**Exploration of Antioxidant and Antimicrobial potential of Agro-Industrial and Spent waste of Camellia sinensis**

**Abstract**

The objective of this research was to assess the value-added potential of agro-industrial and spent black tea waste (SBTW) as a source of antimicrobial and antioxidant phenolic compounds. The influence of extraction solvent (water, ethanol, acetone and Water: ethanol = 60:40) and sample (farm waste [FW], industrial tea waste [ITW] and SBTW) and their relations on the antimicrobial and antioxidant action of the extracts were explored. Among the several extracts of FW, the acetone extract showed the highest antioxidant activity of 72.09 ± 0.35%. Aqueous extract of SBTW gave 59.30 ± 0.354% antioxidant activity. The only extract of ITW that had shown the antioxidant activity was ethanol extract with 1.16 ± 0.25% of this activity. Acetone extract of FW exhibited the highest antimicrobial activity for all investigated pathogens viz. Streptococcus sp (19.3 ± 0.35 mm), Corynebacterium sp (9.3 ± 0.25 mm) and Staphylococcus aureus (3.3 ± 0.25 mm). The antimicrobial activity of acetone extract of SBTW against Corynebacterium sp and Streptococcus sp was found significant (13.33 ± 0.35 mm). The only extracts of ITW that demonstrated antimicrobial activity were ethanol and acetone extract that showed the zone of inhibition of 8.6 ± 0.20 mm and 2.6 ± 0.10 mm for Corynebacterium sp and S. aureus respectively. Flavonoids and phenols were detected in all extracts of FW and SBTW except in the ethanol extracts of latter. Tannins were found in all the extracts of FW, ITW and SBTW except their acetone extracts. Coumarin was detected only in ethanol extracts of FW, ITW and SBTW. The important bioactive components present in the acetone extract of FW of Camellia sinensis were 4-Phenylbut-3-ene-1-yne, 1-Tridecene, Biphenyl, 1-Pentadecene, 8-Pentadecanone, 1-Nonadecene, Neophytadiene, Caffeine, 1-Nonadecene, 4-Oxazolecarboxylic acid, 4,5-dihydro-2-phenyl-, 1-methylethyl ester, Phytol, 1-Heptacosanol, Decanedioic acid, alpha Tocospirone B, Pentatriacontane, Vitamin E, Stigmasta etc. They are known antioxidants and antimicrobials and are of industrial engrossment. Moreover, phenolic compounds hold antimicrobial action against pathogenic bacteria, making them effective preservatives for prolonging the shelf life of foods and inhibiting the growth of foodborne pathogens, as well as anticancer properties, making them valuable assets in several industries, incorporating pharmaceuticals, food, and cosmetics.

**Keywords:** Antimicrobial, bioactive compounds, phenolic compounds, spent tea leaves, waste valorization, flavonoids, antioxidants activity.

**Introduction**

Black tea is one of the popular beverages that is consumed worldwide, besides water. dried and processed leaves of *Camellia sinensis* L produces a herbal beverage popularly known as drinking tea (Basumatary et al. 2018). Exceptional flavour of drinking tea makes this herbal beverage popular, besides outstanding health benefits and copious refreshing effects (Gao et al. 2021). Corresponding to Statista (2024), tea consumption at the global scale was predictable to be 6.7 million metric tons (MMT)/ billion kilograms in 2022 and is anticipated to escalate to 7.4 MMT by 2025. According to the predictions of theFood and Agricultural Organization of the United Nations, the tea production will reach 4.4 MMT by 2027 with the annual growth of 2.2% globally. Various health advantages are connected with drinking tea. Polyphenols are secondary metabolites that functions as a natural antioxidant. Research reveals the existence of antioxidant polyphenols in black teas confers many health benefits like it promotes cardiovascular and metabolic health as it prevents cardiovascular and gastrointestinal diseases (Shen et al. 2019, Tresserra et al. 2014). Anti-ageing, anti-diabetic, anti-microbial activity, and anti-cancer effects are also connected with drinking tea, due to the existence of polyphenols, which are secondary metabolites and functions as a natural antioxidant (Kumar et al. 2023).

