



Research Article

PHYTOCHEMICAL SCREENING, ANTIOXIDANT ACTIVITY AND GC-MS ANALYSIS OF SYZYGIUM DIOSPYRIFOLIUM (WALL. EX DUTHIE) S.N. MITRA: A MEDICINAL PLANT FROM MEGHALAYA, INDIA

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ABSTRACT

Background: *Syzygium diospyrifolium* (Wall. ex Duthie) S.N. Mitra is a traditionally valued but underexplored medicinal plant from Meghalaya, India. This study aimed to assess the phytochemical composition, antioxidant potential, and GC-MS profile of its fruit and leaf extracts to explore their therapeutic prospects. **Methods:** Methanol and ethanol extracts of the fruits and leaves were prepared using a Soxhlet extraction apparatus. Percentage yields were determined, and qualitative phytochemical screening identified secondary metabolites. Total phenolic content (TPC) and total flavonoid content (TFC) were quantified spectrophotometrically, while antioxidant activity was evaluated using DPPH radical scavenging and reducing power assays. GC-MS analysis identified bioactive compounds. All experiments were performed in triplicate (n=3), and results were expressed as mean ± SD with significance at $p < 0.05$ (Student's *t*-test). **Results:** Methanol extracts yielded higher percentages (fruit: 15.2% w/w; leaf: 14.4% w/w) than ethanol extracts. Both extracts contained alkaloids, glycosides, saponins, phenols, tannins, flavonoids, proteins, amino acids, and diterpenes. Fruits exhibited higher TPC (52.23 mg GAE/g) and TFC (152 mg QE/g) than leaves (26.96 mg GAE/g; 96.86 mg QE/g; $p < 0.05$). Antioxidant assays showed stronger activity in fruits (DPPH IC₅₀: 133.95 µg/mL) than leaves (215.11 µg/mL). GC-MS analysis identified sugars, fatty acid amides, sterols, terpenoids, and phenolic derivatives, including DL-Arabinose, D-Allose, and 13-Docosenamamide (Z), reported for the first time. **Conclusion:** This first GC-MS-based phytochemical profiling of *S. diospyrifolium* (Wall. ex Duthie) S.N. Mitra reveals its fruits as rich in phenolic and flavonoid compounds with significant antioxidant potential, supporting its promise for nutraceutical and phytopharmaceutical applications

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INTRODUCTION

Syzygium diospyrifolium (Wall.ex Duthie) S.N. Mitra, a member of the family Myrtaceae, Northeastern India encompasses biodiversity from both the Indo-Burmese and Himalayan regions. A diverse range of vegetation originating in Southeast Asia has therefore been recorded in this part of India as well. The *Syzygium diospyrifolium* (Wall. ex Duthie) S. N. Mitra plant is mainly found in open forests and hill slopes, such as in Assam, Meghalaya, and the Tropical region [1][2]. India harbors around 84 species of the genus *Syzygium*, of which northeastern India contributes 34 [3][4]. Although extensive research has been conducted on various other species of *Syzygium*, like *Syzygium marangense*, *Syzygium jambos*, and *Syzygium cumini*, with demonstrations of potential medicinal properties, *Syzygium diospyrifolium* (Wall.ex Duthie) S.N. Mitra remains understudied. Encouraged by the knowledge gained from these related species, the current study focuses on qualitative phytochemical estimation and antioxidant property investigation of *Syzygium diospyrifolium* (Wall.ex Duthie) S.N. Mitra, a locally entitled medicinal tree native to Meghalaya, India. Our research aims to advance understanding of this unexplored species and assess its potential as an herbal remedy for various health conditions. An evaluation found that *Syzygium jambos* leaves are conventionally used as antiseptic agents. Furthermore, it also indicates extended evaluation of *S. jambos* leaf extracts for antibacterial activity [5]. Seed of *Syzygium cumini* obtained as a by-product due to industrial fruit processing revealed to be carried with certain biologically effective compounds like gallic acid, ellagic acid, corilagin, etc., in consequence, can be considered a potent substitute for chemically derived formulation of dietary supplements and pharmaceuticals. Further, several in-vivo and in-vitro studies have established bioactive properties, including anticancer, gastroprotective, cardioprotective, antimicrobial, hepatoprotective, antidiabetic, and antiobesity, in *Syzygium cumini* [6]. A similar study on *Syzygium marangense* revealed that tender leaves and fruit of the 'Green Giant' variety of *Syzygium marangense* have elevated Total Phenolic Content (TPC) and Total Flavonoid Content (TFC), along with alpha-glycosidase preventive and bactericidal activities, in contrast to other full-growth stages. *Syzygium marangense* was found to be a good source of natural antioxidants with scavenging activity against deleterious free radicals. Moreover, these plant materials exhibit food preservation properties and have promising applications in the pharmaceutical industry [7].

S. diospyrifolium (Wall.ex Duthie) S.N. Mitra, locally known as Maryndm or Ryndm in Meghalaya, India. The fruits of *S. diospyrifolium* (Wall.ex Duthie) S.N. Mitra have two seasons, one in April, which is the start of summer, and the other in November, which is in winter. The fruit is pinkish and is consumed by birds in those areas. Figure 1A & 1B of the fruits and leaves of *S.diospyrifolium* (Wall.ex Duthie) S.N. Mitra is considered a medicinal plant in Meghalaya, is commonly used by traditional healers and local people staying in the area for treating several human diseases and infections, such as diarrhea, food poisoning, wounds, skin infections, ulcers, inflammatory disorders, gastric problems and anti-diabetic. In the context of rising interest in natural bioactive compounds, *S. diospyrifolium* (Wall.ex Duthie) S.N. Mitra has yet to be scientifically examined for its phytochemical composition, particularly through GC-MS, as well as antioxidant activity. Its pharmacological relevance and possible application in the nutraceutical and therapeutic sectors need to be ascertained through a detailed analysis of its chemical profile and biological activity. This work aims to fill the knowledge gap by conducting a detailed phytochemical and antioxidant analysis, providing scientific justification for further research and the use of the plant.

