



Original Research

## *Tinospora cordifolia* (Willd.) Miers: phytochemical composition, cytotoxicity, proximate analysis and their biological activities

Bindu Modi<sup>1,2#</sup>, Niranjan Koirala<sup>2,3##</sup>, Surya Prasad Aryal<sup>1,4</sup>, Jiban Shrestha<sup>5</sup>, Shishir Koirala<sup>6</sup>, Jitendra Upadhyaya<sup>7</sup>, Ram Chandra Basnyat<sup>1</sup>, Mohamed A. Nassan<sup>8</sup>, Mohammed Alqarni<sup>9</sup>, Gaber El-Saber Batiha<sup>9</sup>

<sup>1</sup> Central Department of Chemistry, Tribhuvan University, Kirtipur, Kathmandu, 44600, Nepal

<sup>2</sup> Department of Natural Products Research, Dr. Koirala Research Institute for Biotechnology and Biodiversity, Kathmandu, 44600, Nepal

<sup>3</sup> Laboratory of Biotechnology, Faculty of Science and Technology, University of Macau, Macau SAR, 999078, China

<sup>4</sup> University of Kentucky, Lexington, Kentucky, 40506, USA

<sup>5</sup> National Plant Breeding and Genetics Research Centre, Khumaltar, Lalitpur, 44700, Nepal

<sup>6</sup> Universal College of Medical Sciences, Ranigaon, Siddharthanagar, Bhairahawa, Nepal

<sup>7</sup> McGill University, Macdonald Campus, 21, 111 Lakeshore Road, Ste-Anne-de-Bellevue, Montreal Quebec, Canada

<sup>8</sup> Department of Clinical Laboratory Sciences, Turabah University College, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia

<sup>9</sup> Department of Pharmaceutical Chemistry, College of Pharmacy, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia

<sup>10</sup> Department of Pharmacology and Therapeutics, Faculty of Veterinary Medicine, Damanhour University, Damanhour 22511, AlBeheira, Egypt

\*Correspondence to: [koirala.biochem@gmail.com](mailto:koirala.biochem@gmail.com)

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#These authors contributed equally to this research work and share first authorship.

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**Abstract:** The present research work has been performed to evaluate the phenolic content, flavonoids content, and cytotoxicity of a multidimensional medicinal plant; *Tinospora cordifolia* and as well as to determine nutritive value by proximate analysis. The total phenolic and flavonoids contents of *Tinospora cordifolia* were found to be significantly greater in methanol extract as compared to corresponding hexane extract. Brine shrimp bioassay indicated *Tinospora cordifolia* is pharmacologically active. The percentage composition of different nutrition parameters namely moisture, total ash, crude fat, protein, fibre, carbohydrate, and vitamin C were assessed. The nutritive values of fresh and dried stem samples were evaluated as 156.44 Kcal/100g and 232.61 Kcal/100g respectively. From Gas column mass spectrometry analysis, it can be reported that inositol, 1-deoxy-, trans-sinapyl alcohol, n-hexadecanoic acid were present in the major amount in methanol stem extract. The findings from this study reveal *Tinospora cordifolia* contains an adequate amount of phenolic and flavonoids content, vital bioactive antioxidant compounds, and a good source of carbohydrates and fibers which potentially adds to the overall value of the plant.

**Key words:** Natural products; *Tinospora cordifolia*; Phytomedicine; Phenolic content; Flavonoids content; Cytotoxicity; Proximate analysis; GC-MS analysis.

### Introduction

Natural products have been the origins of most pharmaceutical active compounds (1). Depending on the biosynthetic origin, natural products are categorized into distinct categories like alkaloids, polyphenols, polyketides, terpenoids, steroids, and saponins, etc. (2,3). Research into phytomedicine is among the pioneering fields of research. Human beings benefit greatly from plant natural products and their secondary metabolites throughout every aspect of life from providing oxygen, heat, and water to building shelter, amusement, and travel systems (4,5). Eighty percent of the world, and as per the World Health Organization (WHO), relies on the medicinal plant as a provider of primary health care that is important to maintain the accessibility towards the proper health care (6).

