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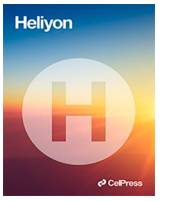

Antioxidant activity study and GC-MS profiling of *Camellia sinensis* Linn

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ABSTRACT

The antioxidant activity of tea leaves extract is a widely researched topic. Tea leaves, particularly those from the *Camellia sinensis* Linn plant, have garnered attention due to their potential health benefits attributed to their antioxidant properties. Antioxidants are compounds that can neutralize harmful free radicals in the body, which can damage cells and contribute to various health issues, including cancer, cardiovascular diseases, and aging. In this research, the matured tea leaves which has been considered as agricultural waste in Moulovibazar area of Bangladesh have been investigated as a potential source of antioxidant. Methanol was used as solvent for the extraction of antioxidant. DPPH (1,1-diphenyl-2-picryl hydrazyl) scavenging free radical assay method was used to assess the antioxidant activity of the extracts and ascorbic acid was used as positive control. Gas chromatography with mass spectrometric (GC-MS) analysis method was conducted on this extract to investigate the principal components. The half inhibitory concentration (IC_{50}) values of methanol extract and ascorbic acid were found to be 69.51 μg/mL and 10.70 μg/mL, respectively. Caffeine is the main compound (74.47%) among the eight bioactive compounds was identify and quantified by GC-MS.

1. Introduction

Plants possess a wide array of bioactive compounds that exhibit significant and remarkable biological activity. Bioactive compounds, sometimes referred to as secondary metabolites, are naturally produced as a result of the normal metabolic pathways in medicinal plants [1–3]. Throughout history, humans have relied on medicinal plants, which contain bioactive compounds, to address a wide range of ailments and health conditions. The use of medicinal plants has persisted from ancient times to contemporary healthcare systems, as these plants offer valuable therapeutic benefits and have found a place in both traditional and modern medical practices [4–6]. The advancement of traditional medicine, considering the dimensions of safety, efficacy, and quality, would not only serve to safeguard traditional heritage but also to rationalize the utilization of herbal medicine in the realm of human healthcare. Nature is widely regarded as a comprehensive source of templates for the development of novel compounds. The investigation of medicinal

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plants referenced in many ancient writings from throughout the world can be undertaken using contemporary scientific methodologies to yield more promising insights in the field of healthcare. Medicinal plant-derived drugs has distinct chemical and biological characteristics, rendering them noteworthy in the field. Their increasing global recognition stems from their ability to provide natural remedies for ailments and contribute to the advancement of healthcare [7,8]. The recognition and exploration of a plant's potential should be appropriately acknowledged and examined, incorporating not only its nutritional significance but also its medicinal attributes, so making a valuable contribution to overall health and the avoidance of diseases [9,10]. The bioactive components extracted from these medicinal plants provide raw materials for the pharmaceutical and cosmetic industries [6]. One of the essential aspects of biomedical research involves conducting comprehensive investigations on the chemical composition and biological mechanisms related to plant species or specific compounds found in plants. The investigative process is of utmost importance in the identification of novel biological resources that may be utilized for the purposes of disease prevention and therapeutic interventions.

An antioxidant is a compounds that can prevent or slow down oxidative damage to cells and tissues in the body. Oxidative damage is caused by free radicals, which are highly reactive molecules that can damage cells, proteins, and DNA. Antioxidants neutralize these free radicals, thereby reducing their harmful effects. The importance of antioxidants lies in their ability to protect the body from various health problems and diseases associated with oxidative stress. Oxidative stress is a condition where there is an imbalance between free radicals and antioxidants in the body. When this balance is disrupted, it can lead to cellular damage, inflammation, and the development of chronic diseases such as cancer, heart disease, diabetes [11–13].

Camellia sinensis Linn is extensively known all over the world as a tea plant and is commercially planted in over 30 countries which is a potential source of antioxidant [14]. It is well-grown around tropical countries and sub-tropical areas because slightly acidic soils are effectively favorable for tea harvesting and also favored by heavy rainfall and rapid drain-out system [14,15]. It is an evergreen plant of the family Theaceae [16]. Tea, the most common, popular, and widely intake beverage, is prepared from buds, tea plant leaves and shoots extract [17]. After water, people all over the world chose to consume tea as their second drink [16]. The popularity of tea as a beverage can be associated to its good health properties, unique flavor and fragrance, and its fundamental significance in social and cultural context [18]. From ancient times, it is used to cure headaches and for improving immune defense and digestion [19]. A previous phytochemical examination indicated the presence of carbohydrates, alkaloids, cardiac glycosides, amino acids, flavonoids, proteins, phenolic acids, reducing sugars, vitamins (A, C, and E), saponins, steroids, tannins, and terpenoids [20,21]. However, the quantity of various phytochemical constituents of tea varies with climate, region or place of cultivation, the process of cultivation, season, position of leaves over the shoot, processing techniques, and storage conditions [16,22,23].