MATERIAL AND METHODS

Biomaterial collection and preparation

Fruits and leaves of *S. diospyrifolium* (Wall.ex Duthie) S.N. Mitra were procured from the wet tropical biome of Shillong, Meghalaya, specifically from Jarain near Pitcher Plant Park, Amlarem block, West Jaintia Hills District, during April and November 2019. The Botanical Survey of India, Eastern Regional Center, located in Shillong, validated the collected plant material (BSI/ERC/6/6/2024-25-Tech/619). Leaves and fruits were thoroughly washed with water, sun-dried, and then pulverized into a fine powder using a grinder. The powdered sample was stored in a clean, dry airtight container for further analysis, as shown in Figures 1C & 1D, which display the fine powder of fruit and leaves.

Crude extract preparation

40g each of powdered leaves and fruits of *Syzygium diospyrifolium* (Wall.ex Duthie) S.N. Mitra were subjected to Soxhlet extraction using methanol and ethanol as solvents. Extraction was performed for 20 hours for each solvent [8][9]. The resulting extracts were then evaporated using a rotary

evaporator to yield dry, solvent-free crude extracts. The following formula calculated the percentage yield:

$$\text{Percentage yield} = \frac{\text{Weight of crude extract}}{\text{weight of used plant parts}} \times 100$$

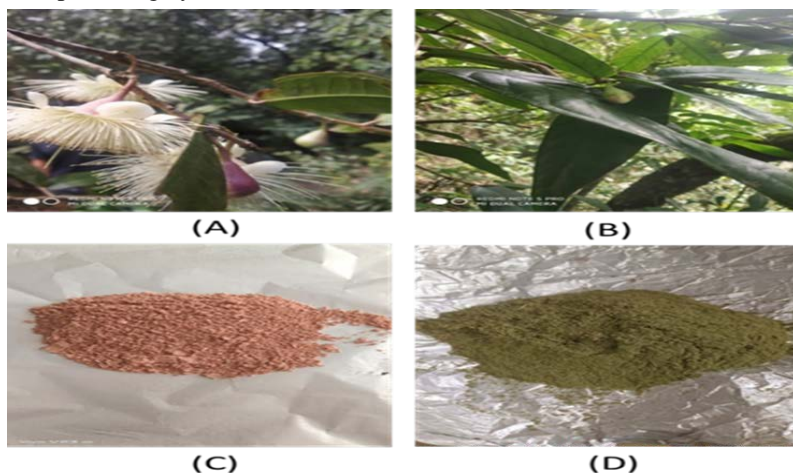


Figure 1: Figure (A) Fruit, (B) Leaves, (C) Dried Fruit powder, and (D) Dried Leaves powder of *Syzygium diospyrifolium* (Wall.ex Duthie) S.N. Mitra

Phytochemical analysis

The presence of secondary metabolites of *Syzygium diospyrifolium* (Wall.ex Duthie) S.N. Mitra of fruit and leaves was carried out using the standard method [10][11]. Methanol and ethanol extracts were used for testing. The following were used to identify the presence of different phytochemical: carbohydrates (Benedict's test), alkaloids (Wagner's & Hager's tests), Glycosides (Modified Borntrager's Test), Saponins (Froth & Foam tests), Phytosterols (Salkowski's test), Phenol (Ferric Chloride Test), Tannins (Gelatin Test), Flavanoids (Alkaline reagent test), Protein and amino acids (Xanthoproteic and Ninhydrin Test), Diterpenes (Copper Acetate Test) [13][14].

Estimation of Total Phenolic Content (TPC)

TPC was estimated for fruits and leaf extracts prepared in methanol using the Folin-Ciocalteu method with Gallic acid as a standard [15][16]. Gallic acid solutions were prepared in Milli-Q water at concentrations of 200, 100, 50, 25, and 12.5 µg/ml. Further, 500 µl of ten-fold diluted Folin-Ciocalteu reagent was mixed with each of the above concentrations, followed by incubation at $22 \pm 2^\circ\text{C}$ for 5 min. Incubation was followed by the addition of 1.5ml of sodium carbonate (20 %) to the above-prepared solutions, then a short vortex. The solutions were then incubated at room temperature for 90 minutes. Absorbance was measured in triplicate, using Milli-Q water as the blank, in a Cary Win UV-Vis spectrophotometer at 765 nm. 100µl of both fruit and leaf extracts (1mg/ml) were subjected to the determination of TPC and recorded as mg GAE/g.

Estimation of Total Flavonoid Content TFC

TFC estimation was performed for both fruits and leaf extracts prepared in methanol, considering quercetin as the reference standard [15][16]. Standard solution preparation was accomplished by adding 10% aluminum trichloride in ethanol to 1 mL of quercetin at concentrations of 100, 80, 60, 40, and 20 µg/mL, followed by the addition of 0.1 mL of 1 M potassium acetate. 5 ml of the final volume was obtained by adding 80% methanol, and the standard solutions were then incubated for 40 minutes at $25 \pm 2^\circ\text{C}$. Similarly, 100 µl of both fruit and leaf extracts (1 mg/ml) were subjected to the determination of TPC. Absorbance was estimated in a Cary Win UV-Vis spectrophotometer at 765 nm by considering Quercetin, potassium acetate, and methanol as blank. Absorbance was expressed as mg QE/g and acquired in triplicate. The following formula calculated both TPC and TFC:

$$\text{TPC/TFC} = x \left(\frac{V}{m} \right)$$

where, V= volume in ml and M= sample mass.

The experiments were carried out in triplicate, and the results are expressed as mean \pm standard deviation (SD). A p-value < 0.05 was considered statistically significant for differences between fruit and leaf by the t-test method.