Plant medicine has been used in human pharmacologic therapy due to the tremendous potential in developing novel therapeutic methods for diseased patients as well as maintaining proper health care of healthy people (7). As antibacterial, antioxidant, anticancer, anti-in-

flammatory, and neuroprotective agents, herbal plants are commonly used (8–10). Plants absorb the sunlight and by photosynthesis produce significant amounts of oxygen and secondary metabolites (11). Plenty of secondary herbal metabolites and spices find potentially vital in a variety of therapeutic compounds that provide an appropriate cure for several human and animal diseases like diabetes, cancer, to main blood pressure, urinary track disease, etc. (12). Free radicals are in reactive stages and comprise an unpaired election. When we consume antioxidants, they play a defense function in our body by removing an excess of free radicals via neutralization. (13,14). Several compounds are observed to have antioxidant potential such as polyphenols, quinones, flavonoids, tannins, etc. (15). which serve as an antioxidant that can scavenge free superoxide radicals, anti-aging, and diminish cancer risk (16,17). Free radicals are produced by the body while operating several biological functions. In order to maintain immunity, the body must preserve the fine line between oxidant and antioxidant. Antioxidant agents neutralized the free radical and protect a body from the harm caused by it

(18).

The plant family Menispermaceae comprises approximately 70 genera and 450 species distributed throughout the lowland tropical regions (19,20). 15 species of *Tinospora* are recognized to exist (21). However, only 4 species have been recorded in India and two species have been reported in Nepal (22). They are prevalent in biologically active alkaloids used in the treatment of a vast array of ailments globally in traditional medicines (23). *Tinospora cordifolia* (Willd.) Miers, usually referred to as Gurjo or Guduchi, is a large, glabrous deciduous shrub of the Menispermaceae family (24) *Tinospora cordifolia*'s stem is succulent in the branches along with thick filiform fleshy aerial roots. The leaves are cordate, alternating, and membranous (25). The composite fruit is purple, fleshy with several drupelets on a stalk with scar subterminal form, colored scarlet (26,27). *Tinospora cordifolia* is referring to as Giloe, which means a divine elixir that has protected and preserved eternally youthful celestial beings from old age in a Hindu mythological term (28). *Tinospora cordifolia* is frequently alluded to as Rasayana and consumed about 1000 tons in Indian medicinal practices (29).

*Tinospora cordifolia* is a widely accepted herbal medicine employed solely as therapeutic drugs in South Asia, with a plethora of pharmacological processes (30). Based on the findings obtained from Ayurveda and ethnobotanical studies, *Tinospora cordifolia* possesses a vast array of pharmacological applications. Several extracts of *Tinospora cordifolia* like aqueous, alcohol, methanol, chloroform, ethanol, acetone, etc are chiefly used in pharmaceutical, pre-clinical and clinical trials (31).

*Tinospora cordifolia* stem is effectively used in health care management, mostly in general fatigue, dyspepsia, fever, urinary disease, constipation, burning pain, diarrhea, blood accumulation, and jaundice treatments. The stem and root of *Tinospora cordifolia* are used in consonance with the several drugs in the treatment of snake-bite and scorpion sting (32). It is also recognized that *Tinospora cordifolia* has immunomodulatory characteristics. *Tinospora cordifolia*'s aqueous extract works by stimulating a macrophage as a first defensive line against pathogens (33). *Tinospora cordifolia* helps to reduce body heat. Thus, consumption of *Tinospora cordifolia* *Tinospora* is very beneficial for jaundice patients. Similarly, mixing the powder of root and stem of *Tinospora* with milk helps in cancer treatment. *Tinospora cordifolia* juice also helps in the treatment of asthma (34).

