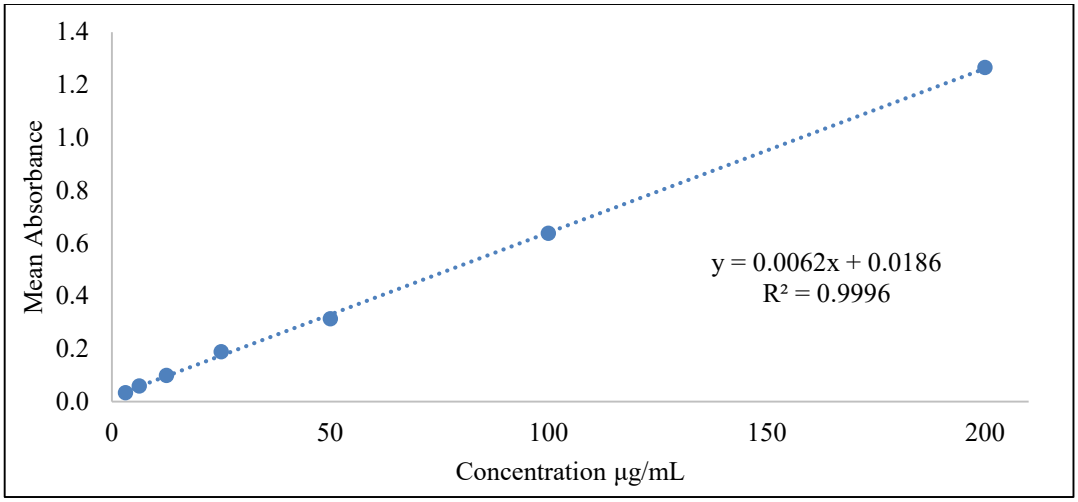

Figure 2 Quercetin calibration curve.

Table 3 Flavonoid content in SRH and SRM.

Name of sample	Total flavonoids content (mg/g of QE) Mean ± SEM	99 % Confidence Interval for mean (lower bound-upper bound)
SRH	7.85 ± 0.71	0.788 - 14.905
SRM	105.16 ± 0.93	95.935 - 114.385

The result of the flavonoid study exhibited that methanol extract has higher flavonoid content (105.16 ± 0.93 mg/g) than hexane extract (7.85 ± 0.71 mg/g). The higher the flavonoid content greater the antioxidant activity, thus SRM has good antioxidant action.

DPPH free radical scavenging assay

The amount of decolourization caused by DPPH accepting an electron given to it by an antioxidant chemical can be calculated from changes in absorbance. **Table 4** and **Figure 3** show the IC_{50} values of the *S. reticulatum* extracts.

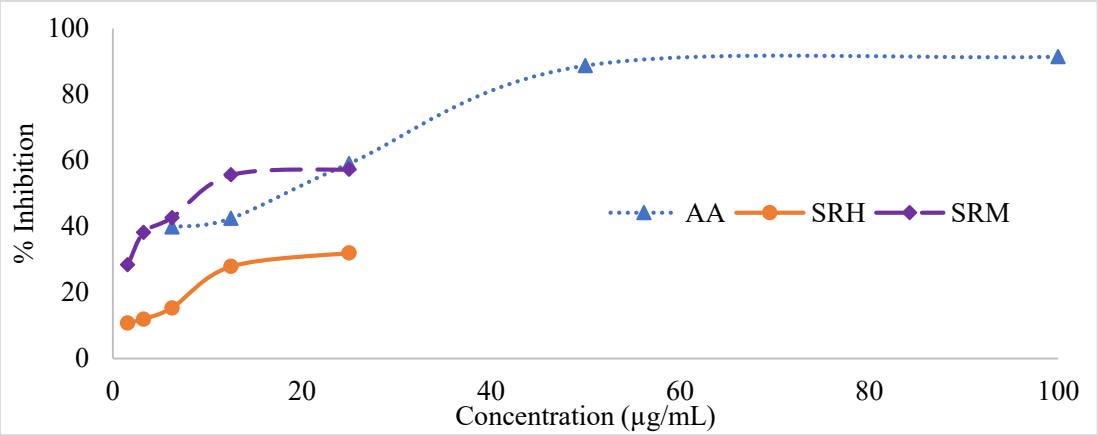

Figure 3 Ascorbic acid and *S. reticulatum* extracts DPPH-free radical scavenging properties.

Table 4 IC_{50} values of the SRH, SRM & Ascorbic acid.