ANTIOXIDANT ASSAYS

DPPH Free Radical Scavenging Assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay extract solution was prepared at 0.1 mM in

methanol. Serial dilutions were performed 4 times from 200 µg/ml of methanol to obtain 4 different concentrations. Crude extracts were prepared at different methanol concentrations by serial dilution of 200 µg/ml four times. Considering ascorbic acid as a standard, both the sample extracts were evaluated at various concentrations. 2 ml of 0.1 M reaction mixture was prepared in Tris-HCl buffer at pH 7.4, followed by dark incubation for 30 minutes. Absorbance was acquired in triplicate at 517 nm in a Cary Win spectrophotometer. Radical scavenging activity was estimated by using the following formula:

$$\text{Scavenger Percentage} = \left(1 - \frac{As - Ab}{Ac}\right) \times 100$$

Where, *As* is Absorbance of the tested extract, *Ac* is Absorbance of Control, *Ab* is Absorbance of blank [17]

Reducing Power Assay (RPA)

The RPA estimation was performed for both fruit and leaf extracts prepared in methanol at various concentrations, using ascorbic acid as a standard. The extracts were prepared for the assay in 0.2 mole/l of phosphate buffer at pH 6.6, followed by the addition of 1% potassium ferricyanide in equal volume. An incubation period of 20 minutes at 50°C was performed, followed by the addition of 10% trichloroacetic acid to an equal volume. The solutions were then centrifuged for 10 minutes at 3000 rpm. Furthermore, 2.5 ml of each of the abovementioned prepared solutions was blended with Milli-Q water and 0.1 % of FeCl₃. The absorbance was measured in triplicate in a Cary Win spectrophotometer at 700 nm [17].

GC-MS analysis

GC-MS analysis was performed in a Thermo Fisher Scientific GC system at the Central Instrumentation Facility (CIF), University of Science and Technology Meghalaya, India. The system was furnished with a split/splitless injection port with a 1/100 split ratio. An electron-impact mode accompanied it on an ISQ7000 mass spectrometer. A column fused with TG-5MS silica capillary with the specification of 30 m × 0.25 mm i.d. and 0.25 µm film thickness was employed for the analysis. The initial temperature setting was 3 min at 60 °C, followed by a ramp to 230 °C at 5 °C/min. Both the transfer line and the injector temperature were maintained at 230°C and 290°C, respectively. The ion source block was directly connected to the column outlet. The flow rate of ultrapure helium as a carrier gas was set at 1 ml per minute with 0.2. µL of injection volume. The Chromeleon™ Software, provided with the instrument, was used to calculate peak area percentages. The qualitative analysis

was performed in dilute solutions of pre-determined concentrations. Confirmation of compound identifications was finalized by comparing the laboratory retention indices with those of NIST library-derived mass spectra of undisputed standards.

RESULTS AND DISCUSSION

Percentage Yield of the Extracts

The percentage yields of the extracts were determined for methanol and ethanol separately using the Soxhlet extraction method. For each extraction, a solvent-to-solvent ratio of 5:1 (v/w) was maintained (200 mL solvent for 40 g of powdered sample). The Soxhlet apparatus was run for several siphoning cycles to ensure exhaustive extraction. Temperature was kept at the boiling points of the respective solvents (~65°C for methanol and ~78°C for ethanol). After extraction, the solvents were concentrated under reduced pressure using a rotary evaporator, and the extracts were dried to constant weight. The final dry weights obtained were used to calculate the extractive yields, which are presented in Table 1 and were calculated using the following formula:

$$\text{Percentage yield (\% w/w)} = \frac{\text{Weight of dried extract}}{\text{Weight of powdered sample taken}} \times 100$$

Table 1: The percentage yield of the extract *Syzygium diospyrifolium* (Wall.ex Duthie) S.N. Mitra fruit and leaf were evaluated in Methanol and Ethanol

S. No	Sample	Solvent	Percentage yield
01	Fruit	Methanol	15.2% w/w
02	Fruit	Ethanol	12.6% w/w
03	Leaf	Methanol	14.4% w/w
04	Leaf	Ethanol	11.7% w/w

The results revealed that the methanol extracts of fruit and leaf yielded the highest extractive percentages, 15.2% w/w (Fruit) and 14.4% w/w (Leaf), followed by 12.6% w/w ethanol (Fruit) and 11.7% w/w ethanol (Leaf). The fruit & leaf extract in methanol shows a higher percentage than in ethanol, which might be because methanol has higher polarity & solubility for polar compounds than ethanol.

Phytochemical analysis

Both fruit and leaves extracts of *Syzygium diospyrifolium* (Wall.ex Duthie) S.N. Mitra, prepared in methanol and ethanol, revealed diverse secondary metabolites. Phytochemical analysis

was limited to qualitative tests, which confirmed the presence of secondary metabolites, including alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenols, tannins, flavanoids, proteins, amino acids, and diterpenes, as shown in Table 2. However, quantitative estimation of individual compounds was not performed. Therefore, the findings should be considered preliminary for compound detection, and future studies incorporating quantitative analysis and standard references are necessary to establish the extract concentration and the potential bioactivity of these metabolites.

Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

The TPC and TFC analysis revealed that both fruit and leaves (*Syzygium diospyrifolium* (Wall.ex Duthie) S.N. Mitra) have convincing amounts of phenolic and flavonoid compounds when compared with other *Syzygium* species. However, in Table 3, the present study observed that both TPC and TFC were higher in fruits than in leaves. TPC was expressed as mg GAE/g and TFC as mg QE/g. Specifically, the total phenolic contents varied from 52.23 mg GAE/g (Fruit) and 26.96 mg GAE/g (Leaves), while the total flavonoid contents varied from 152 mg QE/g (Fruit) and 96.86 mg QE/g (Leaves). In comparison with other *Syzygium* species, the TPC of *Syzygium diospyrifolium* (Wall.ex Duthie) S.N. Mitra (fruit) is 52.23 mg GAE/g, which is similar to that of *Syzygium cumini* (leaves) 52.17 mg GAE/g [32] and higher than that of *Syzygium polyanthum* buds (19.11 mg GAE/g) [33]. However, it is lower than *Syzygium polyanthum* leaves (94.63-122.87 mg GAE/g [34] and *Syzygium jambos* leaves (230.82mg GAE/g [35]). In terms of TFC of *Syzygium diospyrifolium* (Wall.ex Duthie) S.N. Mitra (fruit) 152 mg GAE/g displays significantly higher concentration than those reported for *Syzygium cumini* leaves (2.76 mg QE/g [32], *Syzygium polyanthum* leaves (60 mg QE/g) [34], and *Syzygium jambos* leaves (11.84mg QE/g [35]). This investigation indicates that *Syzygium diospyrifolium* (Wall.ex Duthie) S.N. Mitra (fruit) is a rich source of flavonoids. Both TPC and TFC values between fruit and leaf extract differed significantly ($p < 0.05$), Student's t-test. The investigation reveals that the fruit extract exhibits higher antioxidant potential than the leaf extract. Overall, this comparative analysis highlights the novelty and phytochemical richness of *Syzygium diospyrifolium* (Wall.ex Duthie) S.N. Mitra (fruit) extract, positioning the species as a promising candidate for further exploration as a natural antioxidant property.