Phytochemical screening of *Tinospora cordifolia* indicates the presence of various chemicals, such as alkaloids, diterpenoid, lactones, steroids, glycosides, polysaccharides from different parts of *Tinospora cordifolia* (35). The ethnobotanical survey indicates many people consume *Tinospora cordifolia* plant through the oral route directly or with the combination of other plants (36). When taken orally, there is no undesirable impact on the human reported with *Tinospora cordifolia* plant. No serious effect has been observed when *Tinospora* stem extract was given to rabbits at nominal oral doses of 1.6 g/kg and to rats at 1000 mg/kg of whole plant extract (37). With the increase in inclination and demand from people, the impact of the herbal pharmaceutical in-

dustry is skyrocketing day by day. Since many of these herbal products are used in their natural form through oral routes, it's important to perform proximate and nutrient analysis of these products and their raw materials to have a better idea about how they could potentially play a role in our body after consumption (38).

Fresh, nutritious, and healthy foods are the sole source of energy for proper health care, proper growth, and maintenance of physiological homeostasis in the body. Many of these nutrients help to prevent diseases by boosting the immune system (39). Proximate analysis of food is the assessment of the food components, including moisture, protein, fat, ash, crude fibre, and total carbohydrate (40). In addition to their medicinal benefits, many of the medicinal plant species have been used as food, so assessing their nutritional value can help to realize the worth of these plant species (41). Although the plant *Tinospora cordifolia* has enormous therapeutic potential due to the paucity of information about the proximate composition of *Tinospora cordifolia* and its bioactive compounds, this study was conducted to estimate the chemical composition, phenolic and flavonoids content, the available carbohydrates, dietary fibre, and protein, vitamin and moisture of the plant *Tinospora cordifolia*.

## Materials and Methods

### Collection and identification of plant

For this study, the stem and leaves of *Tinospora cordifolia* were chosen as the plant material. Plant materials were collected from Sangrumba VDC, Ilam District, Nepal during November 2018, and identified at Central Department of Botany, Kirtipur, Kathmandu, Nepal.

### Sample preparation and extraction

Those collected plant materials were washed under tap water to remove contaminations chopped into small pieces, and air-dried in shade. The dried sample was milled into powder and stored in a sealed airtight container. The extract was prepared by Soxhlet extraction using successive solvents; hexane and methanol. In this process, 100 grams powder of *Tinospora cordifolia* stem and leaves were subjected to continuous extraction with hexane and methanol. The hexane extraction was continued until the last extract was nearly colorless. Upon hexane extraction, the residual residue was refluxed with methanol for four hours. After the complete extraction, the solvent was removed by using a rotary vacuum evaporator. Then the crude extract was transferred in a bottle, labeled, and stored in a refrigerator for further use.

### Total phenolic content

We used the Folin-Ciocalteu colorimetric method based on the oxidation-reduction reaction to determine the total phenolic content in plant extract as described by Waterhouse in 2012 (42). Gallic acid was used as a standard. The various concentrations (20, 40, 60, 80 and 100 µg/mL) of different extract solutions were prepared. 1mL of the extract solution from each concentration was poured into test tubes after that 5 mL of 10 % FCR added followed by 4 mL of 7 % sodium carbonate solution (Na<sub>2</sub>CO<sub>3</sub>) to those test tubes to make a total

volume of 10 mL. The mixture was shaken well and then incubated for 30 minutes at 40 °C in a water bath. Finally, the absorbance of the solution was measured at 760 nm wavelength using a spectrophotometer against a blank solution containing all reagents except gallic acid. The total phenol content was calculated by using the standard calibration curve of gallic acid.

### Total flavonoids content

The flavonoids content of the plant extract was determined by Aluminium chloride colorimetric assay (43). The standard compound used for the determination of flavonoids was quercetin. An aliquot of 1 mL of the different extract solution (20, 40, 60, 80, and 100 µg/mL) was poured into a 20 mL test tube containing 4 mL distilled water. Then, at zero-time, 0.3 mL 5% NaNO<sub>2</sub> was poured to the test tube followed by the addition of 0.3 mL of 10% AlCl<sub>3</sub> after 5 minutes. Immediately, 2 mL of 1 M NaOH was added to the mixture after 6 minutes, followed by addition of 2.4 mL distilled water. The absorption was measured at 510 nm wavelength versus blank containing all the reagents except quercetin. The total flavonoids content was determined by using the calibration curve of quercetin.