The leaves of *Camellia sinensis* undergoes various processes likewithering, rolling, fermenting, and drying during the manufacture of tea. Fermentation is one of the crucial steps during tea production that is responsible for the development of polyphenols (Ismail et al. 2018). During this procedure, tea leaves are oxidized to generate multimeric polyphenols with high antioxidant potential (Łuczaj and Skrzydlewska 2005). As mentioned earlier, polyphenols have substantial health gains and therefore the consumers interested in health and wellbeing prefer to drink the hot water infusion yielded from tea leaves, that not only satisfies thirst, but is also considered as a therapeutic beverage (Zhang et al. 2019). dried tea leaves are steeped in hot water for 5–10 min to prepare black tea infusion. As these brewing conditions are mild, they are insufficient to extricate all the accessible polyphenols present in tea and substantial amounts of polyphenols with a high antioxidant power persist in spent black tea leaves (Rajapaksha and Shimizu 2022, Abdeltaif et al. 2018). Spent tea leaves (STL) generated after brewing become a waste product that demands disposal. This residual (STLs) tallies to approximately 90% (Hussain et al. 2018), and this enormous quantity of solid waste is generated in various points that includes houses, restaurants, tea shops, hotels, etc. causing environmental pollution. STL, is a source of significant quantity of bioactive chemicals, antioxidants, and amino acids (Güçlü Üstündag et al. 2016). These residual polyphenols persist as non-extractable polyphenols, which may intricate with proteins and cell wall polysaccharides (Durazzo 2018). Ready-to-drink (RTD) or bottled tea is also very popular and is commercially produced in many parts of the world due to its convenience of consumption. As a result, a huge quantity of spent tea leaves (STL), the post-production residue of this tea product is produced annually (Kondo et al. 2018). Only a small percentage of STL is used like a feedstock or converted to compost and rest of it mostly become waste (Sagar et al. 2018).

Chiefly because of quality considerations, parts of the tea plant like stems, stalks, buds and dust particles are physically removed throughout various stages of tea processing. These are black tea processing waste (farm waste) (Abdeltaif et al. 2018). Even though this tea waste is generated in huge quantity at tea farms and presents a cost-effective raw material for retrieving bioactive phenolic compounds, there is limited research on its characterization and processing as a source of phenolic (Nadiah and Uthumporn 2015). Copious amount of waste is generated by tea industries post tea production. This waste is the unused portion of tea that has been processed in tea production. Consequently, it is similar in composition as processed tea (Çakmak et al. 2024). The industrial waste is underutilized source of natural antioxidants should also be tested for the presence of bioactive components that can have either antioxidant or antimicrobial activity or both (Abdeltaif et al. 2018).

Tea wastes is an abundant source of nutritional and functional components. Fiber and protein are its nutritional components, whereas tannins, phenols, steroids, and saponins are its functional components. Particularly some forms are recognized as an appropriate source of proteins and comprises a diversity of amino acids (Jiang et al. 2019; Kondo et al. 2018; Xu et al. 2021). Furthermore, saturated fatty acids are also found in tea waste (Fadhil and Saeed, 2016). Tea waste can be employed in producing both food and non-food products because of its significant structural and nutritional potential (Geremu et al. 2016). Use of tea waste as a raw compost is very restricted and often dumped in landfills. In recent times, due to increase in nutritional and environmental interests, these residues have been evaluated as biocomponents (Negi et al. 2022).

In the circular economy system, food by-products valorisation can generate bioactive compounds principally antioxidant polyphenols that can be further applied as functional ingredients in the food industry (Rajapaksha and Shimizu, 2021). Hence, utilization of tea production waste such as farm waste, industry waste and spent tea leave waste is a cost-effective and sustainable attractive mode for the recovery of antioxidant phenolic compounds (Rajapaksha and Shimizu 2020). Likewise, the prospective and feasible utility of tea waste is of high research effort.

Taking all these characteristics into the consideration the goal of this research was to explore the potential of tea farm waste (FW), industry tea waste (ITW) and spent black tea waste (SBTW) to produce polyphenols and other bioactive compounds and thereby to evaluate their anti-oxidant and anti-microbial potential.

**Material and Methods**

**Collection and processing of Tea wastes**

In the present investigation, farm-waste (stalks, branches, leaves) (FW), industrial tea waste (ITW) and spent black tea waste (SBTW) of *Camellia sinensis* were selected. The farm-waste was collected from Sarudih Tea Estate of Jashpur (26°13′N 78°11′E﻿ / ﻿26.22°N 78.18°E)﻿ / 26.22; 78.18, Chhattisgarh, India. Industrial tea waste was collected after the tea was produced in tea industry of this tea estate﻿ / 26.22; 78.18. FW and ITW were dried in a hot-air oven at 60ºC for 20 h and pulverized in a hammer mill to disseminate through a 2.27 mm screen. Black tea (expiration date: 30/12/2024) was supplied by this tea industry. Spent black tea waste was collected after brewing of black tea. Black tea leaves (50 g) were brewed in one litre of boiling water (100°C) for 10 minutes. The infusion was then filtered employing a tea strainer, and the residue was dried in an hot-air oven at 45°C for 10 h and then powdered. All these wastes were then preserved in airtight plastic bags at 4°C to avoid any probable spoilage or degradation.

2,2-diphenyl-1-picrylhydrazyl (DPPH) was supplied by Sigma- Aldrich (Taufkirchen, Germany), DMSO (Merck, Germany). All other chemicals and media used were of Qualigen and Hi-media respectively. Deionized water was employed in all the experimentations. Chemicals and solvents used for liquid chromatography–mass spectrometry (LC–MS) were of HPLC grade.