ANTIOXIDANT ASSAYS

DDPH Radical Scavenging

The antioxidant efficacy of both fruit and leaf methanol extracts was evaluated using the DPPH radical scavenging assay. The experiments were performed in triplicate, and data are expressed as mean \pm SD ($n = 3$). In Figure 2, the results demonstrate a concentration-dependent increase in absorbance and reducing power for both fruit and leaves. Antioxidant activity was higher in the fruit extract than in the leaf extract across all concentrations. At 200 $\mu\text{g/mL}$, the fruit extract showed an absorbance of 1.268 ± 0.005 , whereas the leaf extract showed 0.868 ± 0.005 . This difference was statistically significant ($p < 0.05$, Student's t-test), indicating the more substantial antioxidant potential of the fruit extract, likely attributable to its higher phenolic content or other bioactive molecules. Table 4-6 and Figure 2-4 show that DPPH radical scavenging analysis indicates that ascorbic acid exhibited an IC_{50} of $93.11 \pm 0.03 \mu\text{g/ml}$. In contrast, fruit and leaf extracts exhibited IC_{50} values of $133.95 \pm 0.04 \mu\text{g/ml}$ & $215.11 \pm 0.02 \mu\text{g/ml}$, respectively. This indicates that the fruit extract was ~ 1.44 times less effective than ascorbic acid, while the leaf extract was ~ 2.31 times less effective than ascorbic acid. When compared directly, the fruit shows a ~ 1.6 -fold higher scavenging efficiency than the leaf extract ($p < 0.05$), with Student's t-test further supporting its higher phenolic content and superior antioxidant activity. Figure 2-4 represents mean $\text{SD} \pm$ ($n=3$). Statistical significance between treatments was evaluated using an asterisk ($p < 0.05$). Student's t-test, and significance is indicated.

Reducing power activity (RPA)

Table 7 shows that in the present investigation, the reducing power activity of *Syzygium diospyrifolium* (Wall. ex Duthie) S.N. Mitra of both fruit and leaf extracts prepared in methanol demonstrated a concentration-dependent increase in absorbance at 700 nm, indicating a significant enhancement in electron-donating ability with increasing concentration. Fruit extract showed absorbance values increasing from 0.156 at 12.5 $\mu\text{g/ml}$ to 1.268 at 200 $\mu\text{g/ml}$, while the leaf extract rose from 0.103 to 0.868 in the same range, both representing more than an eightfold increase. A significant increase is reflected when the absorbance values rise progressively without overlapping within the standard deviation range, and the differences are statistically supported by ($p < 0.05$, Student's t-test), confirming reliable separation of activity between the extracts. Although ascorbic acid showed the highest reducing power (0.338 at 12.5 $\mu\text{g/mL}$ to

1.84 at 200 µg/mL), the fruit extract showed consistently higher absorbance than the leaf extract at all concentrations. This finding suggests that the fruit contains comparatively higher levels of phenolic and flavonoid compounds, which are likely responsible for its superior antioxidant potential.

Practically, this means that while both extracts exhibit notable reducing power, the fruit extract is significantly more effective than the leaf extract. However, both showed less potency than ascorbic acid, thereby highlighting that the fruit is a richer source of natural antioxidants.

Table 2: Phytochemicals analysis *Syzygium diospyrifolium* (Wall.ex Duthie) S.N. Mitra revealed the presence of phytochemicals in the fruit and leaf extracts prepared in ethanol and methanol

Phytocompounds	Test performed	Fruit extract		Leaves extract	
		Ethanol	Methanol	Ethanol	Methanol
Alkaloids	Wagner's test	+	+	+	+
	Hager's test	+	+	+	+
Carbohydrates	Benedict's test	+	+	+	+
Glycosides	Modified Borntrager's test	+	+	+	+
Saponins	Froth test	+	+	+	+
	Foam test	+	+	+	+
Phytosterols	Salkowski's test	+	+	+	+
Phenols	Ferric chloride test	+	+	+	+
Tannins	Gelatin test	+	+	+	+
Flavanoids	Alkaline reagent test	+	+	+	+
Proteins	Xanthoproteic test	+	+	+	+
Amino acids	Ninhydrin test	+	+	+	+
Diterpenes	Copper acetate test	+	+	+	+

Table 3: Total Phenolic Compounds (TPC) and Total Flavonoid Compounds (TFC) of fruits and leaves extract *Syzygium diospyrifolium* (Wall.ex Duthie) S.N. Mitra prepared in methanol

Sample	TPC (mg GAE/g)	TFC (mg quercetin/g)
Fruit	52.23 ± 0.010	152 ± 0.001
Leaves	26.96 ± 0.005	96.86 ± 0.005

Table 4: Representing mean absorbance, % inhibition & IC₅₀ value of Ascorbic acid for DPPH assay

Concentration	Absorbance			Mean	Std dev	% Inhibition	IC ₅₀
	0.002	0.001	0.003				
Blank	0.002	0.001	0.003	0.002	0.001		93.11
Control	0.739	0.742	0.742	0.741333	0.002082		
12.5	0.652	0.662	0.66	0.658	0.005292	11.24100719	
25	0.606	0.605	0.606	0.605667	0.000577	18.30035971	
50	0.474	0.474	0.473	0.473667	0.000577	36.10611511	
100	0.23	0.233	0.236	0.233	0.003	68.57014388	
200	0.102	0.101	0.102	0.101667	0.000577	86.28597122	

Table 5: Representing mean absorbance, % inhibition& IC₅₀ value of methanoil extract of fruit for DPPH assay