Teas derived from the species Camellia can be classified into three distinct categories according to the tea manufacturing method, namely the degree of fermentation: green tea (unfermented), oolong tea (partially fermented), and black tea (completely fermented). Fresh tea leaves have a significant amount of catechins, which can make up to 30% of their dry weight [23]. The primary catechins found in young tea leaves include epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), gallocatechin (GC), epicatechin (EC), and catechin. The concentration of catechins is subject to variation based on factors such as climate, season, horticultural practices, leaf age, and variety. Chen et al. (2003) [24], investigated that young tea leaves contained higher concentrations of caffeine, EGCG, and ECG compared to mature leaves. Theanine, EGC, and EC were shown to be present in higher concentrations in older leaves. The caffeine content in old leaves was comparatively lower, whereas the levels of EGCG, EGC, EC, and catechin were higher in comparison to young leaves [25]. According to Dufresne and Farnworth (2001), tea contains catechins and other polyphenols that demonstrate significant antioxidant properties [26]. In vitro, they exhibit antioxidant properties through the sequestration of metal ions and the scavenging of reactive oxygen and nitrogen species [27,28]. In addition, it is possible for them to serve as antioxidants indirectly by influencing the activities of transcription factors and enzymes [29]. During the tea processing procedure, the process of fermentation leads to the synthesis of theaflavins and thearubigins. The composition of black tea consists of theaflavins, which make up approximately 2–6% of its content, and thearubigens, which account for more than 20%. In contrast, green tea contains catechins, which make up approximately 30–42% of its composition [30,31].

Moreover, there is an increasing research interest on investigating natural antioxidants as viable alternatives to synthetic antioxidants with carcinogenic properties that are already utilized in the cosmetic, food, and pharmaceutical industries. Numerous studies in the field of epidemiology have demonstrated a clear association between tea flavonoids and various advantageous effects on human health. These benefits encompass the prevention of cardiovascular illnesses, malignancies, diabetes, and microbiological diseases, in addition to the management of obesity. Lipid peroxidation is one of the major problems in food and allied industries, which can cause potential toxic reactions and unfavorable rancidity in products. It is reported that tea flavonoids can prevent this harmful lipid peroxidation [17]. A number of studies have claimed that tea has anti-atherosclerotic, antibacterial, anticarcinogenic, anti-inflammatory, antimutagenic, antitumor, and antiviral activities [16]. Several experiments have been frequently used for the determination of antioxidant activity of tea extract, such as DPPH (1,1-diphenyl-2-picryl Hydrazyl) radical scavenging [32], 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid (ABTS) scavenging activity [33], ferric reducing antioxidant power (FRAP) [34], oxygen radical absorbance capacity (ORAC) [35], superoxide dismutase (SOD) [36] methods. These antioxidants have been analyzed using a range of techniques, including High-Performance Liquid Chromatography (HPLC), Liquid Chromatography Mass Spectroscopy (LC-MS), Gas Chromatography (GC), and Gas Chromatography Mass Spectrometry (GC-MS) [37–47]. Unachukwu et al. using HPLC protocols were optimized and validated for the quantification of 9 phenolic and 3 methylxanthine compounds to examine inter- and intra-variation in white and green tea types and subtypes. A sampling strategy was devised to assess various subtypes procured from different commercial sources. Variation in antioxidant activity and total phenolic content (TPC) of both tea types was further assessed by the 1-1-diphenyl-2-picrylhydrazyl (DPPH) and Folin–Ciocalteau (F–C) assays, respectively. Total catechin content (TCC) for white teas ranged widely from 14.40 to 369.60 mg/g of dry plant material for water extracts and 47.16–163.94 mg/g for methanol extracts. TCC for green teas also ranged more than 10-fold, from 21.38 to 228.20 mg/g of dry plant material for water extracts and 32.23–141.24

mg/g for methanol extracts [48]. Farhoosh et al. investigated the potential antioxidant activity of old tea leaves (OTL) and black tea waste (BTL) compared to green tea leaves (GTL). They found that OTL and BTW, which are often considered as agricultural wastes, can be used as potent natural antioxidative sources [49].