### Brine shrimp bioassay

The brine-shrimp toxicity assay was carried out of a methanol extract of leaves and stem of *Tinospora cordifolia* by using a protocol given by Meyer *et al.*, (1982) (44). Brine shrimp bioassay process involves introducing the newly hatched brine shrimp nauplii to the crude plant extracts. The method determines the LC<sub>50</sub> values (µg/mL) for the crude extracts. LC<sub>50</sub> value is the lethal concentration dose required to kill 50% of the shrimps. Extract solutions of concentration 1000 µg/mL, 100 µg/mL, and 10 µg/mL were prepared. 2 mL solutions from each solution (1000 µg/mL, 100 µg/mL, and 10 µg/mL) were transferred to nine different test tubes, three for same concentration. Similarly, 2 mL methanol was taken in three test tubes (as a blank). After labeling these test tubes, they were kept for 24 hours to evaporate the solvent (methanol). After evaporation, 5 mL of sea water was added to each test tube with gentle shaking. The control was formed by adding the solvent used to dissolve the extract (methanol) in the assay. 10 matured nauplii were introduced in each test tube. After 24 hours, the number of survivors was counted with the help of disposable pipettes and the LC<sub>50</sub> value of the extract was calculated.

### Proximate analysis of fresh and dried stem of *Tinospora cordifolia*

The proximate analysis was carried out by following the standard protocol of Association of Official Analytical Chemists (AOAC, 1990). The nutritional parameters like moisture content, ash content, crude protein, crude fat, fibre, carbohydrates were assessed in the samples and nutritive value of stem was calculated (45).

Nutritive value (Kcal/100g) = (4 × % Protein) + (9 × % crude fat) + (4 × % carbohydrate) (45).

### GC-MS analysis

The analysis was performed by using the GC-MS-QQP2020 instrument (Shimadzu made in Japan) equip-

ped with RTX-5MS fused silica capillary column of 30 m length, 0.25 mm diameter, and 0.25 µm film thickness. Helium (>99.99 % purity), where the linear velocity measured was 50 cm/sec with the total flow 37.7 ml/min along with the column flow 1.70 ml/min, and purge flow 2.0 ml/min. The volume of the injected sample was 1 µl with a split ratio 20. The sample injection process was carried out at the injection temperature of 260°C. The oven temperature was adjusted to 160 °C and hold for 1 min. After holding for a minute, the temperature was raised to 250°C at rate 6 °C/min. The temperature was successively increased to 260°C at rate 2 °C/min, and to 300°C at rate 6 °C/min. The analysis system was adjusted between the ion source temperature at 250 °C and the interface temperature 275 °C. Solvent cut time was 2.50 min and the total run time of the sample was 25.0 min where the mass scan range was between 34 to 45 m/z. The identification of the compounds was done by comparing obtained mass spectra data from analysis with “NIST/EPA/NIH mass spectral library 2017”.

## Results and Discussion

### The total phenolic and flavonoids content

For the determination of total phenolic content (TPC) and total flavonoids content (TFC), gallic acid and quercetin respectively were used as a reference compound for the construction of calibration curve. The average absorbance values (at 760 nm) obtained for specific gallic acid concentrations were used to construct the calibration curve for TPC as shown in figure 1.

For TFC, the plant extracts react with aluminum chloride and form acid liable complexes with yellow fluorescence which was observed under UV spectrophotometer at 510 nm as presented above in figure 2. Using calibration curve and absorbance values of methanol and hexane extract of leaves and stem of different concentrations (20, 40, 60, 80, and 100 µg/mL), total phenolic and flavonoids content were obtained which is represented in table 1.

The comparison of the total phenolic and flavonoids contents of different extract of *Tinospora cordifolia* plant is shown in figure 3. Figure 3, indicates the phenolic and flavonoids contents are found in a maximum amount in the methanol extract of leaves and stem as compared to their corresponding hexane extract.