Concentration	Absorbance			Mean	Std dev	% Inhibition	IC ₅₀
Blank	0.002	0.001	0.003	0.002	0.001		133.95
Control	0.739	0.742	0.743	0.741333	0.002082		
12.5	0.692	0.695	0.696	0.694333	0.002082	6.339928058	
25	0.654	0.658	0.662	0.658	0.004	11.24100719	
50	0.571	0.562	0.577	0.57	0.00755	23.11151079	
100	0.455	0.462	0.465	0.460667	0.005132	37.85971223	
200	0.198	0.203	0.199	0.2	0.002646	73.02158273	

Table 6: Representing mean absorbance, % inhibition& IC₅₀ value of methanoil extract of leaves for DPPH assay

Concentration	Absorbance			Mean	Std dev	% Inhibition	IC ₅₀
Blank	0.002	0.001	0.003	0.002	0.001		215.11
Control	0.739	0.742	0.743	0.741333	0.002082		
12.5	0.712	0.709	0.714	0.711667	0.002517	4.001798561	
25	0.674	0.678	0.672	0.674667	0.003055	8.992805755	
50	0.601	0.61	0.605	0.605333	0.004509	18.34532374	
100	0.555	0.562	0.565	0.560667	0.005132	24.3705036	
200	0.398	0.393	0.399	0.396667	0.003215	46.49280576	

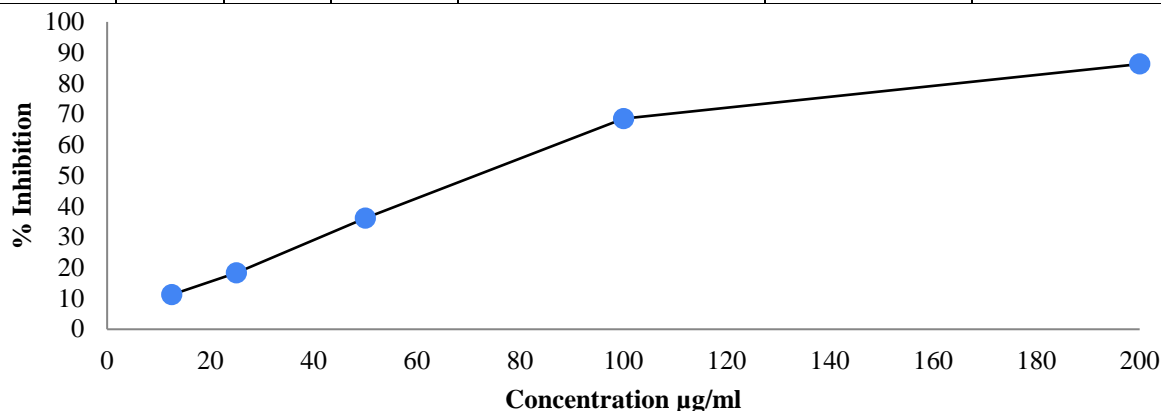


Figure 2: Representing mean absorbance, % inhibition& IC₅₀ value of Ascorbic acid for DPPH assay

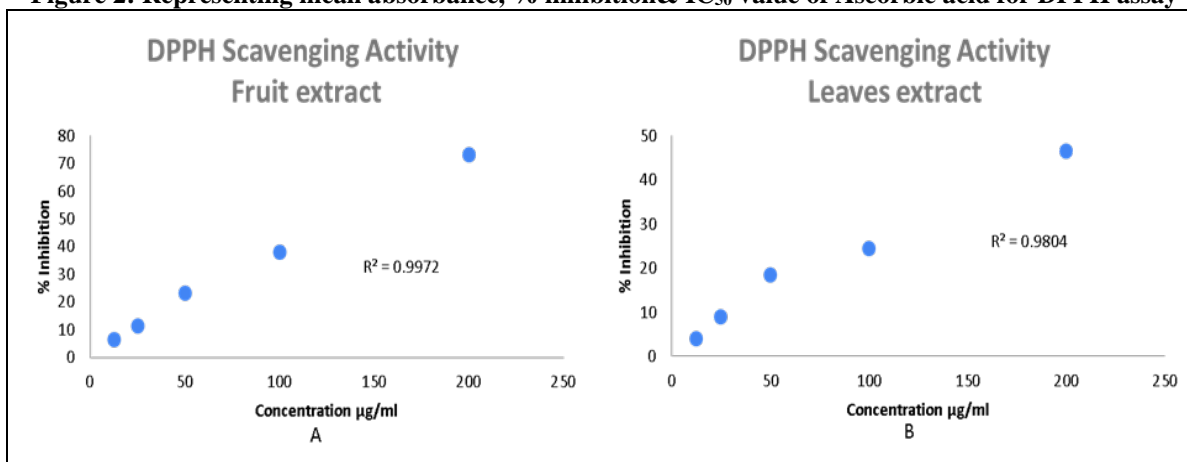


Figure 3: DPPH scavenging activity of *Syzygium diospyrifolium* (Wall.ex Duthie) S.N. Mitra extracts prepared in methanol(A) Fruits and (B) Leaves

Table 7: Antioxidant properties (Reducing Power Assay) of *Syzygium diospyrifolium* (Wall.ex Duthie) S.N. Mitra Fruit and Leaf extracts compared to ascorbic acid (control).