Matured tea leaves, often regarded as agricultural byproducts, hold promise as a potential reservoir of antioxidants. The composition of these tea leaves can vary significantly based on factors such as geographical region, soil characteristics, and precipitation levels. This study sought to explore the antioxidant potential of such tea waste in the context of Bangladesh. The investigation involved the utilization of the DPPH method for antioxidant assessment and GC-MS analysis for compositional profiling of these antioxidants.

2. Materials and methods

2.1. Plant materials and chemicals

The study involved the collection of mature tea leaves from the *Camellia sinensis* Linn plant in the Moulvibazar district of Bangladesh. These leaves were then identified and recognized by the Bangladesh National Herbarium (BNH) in Dhaka (specimen # DACB 45681). All of the chemicals and solvents utilized in this study were of the analytical grade and those were purchased from Sigma Aldrich and E. Merck in Germany.

2.2. Preparation of plant materials

The tea leaves were carefully dried in a controlled environment to prevent exposure to direct sunlight. Following this, they were finely ground using a mechanical grinder. The powdered leaves obtained were carefully stored in a dry, airtight container located in a deep freezer maintained at a temperature of −15 °C, in preparation for subsequent processing and analysis.

2.3. Preparation of tea extracts

10 g of the collected matured tea leaves were washed under running tap water to remove adherent impurities and placed within a closed container. Methanol was subsequently added at a ratio of 1:20. The samples were immersed in the methanol solvent for a duration of 72 h, thereby performing a cold extraction process. It was then filtered. The filtrate containing antioxidant was then processed using a rotary evaporator to obtain extracts.

2.4. Gas chromatography mass spectrometry (GC-MS) analysis

Tea leaves extracts were analyzed using a SHIMADZU GC-MS QP-2020 equipped with an autosampler (AOC-20s) and autoinjector (AOC-20i) utilizing a SH Rxi 5MS Sill column (30 m 0.25 mm; 0.25 m). The temperature in the column oven was programmed at 80.0–280 °C. The initial temperature was 80 °C (hold time: 2 min), then increased at a rate of 5 °C/min up to 150.0 °C (holding time: 5.00 min), and the final temperature was achieved at 280.0 °C (hold for another 8 min). The mobile phase was helium with a flow rate of 1.72 mL/min. The sample injection volume was 6.0 μL at a 20:1 split ratio (with the splitless injection mode), the sample injector performed at temperature 230.0 °C, the ion source temperature was 280.0 °C, and so forth. The ionization mass spectroscopic study utilized an energy of 70 eV. The mass spectrum was acquired over a range from 45 to 350 *m/z*, with the entire run lasting 55.0 min. A solvent cut time of 3.2 min was implemented during the analysis. A small quantity of the crude sample, *Camellia sinensis*, was introduced into a 50 mL Falcon tube. Subsequently, methanol of GC Grade (Sigma-Aldrich) was introduced into the tube until the sample achieved a colorless state. A 2 mL portion was extracted from the upper layer and transferred to a GC vial for analytical purposes. The compounds were characterized by contrasting their mass fragments with those available in the NIST08s, NIST08, and NIST14 libraries.

2.5. UV-visible spectroscopic analysis

The antioxidant activity of the obtained extracts were measured using a UV-VIS spectrophotometer, PerkinElmer Lambda-35 (USA) were performed in a quartz cuvette with an optical pathway of 1 cm. The measurement of absorbance of the sample and the control of various concentrations were carried out for estimation of antioxidant activity of tea leaves extracts.