The total phenolic content (TPC) of methanol and hexane extracts of stem and leaves of *Tinospora cordifolia* is calculated by using the calibration curve and absorbance values (Figure 1). The result shows TPC of methanol extract for leaves and stem is 154.54 and 179.074 mg per gram Gallic acid equivalent (mg/g GAE) respectively. Likewise, leaves and stem hexane

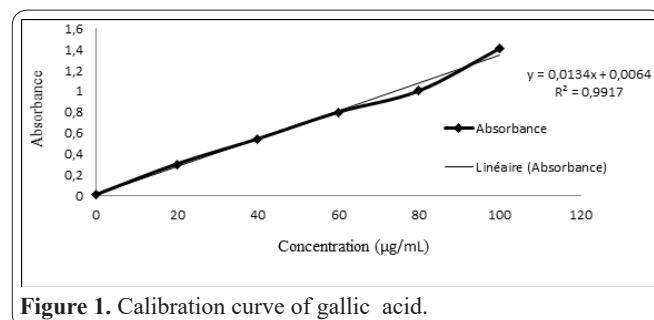


Figure 1. Calibration curve of gallic acid.



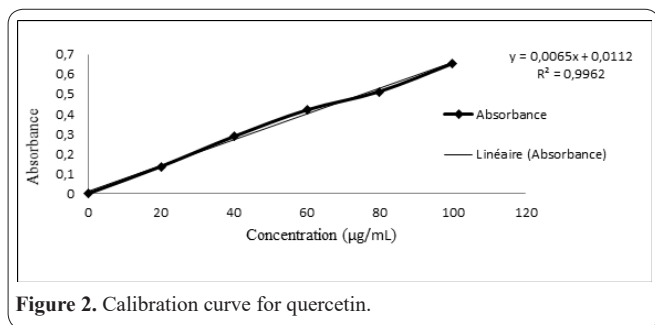


Figure 2. Calibration curve for quercetin.

extracts TPC is calculated as 27.520 and 35.502 mg per gram Gallic acid equivalent (mg/g GAE) respectively. Also, the total flavonoid content (TFC) of leaf and stem methanol extract is quantified by using the calibration curve as well as the absorbance values (Figure 2). TFCs of methanol and hexane extract of leaves and stem are estimated at 145.21 and 40.87 mg per gram quercetin equivalent (mg/g QE) and 19.705, and 9.1725 mg/g QE respectively.

Our present study revealed that both the TPC as well as TFC in the methanol extract were the highest among the analyzed samples. Those findings are similar to the results obtained from Gangadeep et al., which also indicates the presence of highest phenolic and flavonoids content in the methanol extract followed by ethyl acetate and chloroform and least in the hexane extract (46).

The study recorded the methanol extract of leaves and stem have  $IC_{50}$  values lower than their corresponding hexane extracts, indicating the spectacular antioxidant activity in methanol extract as compared to hexane extract (47). In general, those plant extract which has the higher antioxidant potential also contains a higher amount of phenolic and flavonoids. Because of its free radical scavenging properties, phenolic compounds have been widely recognized to exert significant antioxidants. The extract containing considerable amounts of polyphenol content has been believed to enhance a significant antioxidant potential (48).

### Brine shrimp lethality test

Brine shrimp lethality analysis is an outstanding

technique for analyzing cytotoxicity and determining the biological activities of different plant species (49).  $LC_{50}$  value of methanol extract of *Tinospora cordifolia* stem and leaves were calculated to be 462.8  $\mu$ g/mL and 676.08  $\mu$ g/mL. The  $LC_{50}$  value signifies both extracts are toxic towards *Artemia salina* larvae.  $LC_{50}$  values along with the mortality rate are interpreted in table 2.