Concentration ($\mu\text{g/ml}$)	Absorbance at 700nm (Mean \pm SD)		
	Control	Fruits	Leaves
Blank	0.002 \pm 0.001	0.002 \pm 0.001	0.002 \pm 0.001
12.5	0.338 \pm 0.003	0.156 \pm 0.005*	0.103 \pm 0.003
25	0.455 \pm 0.003	0.244 \pm 0.008*	0.151 \pm 0.008
50	0.753 \pm 0.009	0.505 \pm 0.010*	0.320 \pm 0.005
100	1.121 \pm 0.004	0.846 \pm 0.019*	0.569 \pm 0.013
200	1.84 \pm 0.020	1.268 \pm 0.005*	0.8680.005

Notes: Data represent mean \pm SD(n=3). Asterisks (*) indicates statistically significant difference between fruit ad leaf extract at the same concentration (Student's t-test,p<0.05)

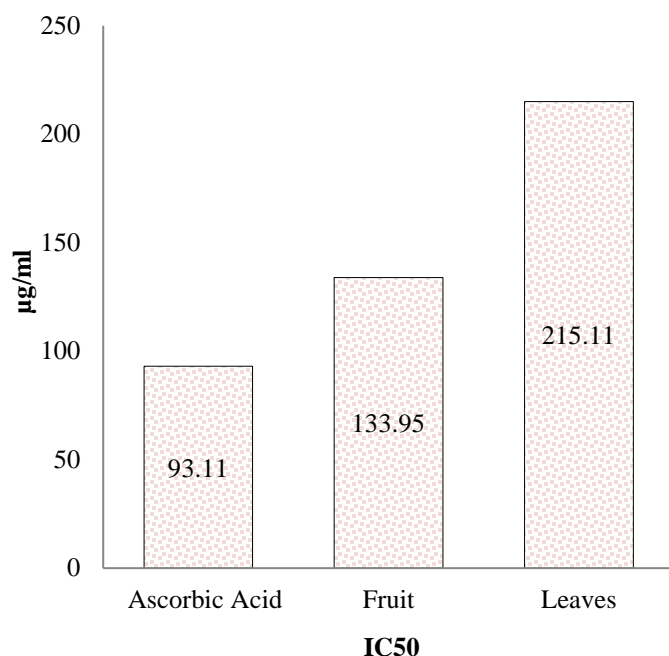


Figure 4: Graphical representation of 50% scavenging activity (IC_{50}) of Ascorbic acid (control), Fruit (methanol extract), and Leaves (methanol extract)

GC-MS Analysis of Methanolic Extract of fruits and leaves of *Syzygium diospyrifolium* (Wall.ex Duthie) S.N. Mitra

The GC-MS chromatogram of both the fruits and leaves extract of *S. diospyrifolium* (Wall.ex Duthie) S.N. Mitra, prepared in methanol, and peak identification was carried out based on retention times, mass spectral fragmentation patterns, and comparison with the NIST library database. The results were expressed as relative peak area percentages, which provide an estimate of the abundance of individual metabolites in the extracts. However, no calibration curves or internal standards were employed & the results are therefore considered qualitative & preliminary, with possible co-elution of metabolites. This

limits direct comparison of compound concentration with other published reports. Despite this limitation, the findings provide valuable preliminary insight into the phytochemical profile of *S. diospyrifolium* (Wall.ex Duthie) S.N. Mitra, identifying several bioactive metabolites. In future authentic standards, quantitative calibration and advanced deconvolution will strengthen the reliability of this observation, potentially enabling the detection of the antioxidant. Comprehensive analysis of compounds present in fruits & leaves by GC-MS analysis is shown in Table 8 & Figure 5, 6 & phytochemical compounds in leaves & fruits of *Syzygium diospyrifolium* (Wall.ex Duthie) S.N. Mitra: retention times, % detection, and reported occurrence in other *Syzygium* species in Table 9.

Common compounds that have been documented in *Syzygium* species are presented in Table 10. Fruits are dominated by sugars & fatty acid amides; whereas leaves contain more sterols, terpenoids & phenolic compounds. In the present study, GC-MS analysis identified several common compounds commonly reported in plant studies, including squalene, tocopherol (vitamin E), and β -sitosterol. While these compounds are widespread, their levels in *Syzygium diospyrifolium* (Wall.ex Duthie) S.N. Mitra are noteworthy due to potential contribution to antioxidant activities.

Interestingly, GC-MS analysis identified several unique compounds in GC-MS profiling that are reported in *Syzygium diospyrifolium* (Wall.ex Duthie) S.N. Mitra, including rare sugar (DL-Arabinose, D-Allose, 6-Acetyl- β -D-mannose), fatty acid amides (Palmitoleamide, 13-Docosenamide (Z), long chain alcohol(1-Heptatriacotanol), phenolic derivatives ((Z)-3-(Heptadec-10-en-1-yl)phenol). These compounds exhibit diverse bioactivities, including anti-diabetic, antioxidant,

antimicrobial, anti-inflammatory, neuroprotective, and cytoprotective effects. These unique compounds not only highlight but also underscore the plant therapeutic potential and open exciting opportunities for developing future nutraceuticals, cosmeceuticals, and innovative phyto-pharmaceutical formulations.

Table 8: GC-MS Analysis of Compounds present in methanol extracts of fruits and leaves of *Syzygium diospyrifolium* (Wall.ex Duthie) S.N. Mitra

S. No	Compound Name	Category	% Peak Area (Fruits)	% Peak Area (Leaves)	Common/Unique
1	Cyclohexene-3,5-diol, cis-	Hydrocarbon	0.31%	-	Unique to leaves
2	Melibiose	Sugar	0.40%	-	Unique to leaves
3	Cyclopentane, 1-acetyl-1,2-epoxy-	Epoxide	3.28%	0.57%	Unique to leaves
4	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	Phenolic Compound [27]	3.85%	0.96%	Common
5	5-Hydroxymethylfurfural (HMF)	Sugar Derivative	15.30%	1.05%	Common
6	6-Acetyl- β -D-mannose	Sugar	0.13%	-	Unique to leaves
7	1,2,4-Benzenetriol / 1,2,3-Benzenetriol	Phenolic Compound	4.53%	12.23%	Common
8	Melezitose	Sugar [25]	13.91%	0.52%	Common
9	Maltose	Sugar	1.52%	-	Unique to leaves
10	Lactose	Sugar	4.66%	-	Unique to leaves
11	1-Heptatriacotanol	Long-Chain Alcohol	0.18%	-	Unique to leaves
12	n-Hexadecanoic acid (Palmitic acid)	Fatty Acid	0.62%	1.92%	Common
13	Linoelaidic acid	Fatty Acid [24]	0.47%	-	Unique to leaves
14	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	Fatty Acid Derivative	3.18%	-	Unique to leaves
15	(Z)-3-(Heptadec-10-en-1-yl) phenol	Phenolic Compound	8.97%	29.21%	Common
16	Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	Fatty Acid Derivative	0.91%	-	Unique to leaves
17	Palmitoleamide	Fatty Acid Amide (Major)	24.71%	-	Unique to leaves
18	β -Sitosterol	Sterol [22]	0.89%	1.96%	Common
19	dl- α -Tocopherol (Vitamin E)	Antioxidant Sterol[21]	-	3.55%	Unique to fruits
20	Squalene	Terpenoid [20]	-	2.14%	Unique to fruits
21	Friedelan-3-one	Triterpenoid [23]	-	2.40%	Unique to fruits
22	13-Docosamide (Z)	Fatty Acid Amide	-	1.39%	Unique to fruits
23	9,12,15-Octadecatrienoic acid (Linolenic acid, Z, Z, Z)	Fatty Acid	-	2.00%	Unique to fruits
24	Neophytadiene	Hydrocarbon	-	2.21%	Unique to fruits
25	DL-Arabinose	Sugar	-	0.64%	Unique to fruits
26	D-Allose	Sugar [26]	-	1.41%	Unique to fruits
27	5-(3-Hydroxypropyl)-2,3-dimethoxyphenol	Phenolic Compound	-	1.55%	Unique to fruits
28	Phenol, 3-pentadecyl-	Phenolic Compound	-	1.57%	Unique to fruits