2.6. Test for antioxidant activity of the extract of matured tea leaves using DPPH radical scavenging

DPPH (1,1-diphenyl-2-picrylhydrazyl) has been frequently used to assess the capacity of compounds to function as scavengers of free radicals and for evaluating the antioxidant properties of substances. DPPH exhibits stability as a free radical owing to the presence of delocalized electrons. The methanol solution of DPPH exhibits a purple color and exhibits a pronounced absorption peak at 517 nm, which can be attributed to the existence of an unpaired electron. As soon as the DPPH free radical is mixed with an antioxidant that can donate a proton, the reduced form will be generated. This phenomenon may be noticed as the DPPH radical in a methanol solution undergoes a color change from purple to yellow. This change occurs due to the pairing of the odd electron of the DPPH radical with a hydrogen atom from the antioxidant, resulting in the formation of the reduced DPPH-H species [50]. The DPPH radical scavenging assay was determined as previously described method with few modification [48]. Ascorbic acid was used as control and methanol was

used as blank sample. 5, 10, 15, 20, 25 and 30 μg/mL solution of control and test sample (tea extract) were prepared in methanol. 0.1 mM solution of DPPH in methanol was prepared. 0.5 mL freshly prepared DPPH solution was added with the different concentration of control and test samples. The mixtures were incubated for 30 min at 37 °C and the absorbance of the mixtures were measured at 517 nm using a UV-VIS spectrophotometer. The percentage of inhibition of free radicals in DPPH was estimated as follows: The experiments were conducted in triplicate.

$$\text{Inhibition } (\%) = \left[\left(\text{ABS}_{\text{control}} - \text{ABS}_{\text{sample}}\right)/\text{ABS}_{\text{control}}\right] \times 100$$

where,

$\text{ABS}_{\text{sample}}$ = the measured absorbance of the test samples,

$\text{ABS}_{\text{control}}$ = the measured absorbance of the controlled solution.

The IC_{50} value of the sample indicate the concentration that required to inhibit 50% of the DPPH free radical, was determined from log dose inhibition curve. This value indicate the DPPH activity. The lower absorbance of the reaction mixture indicated higher free radical activity [51].

3. Results and discussion

3.1. Antioxidant activity of matured tea leaves extract

The IC_{50} value of the methanol extract of matured tea leaves and the standard ascorbic acid which is considered as positive control were found to be 69.5 51 μg/mL and 10.70 μg/mL, respectively has been tabulated in Table 1. These results reveal that the extract possesses significant free radical scavenging capabilities. A lower IC_{50} value suggests higher potency because it means that a lower concentration of the substance is required to achieve a 50% inhibition of the biological function. Conversely, a higher IC_{50} value indicates lower potency, requiring a higher concentration for the same level of inhibition [52]. Unachukwu et al. [48] investigated that green tea exhibit higher antioxidant activity compared to white tea. They found that the IC50 values of 36.07 μg/mL and 23.26 μg/mL for white teas and green tea respectively. Gallic and ascorbic acids were used as positive controls resolving scavenging activity with mean IC_{50} values of 3.68 μg/mL and 11.56 μg/mL respectively. Saito et al. [53] found high antioxidant activity of green teas where the IC_{50} values were found to be ranging from 8.33 to 10.10 μg/m. This differ in IC_{50} values of the same extract is due to the different method of extraction. This has been supported by Manian and coworkers [54], they investigated the antioxidant activity of green tea which were extracted with methanol and 70% acetone. They found that DPPH IC_{50} values of green tea extract of 19.50 μg/mL with a slightly different extraction method. In modern times, there is an increasing dependence on synthetic antioxidants, such as butylatedhydroxy anisole (BHA) and butylatedhydroxy toluene (BHT), despite the possible adverse effects on human health [55]. The increasing prevalence of this phenomenon emphasizes the importance of natural antioxidants, namely those found in extracts of plants. The key active ingredients responsible for the antioxidant activity have been identified as polyphenolic chemicals, specifically tannins and flavonoids [56,57]. The aforementioned findings emphasize the importance of investigating antioxidants derived from plants as a means of discovering alternative sources that are both natural and beneficial for human health.