The result obtained from brine shrimp bioassay indicated that the degree of lethality is directly correlated with the concentration of the extracts; that is maximum mortalities of the brine shrimp larvae took place at the concentration of 1000  $\mu$ g/mL and least mortalities were at 10 $\mu$ g/mL. Those having  $LC_{50}$  values less than 1000  $\mu$ g/mL are regarded as pharmacologically active or likely candidates for anticancer or antitumor drugs. The plant extracts have indicated promising results, implying the samples are biologically active. The study by Ishlam and Ashalkn, 2011, also reported a significant cytotoxicity of *Tinospora cordifolia* extract against the brine shrimp nauplii larvae. Further findings indicate that the mortality rate is directly in line with the concentration of extract (50).

The plant extracts toxicity test is specifically associated with the cytotoxic and anti-tumor properties (51). The outcome of this experiment shows that *Tinospora cordifolia* acts as an effective anti-tumor agent. Brucine is a natural alkaloid present in *Tinospora cordifolia*, responsible for the antitumor agent (52). Furthermore, inositol and its derivative show some effectiveness against tumors (53).

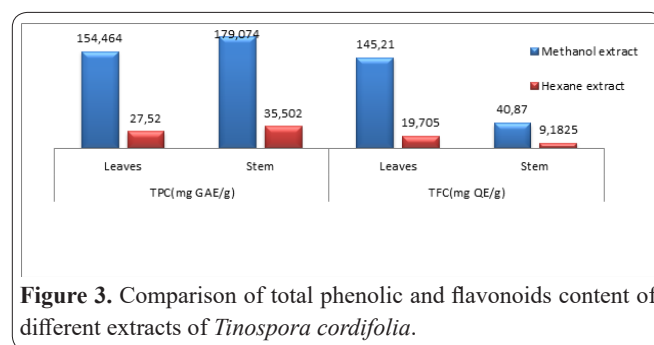


Figure 3. Comparison of total phenolic and flavonoids content of different extracts of *Tinospora cordifolia*.

Table 1. Total phenolic and flavonoids content of *Tinospora cordifolia* extract.

S.N.	Parameters		Methanol extract	Hexane extract
1.	TPC(mg GAE/g)	Leaves	154.464	27.520
		Stem	179.074	35.502
2.	TFC (mg QE/g)	Leaves	145.21	19.705
		Stem	40.87	9.1825

Table 2. Brine shrimp lethality test to determine the  $LC_{50}$  values of the different plant extract.

S.N.	Plant extract	Concentration $\mu$ g/mL	Total number of larvae	Number of survivor	Mortality%	$LC_{50}$ $\mu$ g/mL
1.	<i>Tinospora cordifolia</i>	10		8	20	462.38
		100		7	30	
	Stem	1000	10	4	60	
2.	<i>Tinospora cordifolia</i>	10		8	20	676.08
		100	10	6	40	
	Leaves	1000		5	50	

**Table 3.** Percentage composition of different nutritional parameters in fresh and dried sample of *Tinospora cordifolia* stem.

S.N.	Parameters	% in fresh sample	% in dried sample
1.	Moisture	23.11	10.01
2.	Total ash	6.86	7.05
3.	Crude fat	6.68	1.77
4.	Protein	3.30	8.06
5.	Fibre	39.26	26.99
6.	Carbohydrate	20.78	46.11
7.	Vitamin C	5.562mg/100g sample	3.17 mg/100g sample
	Nutritive Value (Kcal/100 g)	156.44	232.61

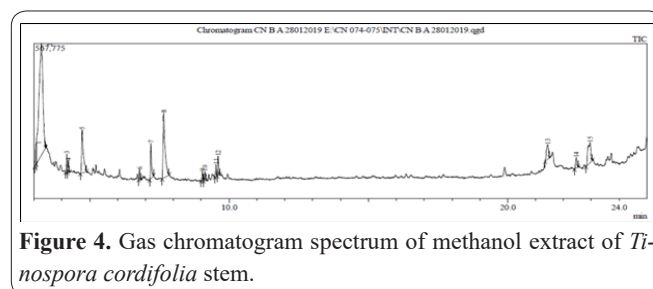
### Proximate analysis of stem of *Tinospora cordifolia*

The proximate study revealed the presence of carbohydrate, protein, fibre, fat, and moisture which helps to determine the nutritive value of the medicinal plant. The findings derived from the proximate assessment of the fresh and oven dried stem sample's nutritional values are summarized in table 3.