Notes: “-” indicated the compound was not detected in that extract; Peak area (%) represents the relative abundance in the GC-MS chromatogram; Compounds are classified as common(present in both extracts)or unique (present only in one extract).

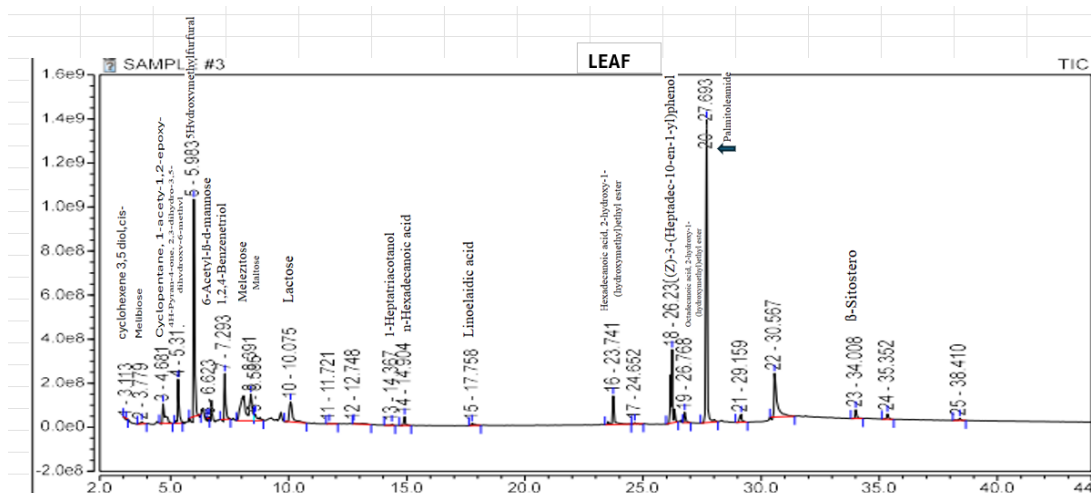


Figure 5:GC-MS chromatogram of *Syzygium diospyrifolium* (Wall.ex Duthie) S.N. Mitra Leaves extract prepared in methanol

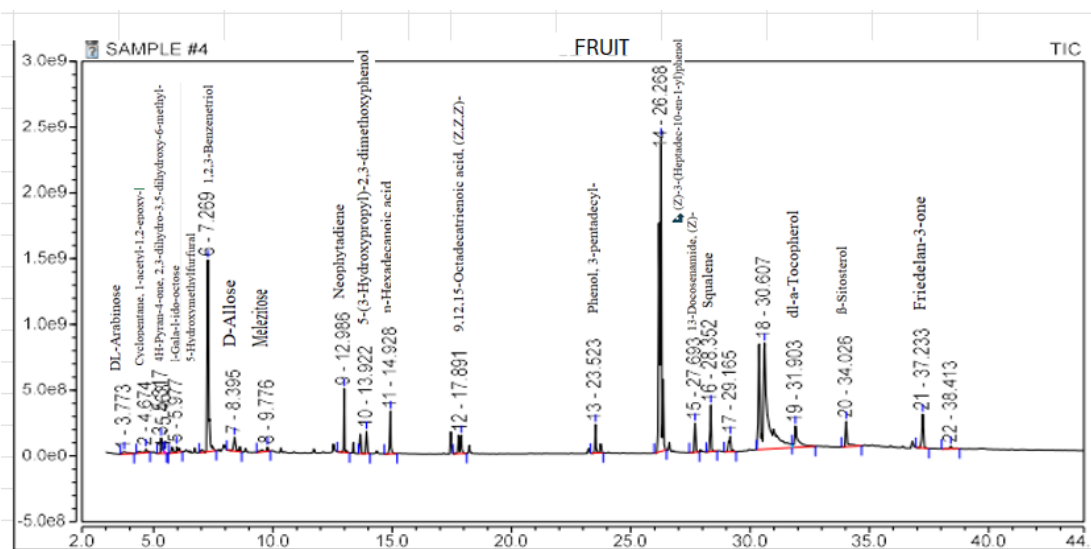


Figure 6: GC-MS chromatogram of *Syzygium diospyrifolium* (Wall.ex Duthie) S.N. Mitra Fruit extract prepared in methanol

Table 9: Phytochemical compounds in leaves and fruits of *Syzygium diospyrifolium* (Wall.ex Duthie) S.N. Mitra: retention times, % detection, and reported occurrence in other *Syzygium* species