Sample/Standard	IC_{50} (µg/mL)
Ascorbic acid	14.08
SRH	41.26
SRM	14.67

DPPH scavenging is a popular technique to assess a compound's capacity to neutralize free radicals [38]. A lower IC_{50} value indicates more antioxidant activity and a greater decolorizing impact. The *S. reticulatum* leaves methanol extract demonstrated notable antioxidant power (IC_{50} = 14.67 µg/mL), comparable to conventional antioxidants. Hexane extract has a weaker antioxidant action (IC_{50} = 41.26 µg/mL) than methanol and ascorbic acid (IC_{50} = 14.08 µg/mL). The extract's level of phenolic and flavonoid compounds responsible for good free radical scavenger, which is higher in methanol extract, maybe the cause of its ability to scavenge DPPH.

Total antioxidant capacity

The total antioxidant capacity of the SRH and SRM was calculated using the ascorbic acid calibration graph and represented ascorbic acid equivalents (AAE) per gram of the plant extracts (y = 0.0035x + 0.0404, R^2 = 0.9926). The findings of the total antioxidant capacity are shown in **Tables 5**, **S3** and **Figure 4**.

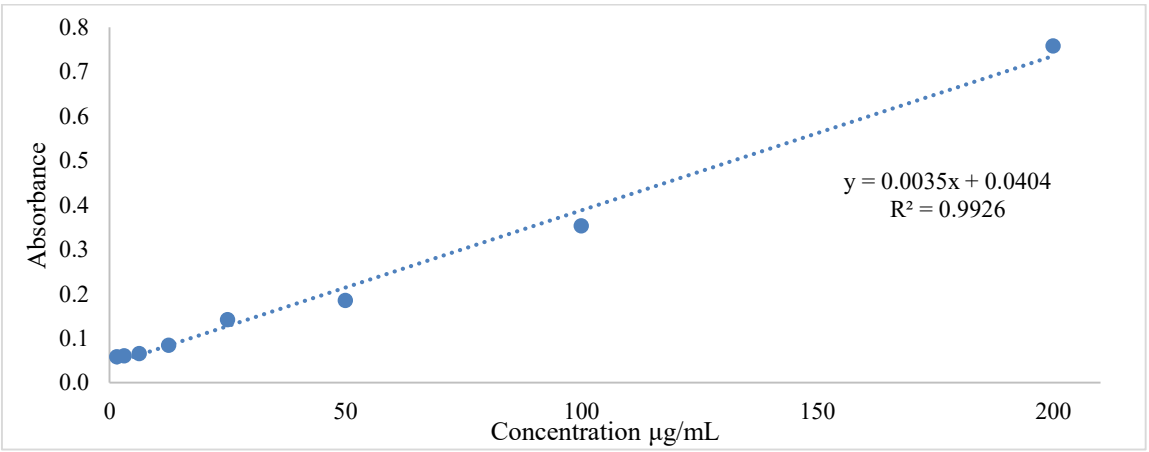

Figure 4 Calibration curve for ascorbic acid.

Table 5 Total antioxidant capacity mg/g of AAE.

Sample Name	Total antioxidant capacity mg/g of AAE (Mean±SEM)	99 % Confidence Interval for mean (lower bound-upper bound)
SRH	21.33 ± 2.52	−3.675 - 46.342
SRM	353.71 ± 4.59	308.137 - 399.291

In this study, methanol extract showed good antioxidant capacity (353.71 ± 4.59 mg/g) as AAE and hexane extract exhibited lower antioxidant capacity.

Cytotoxicity study

The results of the cytotoxicity study are presented in **Figure 5** and **Table 6**. LC_{50} values of the cytotoxicity study indicate that hexane extract is less cytotoxic (370.348 µg/mL) compared to standard vincristine sulfate (0.534 µg/mL). In contrast, methanol extract is good cytotoxic (49.99 µg/mL) compared to standard.

Table 6 LC_{50} value of SRH, SRM and vincristine sulfate (VS).