3.2. Gas chromatography-mass spectrometry (GC-MS) analysis

Gas chromatography-mass spectrometry (GC-MS), a powerful analytical method, integrates gas chromatography and mass spectrometry. This technique is employed to identify a wide range of substances in a test sample, encompassing hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino compounds, nitro compounds, and more [58]. GC-MS is a valuable analytical technique utilized for the detection and identification of trace amounts of substances within various materials. It is widely recognized as the "gold standard" in forensic material identification due to its ability to perform targeted analysis of a given sample [59–61]. In this study, the GC-MS spectrum analysis reveals the identification of eight compounds shown on the chromatogram depicted in Fig. 1, which corresponds to the extract being analyzed. The relative area percentages of the individual peaks of compounds were determined and are presented in Table 2. The quantified percentages for each compound are as follows: caffeine (74.47%), hexadecanoic acid-methyl ester (14.02%), 9,12,15-Octadecatrienoic acid-methyl ester (3.95%), oxirane, tetradecyl (2.04%), phenol-2,4-bis (1,1-dimethylethyl) (1.58%), heneicosanoic acid-methyl ester (1.48%), 9,12-Octadecadienoic acid-methyl ester (1.40%), and 2-pentadecanone, and 6,10, 14-trimethyl (1.06%). The primary compound identified in the analysis is caffeine, as indicated by the highest percentage composition. Additionally, the analysis revealed the presence of several minor compounds, including hexadecanoic acid methyl ester, 9,12,15-Octadecatrienoic acid methyl ester, Oxirane, tetradecyl, Phenol-2,4-bis(1,1-dimethylethyl), heneicosanoic acid methyl ester, 9,12-Octadecadienoic acid methyl ester, and 2-Pentadecanone-6,10,14-trimethyl. Furthermore, the spectrum indicated the presence of a minor

Table 1
The antioxidant DPPH activity of IC_{50} values obtained from the methanol extract of matured tea leaves of *Camellia sinensis* Linn.

Sl No.	Sample	IC_{50} Value (μg/mL)
01.	Ascorbic acid	10.70
02.	Methanol extract of matured tea leaves	69.51

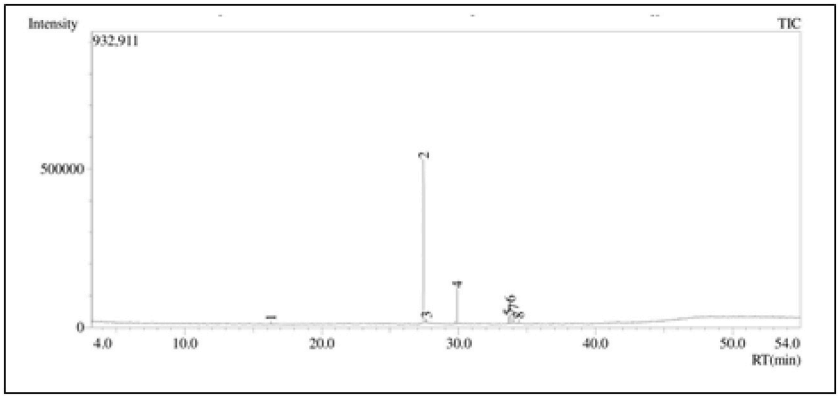

Fig. 1. GC-MS chromatogram of methanol extract of matured tea leaves with retention time.

Table 2
The bioactive compounds isolated from methanol extract of matured tea leaves identified and quantified by GC-MS analysis with retention time and percent yield.

Serial No.	Name of the compounds	Retention time (min)	Yield (%, w/w)
1	Caffeine	27.44	74.47
2	Phenol, 2,4-bis (1,1-dimethylethyl)-	16.29	1.58
3	2-Pentadecanone, 6, 10, 14-trimethyl	27.66	1.06
4	Hexadecanoic acid, methyl ester	29.89	14.02
5	9,12-Octadecadienoic acid, methyl ester	33.675	1.40
6	9, 12, 15-Octadecatrienoic acid methyl ester (Z, Z, Z)	33.776	3.95
7	Oxirane, tetradecyle-	34.016	2.04
8	Heneicosanoic acid, methyl ester	34.394	1.48

proportion of other constituents. The subsequent section provides a concise overview of the typical behavior shown by the compounds.

3.2.1. Caffeine

Caffeine is a natural alkaloid compound that is widely recognized for its stimulant properties. It belongs to a class of chemicals known as xanthines. Caffeine is naturally found in coffee beans, tea leaves, cacao, and some other plants. It is consumed globally, primarily for its stimulating effects. It is widely recognized as the most often taken pharmacologically active metabolite on a global scale. The molecule under consideration is a xanthine alkaloid, which exists in a white crystalline form [62–64]. It possesses a moderate bitterness and is recognized as a potent metabolic stimulant [65]. Additionally, it is often utilized in medicinal treatments with stimulating properties. It is commonly employed in both medical and recreational contexts to alleviate fatigue and sleepiness, as well as to enhance performance in a cost-effective manner, hence reducing the overall likelihood of developing cancer. Furthermore, it is more effectively acknowledged as a non-enzymatic antioxidant [66–68].