The study indicated the nutritive value of the dried stem sample is a higher than the fresh stem sample. Further, the higher quantity of carbohydrates and crude fibre had adequately been found in both samples. Carbohydrates are prevalent in the fresh sample in a small proportion (20.78%) as compared to the dry sample (46.18%). Carbohydrates vary from basic sugar to more complex compounds, like starch and fibre. Carbohydrates are important in food as a dominant source, their derivatives in the functioning process of the immune system, fertilization, blood clotting, imparting essential textural properties, and dietary fibres that contribute to the overall health (54,55). The fibre softens the stools, preventing constipation (56).

Also, epidemiological evidence has shown that intake of moderate quantities of dietary fiber (20 to 35 g/day) reduces the risk of several chronic dietary diseases, such as diverticular disease, heart disease, type 2 obesity (57). Protein in the fresh method was determined to be 3.30% and in the dry sample 8.06. Each creature, including humans, must have a proper protein supply to grow or stabilize itself (58). We are made up of elements that include hydrogen, carbon, nitrogen, oxygen, and sulfur. Protein which is the major compound containing nitrogen in any food sample is used as an index of "protein termed crude protein" as a distinct form of the true protein (59). Ash content reflects the total volume of minerals in foods. Ashing remains a vital part of the proximate analysis and is a key step in ensuring comprehensive mineral analysis (60,61). Further, our finding exhibited a significant presence in both stem samples of moisture, ash content, and vitamin C.

An earlier study suggested a nutrient value of *Tinospora cordifolia* dry stem as 292.54 Kcal/100 g (62). The former study by Kavya B. *et al.*, 2015 found out plant stem possessed high fibre (15.9%), protein (4.5-11.2%), carbohydrates (61.66%), and low fat (3.1%) (63). The results are quite relatable with the above experimental data. The moisture contained in our study was found to be 23%, which is found to be similar in the study by Mahima, *et al.*, 2014. In their study, moisture was assessed as 34.390±4.412, which is closed to our experimental moisture content value (64). Variations in the content of components of the same class may be related to genetic

**Figure 4.** Gas chromatogram spectrum of methanol extract of *Tinospora cordifolia* stem.

origin, geographical location, source, handling, extraction solvent, time of extraction, and conditions of cultivation. Furthermore, the analytical techniques used may also be responsible for the slight variations in the final results.

### GC-MS analysis

From the chromatograms and mass spectra obtained of methanol stem extract of *Tinospora cordifolia* (figure 4), 15 compounds are identified based on the m/z ratio and are depicted with corresponding retention time. The compounds detected were listed along in table 4 with retention time, percentage area, and percentage height, molecular weight, and molecular formula covered by chromatogram. From the GC-MS study, it have been found that the compound obtained have remarkable biological activities such as antimicrobials, antioxidants has been established. The various mass spectra of compounds found from GC-MS analysis are listed in the supplementary section.

The results from GC-MS analysis of the methanolic extract of the experimental plant show the presence of a variety of bioactive compounds (65). GC-MS analysis of the plant's methanol extract revealed the presence of 15 bioactive compounds. Accessible compounds were displayed in Table 3 along with retention time, area, and area percent, which show the presence of inositol, 1-deoxy-, trans-Sinapyl alcohol, (E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol, brucine, etc. in the stem of *Tinospora cordifolia* in the higher proportion. As shown in Table 3, the Inositol, 1-deoxy with 50.10 percentage area appears as the amplest chemical compound in the *Tinospora cordifolia* stem.