Sample/Standard	LC_{50} (µg/mL)
SRH	370.348
SRM	49.99
Vincristine sulfate	0.534

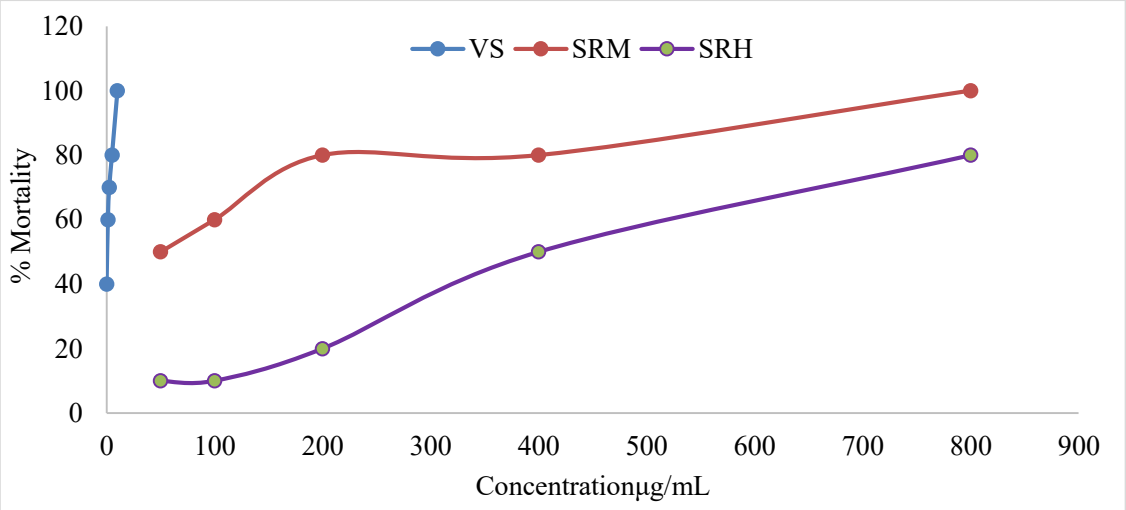

Figure 5 Percent of mortality of Vincristine sulfate (VS), SRM, and SRH.

Acute toxicity test

During the observation period, mice at dosages of 10, 100, 500, and 1,000 mg/kg, p.o., showed no adverse effects or death. The dose for analgesic activity was set at 250 and 500 mg/kg b.w. for a dose-dependent study based on the findings of this investigation.

Analgesic activity study

Hot plate method test

The central analgesia of the methanolic extract in mice was assessed by the hot plate technique. **Table 7** lists the outcomes of the hot plate test. The maximum analgesic activity was observed at 90 min with SRM (250 mg/kg) and was 110.34 %. But standard drug (Aspirin-10 mg/kg) produced the highest 64.29 % at 30 min. One Way ANOVA test analysis and elongation percentage showed that the reaction latency significantly decreased from 30 to 120 min (**Table 7**). The latency was delayed significantly by methanol extract at different periods ($p < 0.05$, $p < 0.01$) compared to the control. SRM 250 showed a significant %

of elongation or latency from 60 to 120 min 55.76, 110.34, 74.76 %. SRM 500 also showed good analgesic activity, which is very close to significant; the highest % of elongation is 78.78 % at 90 min.

Table 7 Analgesic activity of SRM and standard Aspirin.

Groups/ Time	**Mean latency (s) before and after drug administration(s)**					
	0 min	30 min	60 min	90 min	120 min	150 min
Control	5.97 ± 0.44	5.83 ± 0.62	6.37 ± 0.61	6.28 ± 0.76	6.93 ± 0.48	7.43 ± 0.68
STD	5.90 ± 0.58	**9.58 ± 1.29***	8.53 ± 0.43	7.95 ± 0.43	8.50 ± 0.76	6.58 ± 0.84
SRM 250	5.68 ± 0.54	7.92 ± 0.73	**9.92 ± 0.68****	**13.22 ± 2.17****	**12.17 ± 1.97***	9.05 ± 1.61
SRM 500	6.42 ± 0.34	7.70 ± 0.41	8.43 ± 0.85	11.23 ± 1.65	10.68 ± 1.17	10.13 ± 1.61
***elongation percentage* (*Percentage increase in reaction time or pain threshold inhibition*)**						
STD		64.29 %	34.03 %	26.53 %	23.32 %	−11.43 %
SRM 250		35.71 %	55.76 %	110.34 %	74.76 %	21.75 %
SRM 500		32.00 %	32.46 %	78.78 %	53.85 %	36.32 %

Note: Results are expressed as Mean ± SEM. Control = Water Control 10 mL/kg; STD = Aspirin treated group with a dose 100 mg; SRM 250 & 500 = *S. reticulatum* with dose 250 & 500 mg/kg.

Acetic induced writhing test

Compared to the control, the methanolic extract of *S. reticulatum* at a dose of 250 mg demonstrated statistically significant peripheral analgesic efficacy ($p < 0.01$). (**Table 8**). A one-way ANOVA test analysis revealed a considerably lower writhing inhibition. The maximum percentage of writhing numbers was inhibited by standard medication with corresponding percentages of *S. reticulatum* extract (**Table 8**). Inhibition percentages for SRM250 and SRM500 were significantly lower than those for the control SRM250 (63.04 %), SRM500 (28.80 %), and STD (41.85 %) for the acetic acid-induced writhing. When compared to Aspirin, SRM250 significantly ($p < 0.01$) inhibited writhing.