3.2.2. Phenol-2,4-bis(1,1-dimethylethyl)

Phenol-2,4-bis(1,1-dimethylethyl) is alternatively referred to as 1-Hydroxy-2,4-di-tert-butylbenzene, 2,4-Di-tert-butylphenol, or 2,4-bis(1,1-dimethylethyl)-phenol. The substance in question is a widely accessible organic compound (secondary metabolite) that has been detected in diverse species of organisms. According to Ajayi et al. [69], it is commonly observed that this substance belongs to the alkyl benzene group and is generally seen as a member of phenols. Furthermore, it is classed as a lipophilic phenol. Each member of this group of phenols typically exhibits di-tert-butyl substituents at the 2 and 4 positions. The study conducted by Zhao et al. (2020) demonstrated noteworthy properties of the substance, including antioxidant, anti-inflammatory, cytotoxic, adulticidal, ovicidal, larvicidal, repellant, antibacterial, antiviral, and antifungal activity [70].

3.2.3. 2-Pentadecanone, 6,10,14-trimethyl

The chemical compound 2-Pentadecanone, 6,10,14-trimethyl is alternatively referred to as Hexahydrofarnesyl acetone, 6,10,14-Trimethyl-2-pentadecanone, 6,10,14-Trimethylpentadecan-2-one, or Perhydrofarnesyl acetone. The chemical in question is classified as an aliphatic ketone [71,72]. Specifically, it can be more accurately categorized as a fatty acid-ketone, as discussed by Prakasia and Nair (2015). This substance is commonly referred to as hexahydrofarnesyl acetone. This particular molecule belongs to the esteemed category of sesquiterpenoids, renowned for their legendary status. Sesquiterpenoids are a group of terpenes characterized by the presence of three successive isoprene units. According to Amudha et al. (2018), the specific phytocomponent known as 2-pentadecanone-6,10,14-trimethyl- exhibits various beneficial properties, including hypocholesterolemic, anti-inflammatory, antibacterial, anti-nociceptive, antioxidant, and lubricating activities [73].

3.2.4. Hexadecanoic acid methyl ester

The compound hexadecanoic acid methyl ester is alternatively referred to as methyl hexadecanoic acid, methyl palmitate, hexadecanoate methyl ester, n-hexadecanoic acid methyl ester, methyl hexadecanoate, and palmitic acid methyl ester. It is included within the category of chemical compounds referred to as fatty acids methyl esters (FAME). Fatty acid methyl esters (FAMEs) are a group of chemicals formed through the esterification of fatty acids with methanol, a process commonly referred to as methylation. The chemical in question plays a significant function as a metabolite and has antifungal properties, as noted by Abubacker and Deepalakshmi (2013) [74]. Palmitic acid is a type of fatty acid characterized by a carbon chain that is fully saturated. Krishnaveni et al. (2014) reported that esters containing a significant amount of palmitic acid are extensively utilized as a primary raw material in the production of laundry soap and toiletry items [75].

3.2.5. Linoleic acid methyl ester

Linoleic acid, also known as 9,12-Octadecadienoic acid (Z,Z), is a molecule derived from fatty acids that has two doubly unsaturated linkages. Omega-6 unsaturated fatty acid is widely recognized and predominantly found in plant glycosides. Methyl-linoleate is a chemically neutral molecule. Linoleic acid holds significant importance as an essential fatty acid crucial for maintaining human health, as it is not endogenously synthesized and necessitates dietary intake. Methyl-linoleate is derived from various plant sources, with notable concentrations found in the bud and fruit of cloves, bulb of garlic, jasmin oil, white mustard oil, parsley leaf oil, and witch-hazel leaf oil [75]. The molecule known as 9,12-Octadecadienoic acid is a cost-effective and readily available source of polyunsaturated fatty acids for human dietary needs. Additionally, it is employed as a crucial pharmaceutical agent in the management of atherosclerosis and hyperlipidemia [76].