*Tinospora* contains inositol in a significant amount, which is exclusively used to cure diabetes, nerve pain, panic disorder, high cholesterol, insomnia, cancer, depression, tumor (66). Brucine possesses anti-inflammatory, anti-tumor, and analgesic properties (67). Similarly, hexadecanoic acid is used for the treatment of rheumatic symptoms in the conventional treatment therapy (68). Likewise, 9,12-Octadecadienoic acid (Z, Z)- can be

**Table 4.** Compounds detected in GC-MS analysis of methanol extract of *Tinospora cordifolia* stem.

S.N.	Compounds	Retention Time	Area %	Height %	Molecular Formula	Molecular weight
1.	Inositol, 1-deoxy	3.055	2.16	5.07	C <sub>6</sub> H <sub>12</sub> O <sub>5</sub>	164
2.	Inositol, 1-deoxy-	3.256	50.10	27.21	C <sub>6</sub> H <sub>12</sub> O <sub>5</sub>	164
3.	Ethylene diacrylate	4.173	1.64 1.05	4.38	C <sub>8</sub> H <sub>10</sub> O <sub>4</sub>	170
4.	Propanoic acid, decyl ester	4.230		2.84	C <sub>13</sub> H <sub>26</sub> O <sub>2</sub>	214
5.	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol	4.721	8.43	10.57	C <sub>10</sub> H <sub>12</sub> O <sub>3</sub>	180
6.	Hexadecanoic acid, methyl ester	6.786	0.97	2.44	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270
7.	n-Hexadecanoic acid	7.194	3.89	8.56	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256
8.	trans-Sinapyl alcohol	7.653	14.51	15.82	C <sub>11</sub> H <sub>14</sub> O <sub>4</sub>	210
9.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	9.039	0.84	2.22	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294
10.	8,11,14-Docosatrienoic acid, methyl ester	9.218	1.00	2.23	C <sub>23</sub> H <sub>40</sub> O <sub>2</sub>	348
11.	9,12-Octadecadienoic acid (Z,Z)-	9.529	1.27	2.95	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	280
12.	Dichloroacetic acid, tridec-2-ynyl ester	9.606	2.06	4.74	C <sub>15</sub> H <sub>24</sub> Cl <sub>2</sub> O <sub>2</sub>	306
13.	Brucine	21.435	3.46	3.57	C <sub>23</sub> H <sub>26</sub> N <sub>2</sub> O <sub>4</sub>	394
14.	4-Benzyl-1,2,-dihydro-1-oxophthalazine	21.460	1.61	2.48	C <sub>15</sub> H <sub>12</sub> N <sub>2</sub> O	236
15.	Cholest-14-en-3-ol, 4,4-dimethyl-, (3.β.,5.α.)-	22.956	7.03	4.92	C <sub>29</sub> H <sub>50</sub> O	414
			100.00	100.00		

used to make lipid-lowering drugs for the treatment and prevention of atherosclerosis (69). Hexadecanoic acid, methyl ester (methyl palmitate), 9,12-Octadecadienoic acid (Z,Z)- or linoleic acid are the common fatty acids which are effective in coronary heart disease (70).

The plant *Tinospora cordifolia* contains a considerable amount of overall phenolic and flavonoids which exhibit several chemical and biological activities. The methanolic extract of both leaves and stem of *Tinospora cordifolia* has a higher amount of phenolic content as well as flavonoids content, which justifies why the plant is ethnomedicinally important. The brine shrimp bioassay of the plant extracts shows the stem and leaves of *Tinospora cordifolia* are mild cytotoxic and pharmaceutically active. It can be ascertained that *Tinospora cordifolia* stem is very rich in nutritional value; its lower percentage of fats and a higher percentage of crude fibre depicting it to be very healthy if consumed properly and regularly. Further, GCMS analysis shows that it contained various bioactive chemical compounds that have important biological, pharmacological values.

#### Authors contributions

BM did the experimental works and chemical preparations; SPA contributed to the overall data analysis and writing of the manuscript; JS contributed to the editing of the article; SK, and JU analyzed the data and managed the publication process; RCB and NK supervised and guided the execution of the experiments. Funding acquisition was done by G.E.-S.B, M.N, M.A and N.K. Final approval of manuscript was done by all the authors.

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#### Conflicts of interest

The authors declare there is no conflict of interest.

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