Table 8 Effect of aspirin and SRM on mice's writhing after exposure to acetic acid.

Treatment groups	**No. of writhing**	**Percent of inhibition**	***p*-value**
Control	30.67 ± 2.99	0.00	-
STD (Aspirin)	17.83 ± 4.96	41.85 %	0.100
SRM 250	**11.33 ± 5.61****	**63.04 %**	**0.010**
SRM 500	21.83 ± 1.83	28.80 %	0.325

Note: Results are expressed as Mean ± SEM. Control = Water Control 10 mL/kg; STD = Aspirin treated group with a dose 100 mg; SRM 250 & 500 = S. *reticulatum* with dose 250 & 500 mg/kg.

Antidiarrheal test

Diarrhea was caused by magnesium sulfate and castor oil

The percentage of feces and diarrhea inhibited by a methanolic extract of *Syzygium reticulatum* (leaves) was used to measure the anti-diarrheal activity. The findings revealed that a higher dose of the *Syzygium reticulatum* extract in test animals resulted in a statistically negligible decrease in the incidence total feces in castor oil-induced diarrhea, but good % inhibition (68.00 %) of severity of diarrhea compared to the loperamide (76 %) by reducing mean diarrheal feces. The SRM 250 lacked any defense mechanisms against episodes of diarrhea. Magnesium sulfate has laxative properties. Insignificantly, at lower dose, the methanolic extract of *Syzygium reticulatum* decreased the overall number of stools and diarrheal episodes in $MgSO_4$-induced diarrhea. On the other hand, SRM500 exhibited better percent of defecation of diarrheal feces 71.88 % and loperamide (46.88 %). In comparison to the control SRM 250 and standard, models SRM 500 exhibit good anti-diarrheal activity in both cases. **Tables 9** and **10** present the findings of the antidiarrheal study.

Table 9 Effect of Loperamide and SRM on castor oil-induced diarrhea in mice.

Groups	Mean of total feces	% Inhibition of defecation	Mean of diarrheal feces	% Inhibition of defecation
Control	8.5 ± 2.25	-	6.25 ± 2.29	-
STD	5.00 ± 0.71	41.18 %	1.50 ± 0.5	76.00 %
SRM 250	13.5 ± 3.59	−58.82 %	7.00 ± 3.85	−12.00 %
SRM 500	7.5 ± 3.01	11.76 %	2.00 ± 1.23	68.00 %

Note: Results are expressed as Mean ± SEM. Control = Water Control 10 mL/kg; STD = Loperamide treated group with a dose 10 mg/kg p.o.; SRM 250 & 500 = *S. reticulatum* with dose 250 & 500 mg/kg p.o.

Table 10 Effect of Loperamide and SRM on magnesium sulfate-induced diarrhea in mice.

Groups	Mean of total faeces	% Inhibition of defecation	Mean of Diarrheal faeces	% Inhibition of defecation
Control	18.25 ± 3.61	-	8.00 ± 4.24	-
STD	14.05 ± 1.85	20.55 %	4.25 ± 0.85	46.88 %
SRM 250	16.75 ± 2.78	8.22 %	5.00 ± 1.96	37.50 %
SRM 500	13.50 ± 4.25	26.03 %	2.25 ± 1.32	71.88 %

Note: Results are expressed as Mean ± SEM. Control = Water Control 10 mL/kg; STD = Loperamide treated group with a dose 10 mg/kg p.o.; SRM 250 & 500 = *S. reticulatum* with dose 250 & 500 mg/kg p.o.

Conclusions

The current study reveals that *Syzygium reticulatum* leaf extracts had strong antioxidant activity. Hexane extract does not perform as well as methanol extract. Good cytotoxic action is also detected in the methanol extract of *Syzygium reticulatum* leaves against the brine shrimp test. Analgesic and anti-diarrheal studies were carried out with methanol extract. Studies on analgesia using a hot plate and the acetic acid writhing test revealed significant analgesic effectiveness at a lower dose (250 mg/kg) than the standard drug aspirin. The anti-diarrheal activity of the extract showed good activity at the higher dose (500 mg/kg) compared to the standard in both models. GC-MS analysis also indicated the presence of several phytochemicals; the chemicals also reported several biological activities such as antioxidant, hypoglycemic, etc. Thus, we can conclude that leaves of *Syzygium reticulatum* may use in the management of different diseases.

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Supplementary file

Table S1 Analysis of phytochemicals' quality.