S. No	Compound name	Retention time (leaves)	Retention time (fruits)	Detection in leaves (%)	Detection in Fruits (%)	Reported in other <i>Syzygium</i> species
1	Cyclohexene-3,5-diol, cis	3.113	-	0.31%	-	<i>S. cumini</i> & <i>S.aromaticum</i> [9]
2	Melibiose	3.779	-	0.40%	-	<i>S. jambos</i> [13]
3	Cyclopentane, 1-acetyl-1,2-epoxy-	4.681	4.674	3.28%	0.57%	<i>S.aromaticum</i> [9]
4	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	5.317	5.317	3.85%	0.96%	<i>S.malaccense</i>
5	5Hydroxymethylfurfural	5.983	5.977	15.30%	1.05%	<i>S.cumini</i> [29]
6	6-Acetyl-β-d-mannose	6.623	-	0.13%	-	-
7	1,2,4-Benzenetriol	7.293	7.269	4.53%	12.23%	<i>S. cumini</i> [29]
8	Melezitose	8.391	9.776	13.91	0.52%	<i>S.aromaticum</i> [9]
9	Maltose	8.585	-	1.52%	-	-

10	Lactose	10.075	-	4.66%	-	-
11	1-Heptatriacotanol	14.367		0.18%		<i>S. cumini</i> [29] <i>S. aromaticum</i> [9]
12	n-Hexadecanoic acid	14.904	14.928	0.62%	1.92%	<i>S. malaccense</i> <i>S. cumini</i> [9]
13	Linoelaidic acid	17.758	-	0.47%	-	-
14	(Z)-3-(Heptadec-10-en-1-yl)phenol	26.230	26.268	8.97%	29.21%	-
15	Palmitoleamide	27.693	-	24.71%	-	-
16	β -Sitosterol	34.008	34.026	0.89%	1.96%	<i>S. polyanthum</i> (leaves), <i>S. jambos</i> (leaves)[28]
17	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	23.741	-	3.18%	-	-
18	DL-Arabinose	-	3.773	-	0.64%	-
19	l-Gala-l-ido-octose	-	5.463	-	0.03%	-
20	D-Allose	-	8.395	-	1.41%	-
21	Neophytadiene	-	12.986	-	2.21%	<i>S. polyanthum</i> (leaves), <i>S. cumini</i> (leaves)[28]
22	5-(3-Hydroxypropyl)-2,3-dimethoxyphenol	-	13.922	-	1.55%	-
23	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	-	17.891	-	2.00%	<i>S. cumini</i> (leaves and bark)[31]
24	Phenol, 3-pentadecyl-	-	23.523	-	1.57%	<i>S. polyanthum</i> (leaves), <i>S. jambos</i> (leaves)[28]
25	13-Docosamide, (Z)-	-	27.693	-	1.39%	-
26	Squalene	-	28.352	-	2.14%	<i>S. polyanthum</i> (leaves)[28]
27	dl- α -Tocopherol	-	31.903	-	3.55%	<i>S. polyanthum</i> (leaves)[28]
28	Friedelan-3-one	-	37.233	-	2.40	<i>S. jambos</i> (leaves)[28]

Table 10: Common compounds identified by GC-MS that have been previously reported in *Syzygium species*

S.No	Compound name	<i>Syzygium species</i> (Documented)
1	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	<i>Syzygium cumini</i> (Leaf) [28]
2	5-Hydroxymethylfurfural (HMF)	<i>S. cumini</i> (seed and bark)[29]
3	n-Hexadecanoic acid (Palmitic acid)	<i>S. polyanthum</i> (leaves), <i>S. cumini</i> (bark and leaves)[28]
4	1,2,4-Benzenetriol / 1,2,3-Benzenetriol	<i>S. polyanthum</i> (leaves),[30]
5	9,12,15-Octadecatrienoic acid (Linolenic acid, Z,Z,Z)	<i>S. cumini</i> (leaves and bark)[31]
6	Neophytadiene	<i>S. polyanthum</i> (leaves), <i>S. cumini</i> (leaves)[28]
7	β -Sitosterol	<i>S. polyanthum</i> (leaves), <i>S. jambos</i> (leaves)[28]
8	dl- α -Tocopherol (Vitamin E)	<i>S. polyanthum</i> (leaves)[28]
9	Squalene	<i>S. polyanthum</i> (leaves)[28]
10	Friedelan-3-one	<i>S. jambos</i> (leaves)[28]
11	Phenol, 3-pentadecyl-	<i>S. polyanthum</i> (leaves), <i>S. jambos</i> (leaves)[28]

CONCLUSIONS

Syzygium diospyrifolium (Wall.ex Duthie) S. N. Mitra, a less investigated plant used by the Khasi tribes of Meghalaya, India.

The present study demonstrates that phytochemical analysis has identified that both fruits and leaves are rich in secondary metabolites, indicating higher yields and greater antioxidant potential in the methanol extract. Among the samples tested, fruit extract exhibited notably higher total phenolic contents (52.23 mg GAE/g) and total flavonoid content (152 mg QE/g) compared to leaves (26.96 mg GAE/g and 96.89 mg QE/g, respectively). These phytochemical levels corresponded to stronger in vitro antioxidant activity, as evidenced by DPPH radical scavenging and reducing power assays.

GC-MS analysis revealed a range of bioactive compounds, including sugars, fatty acids, phenolic derivatives, sterols, and terpenoids. Some compounds, such as dl- α -Tocopherol (Vitamin E), 9,12,15-Octadecatrienoic acid (Linolenic acid), and β -Sitosterol, are present at levels that may contribute significantly to observed antioxidant potential. Several unique compounds were identified in this species for the first time, underlining the novelty and phytochemical richness of *Syzygium diospyrifolium* (Wall.ex Duthie) S.N. Mitra. These findings support further investigation of *Syzygium diospyrifolium* (Wall.ex Duthie) S.N. Mitra, which could be a promising source of natural antioxidants. Claims regarding nutraceutical, cosmeceutical, or therapeutic applications remain preliminary. This study is limited to in vitro analyses, and no in vivo or toxicology evaluations have been performed. Therefore, further research, including safety assessments, bioavailability studies, and mechanistic investigations, is required to validate its potential health benefits fully.

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NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Probin Phanjom provided overall supervision and direction to the study. Amaryllis Langbang was primarily responsible for conducting the research, analyzing the data, and preparing the manuscript. Neelakshi Sharma and Jayashree Majumdar contributed significantly by collecting samples and preparing them for analysis, ensuring the accuracy and reliability of the experimental work. Manas Jyoti Kapil played an essential role in supporting the publishing process.

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