3.2.6. α-Linolenic acid methyl ester

The compound denoted as 9,12,15-Octadecatrienoic acid methyl ester (Z,Z,Z) or α-Linolenic acid methyl ester holds significant importance as a form of fatty acid, specifically recognized as a natural source of omega-3. Dietary intake of this nutrient is crucial for maintaining optimal human health, and it is abundantly present in many sources such as seeds and leafy green vegetables. It belongs to the group of necessary fatty acids that cannot manufacture in the human body and essentially be received through diet. The role of α-linolenic acid in reducing the likelihood of cardiovascular disease is widely recognized. As a result of its significant unsaturation, the substance exhibits a pronounced vulnerability to oxidation, leading to rapid rancidity. It is also shown in the literature that α-linolenic acid performs the highest antioxidant efficacy among other α-eleostearic acids against oxidative damage of DNA. This chemical has several bioactive and therapeutic properties, such as antiarthritic, antihistaminic, anticoronary, antiandrogenic, antinematicidal, anticancer, and antibacterial activities [77,78].

3.2.7. Oxirane tetradecyle

The compound oxirane-tetradecyl is referred to by various names in academic literature, including 1,2-Epoxyhexadecane, 1,2-Hexadecene epoxide, 1,2-Hexadecane oxide, 2-Tetradecyloxirane, Hexadecene epoxide, Hexadecylene oxide, Oxirane, 2-tetradecyl-, and Tetradecyloxirane. The presence of tetradecyloxirane, a molecule with significant bioactive and antibacterial properties, has been observed and confirmed in the extract. According to Musa et al. (2015), it has been said that this particular component can be found in the red algae *Laurenciabrandenii* and *Seneciopedunculatus* [79–81].

3.2.8. Heneicosanoic acid-methyl ester

The molecule heneicosanoic acid-methyl ester is alternatively referred to as heneicosylic acid-methyl ester, methyl-meneicosanoate, and methyl-heneicosylate. Methyl-heneicosanoate is a naturally occurring compound that has been identified in several plant tissues. It has been reported in previous studies [82–84] that this compound functions as a metabolite in plants.

4. Conclusions

Tea leaves, particularly from the *Camellia sinensis* Linn plant, are known for their antioxidant properties, which helps to neutralize harmful free radicals in the body. This antioxidant activity is widely studied due to its potential health benefits, including reducing the risk of diseases like cancer and cardiovascular issues. The main goal of this study was to examine the potential of utilizing matured tea leaves, which are typically regarded as agricultural byproducts in the Moulovibazar region of Bangladesh, as a prospective reservoir of antioxidants. Methanol was utilized as the extraction solvent for the purpose of isolating antioxidants. Antioxidant activity was tested using the DPPH (1,1-diphenyl-2-picryl hydrazyl) scavenging free radical assay, with ascorbic acid as a positive control. Additionally, an examination utilizing gas chromatography with mass spectrometry (GC-MS) was performed in order to determine the main compounds of the extract. The IC_{50} values for the methanol extract and ascorbic acid were found to be 69.51 μg/mL and 10.70 μg/mL, respectively. Within the sample, a total of eight bioactive compounds were detected and evaluated by GC-MS. The quantified percentages of the eight major compounds were caffeine (74.47%), hexadecanoic acid-methyl ester (14.02%), 9,12,15-Octadecatrienoic acid-methyl ester (3.95%), oxirane, tetradecyl (2.04%), phenol-2,4-bis (1,1-dimethylethyl) (1.58%), heneicosanoic acid-methyl ester (1.48%), 9,12-Octadecadienoic acid-methyl ester (1.40%), and 2-pentadecanone, and 6,10,14-trimethyl (1.06%). The exploration of bioactive compounds within matured tea leaves, often overlooked as agricultural waste, carries significant importance. Given the variations in tea plants across regions due to soil conditions and rainfall patterns, studying the potential antioxidant source in matured tea leaves becomes crucial. The finding of the hidden wealth within waste materials provides the potential to significantly enhance the sustainability of tea farms.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Md. Rashed Hasan: Writing - original draft, Methodology, Investigation, Formal analysis, Data curation. **Mohammad Majedul Haque:** Methodology, Investigation, Formal analysis, Data curation. **Md. Amirul Hoque:** Writing - review & editing, Methodology, Investigation, Formal analysis, Data curation. **Shahin Sultana:** Writing - review & editing, Methodology, Investigation, Formal analysis, Data curation. **Mohammad Mahbubur Rahman:** Writing - review & editing, Writing - original draft, Supervision, Methodology, Investigation, Conceptualization. **Md. Aftab Ali Shaikh:** Writing - review & editing, Supervision, Funding acquisition. **Md. Khabir Uddin Sarker:** Writing - review & editing, Supervision, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

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

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