Investigated phytochemical	SRH	SRM
Alkaloids	+	+
Tannins	-	+
Flavonoids	+	+
Saponins	-	+
Carbohydrates	+	-
Glycosides	-	+

The presence of the phytochemical is indicated by the (+) symbol and its absence by the (-) symbol.

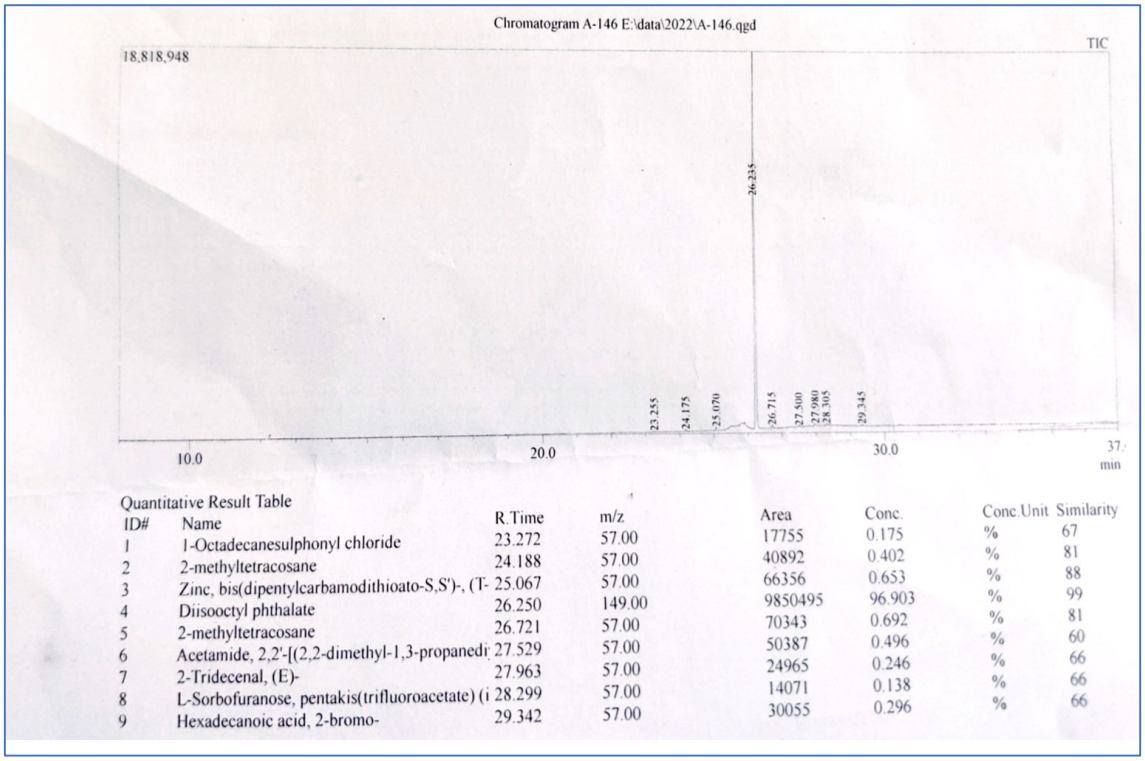

Figure S1 GC-MS chromatogram of SRM.

Table S2 Three consecutive absorption of gallic acid at different concentration.

Concentration (µg/mL)	Absorbance-1	Absorbance-2	Absorbance-3	Mean Absorbance
12.5	0.164	0.153	0.161	0.159
25	0.395	0.342	0.339	0.359
50	0.521	0.671	0.674	0.622
100	1.048	1.108	1.105	1.087
200	2.261	1.813	1.783	1.952

Table S3 Three consecutive absorption of quercetin at different concentration.

Concentration (µg/mL)	Absorbance-1	Absorbance-2	Absorbance-3	Mean Absorbance
3.125	0.036	0.032	0.035	0.034
6.25	0.06	0.056	0.061	0.059
12.5	0.104	0.097	0.095	0.099
25	0.194	0.189	0.184	0.189
50	0.37	0.285	0.287	0.314
100	0.657	0.624	0.631	0.637
200	1.241	1.317	1.239	1.266

Table S4 Two consecutive absorption of ascorbic acid at different concentration.

Concentration (µg/mL)	Absorbance-1	Absorbance-2	Mean absorbance
1.56	0.066	0.051	0.059
3.125	0.067	0.054	0.061
6.25	0.072	0.06	0.066
12.5	0.097	0.072	0.085
25	0.122	0.162	0.142
50	0.187	0.184	0.186
100	0.317	0.389	0.353
200	0.697	0.819	0.758