**Solvent extraction**

One gm of powdered tea waste (FW, ITW and SBTW) was separately taken and was dissolved in 20 ml of organic solvents of different polarities. The solvents selected for the research were of analytical grade (AR grade). The extract was prepared by employing various solvents such as 100% Water (Aqueous extract), Water:Ethyl Alcohol in ratio of 60:40 (Aqueous ethanol extract), 100% Ethyl alcohol (Ethanol extract), 100% acetone (Acetone extract). One part of tea waste was dissolved in 20 parts of organic solvent. The sample-solvent mixture was allowed to stand at 30℃ for 48 hours with gentle agitation. After incubation, the tea waste-solvent mixture was filtered with regular filter paper and then the filtrate was re-filtered with Whatman no. 1 filter paper. The filtrate was then stored in an amber-colored glass bottle for further analysis.

The filtered extracts were then centrifuged to remove any precipitates. These extracts were then reduced using a rotatory evaporator. The extract was gathered from the distillation flask (evaporating flask) with the help of a scalpel. The dried desiccated extracts were weighed and kept in desiccator for 6 hours. After 6 hours it was again weight until we get constant weight of the desiccated sample. The total weight of the extract was weighed using a calibrated weight machine. The extracts were dissolved in Dimethyl Sulphoxide (DMSO) so as to gain a particular concentration (0.1mg/ml) (Miao et al. 2022; Serdar et al. 2017).

**Qualitative analysis of Phytochemical**

All four extracts viz. aqueous extract, aqueous ethanol extract, ethanol extract, acetone extract of FW, ITW and SBTW were tested for the presence of Alkaloid (Mayer's test and Wagner test), Flavonoid (Alkaline reagent test and ferric chloride test), Phenol (Aqueous ferric chloride test), Glycosides (Keller kiliani test), Saponin (Honeycomb test and Foam test), Quinone (Conc. HCl test), Resin (Turbidity method), Carbohydrate (Molisch test and Fehling's test), Tannin (Braymer's test), Terpenoids and Sterols (Salkowski's test), Coumarins (Alkaline test), Amino acids & Proteins (Ninhydrin test), Gums & Mucilage (Precipitation test) (Harborne 1998; Abeysekera et al. 2018; Doss 2009; González Mera et al. 2021)

**Determination of Antioxidant Activity**

The scavenging activity of the diphenyl-2-picrylhydrazyl (DPPH) radicals of extract was measured in accordance to the method that was reported by Chang et al. (2001), which was cited in Shyur et al. (2005). Assays were performed in 3 mL reaction mixtures containing 2.0 mL of 0.1 mM DPPH-ethanol solution, 0.9 mL of 50 mM Tris-HCl buffer (pH 7.4), and 0.1 mL of deionized water or sample extract. The absorbance was measured at 517 nm in UV-Vis Spectrophotometer (Shimadzu Corporation). The capacity to scavenge for the DPPH radical was calculated using the following equation:

Scavenging of DPPH% = [(Absorbance control - Absorbance sample)/Absorbance control]X 100

(Abdeltaif et al. 2018; Brand-Williams et al. 1995; Derewiaka et al. 2022; Gouda and Das 2023)

**Antimicrobial analysis**

Important throat infection causing microorganisms including *Streptococcus sp., Corynebacterium sp. and Staphylococcus aureus* were chosen to examine the antimicrobial activityof all the extracts. All these pathogenic bacteria were isolated from throat sample.The swab sample was collected from the throat of infected person (person with cough and cold). Then the swab was put into 0.9% saline media and kept for 5-6 hours at 37ºC. Then 0.1 ml of the saline diluent were transferred to Nutrient agar media (Peptone: 5gm, Sodium chloride: 5gm, beef extract: 3gm, agar: 18gm, distilled water: 1000 ml, pH: 7.0), and was incubated in bacteriological incubator at 37ºC for 24-48 hours. The bacterial identity was verified bybiochemical assays and Gram’s staining. Bacterial cultures were stored at −70 °C in thesuitable growth medium comprising 30 % glycerol.

The antimicrobial activities of all extracts were tested by the agar well diffusion assay method. The bacteria were cultured separately in Tryptic Soy Broth (Hi-media) at 36 °C for 18 h. Cultures were then centrifuged (Remi) at 3000 g for 15 min. The supernatant was removed, and the pellet formed was dissolved in saline solution to produce 0.5 McFarland standards, comprising nearly 108 CFU/ml bacteria. Final concentration of the bacterial culture was adjusted to 106 CFU/ml, by streaking onto suitable agar with sterilized cotton swab to produce a bacterial lawn.

As mentioned above, the extract solvent was removed by rotatory evaporator. The reduced extract without solvent was carefully transferred to silica gel desiccator for final removal of residual solvent. As the solubility of the extract is low in water, desiccated extract without solvent was dissolved in 20 % DMSO to enhance the extract diffusion through agar. The final concentration of the extract was adjusted to 100 µg/ml (0.1 mg/ml). The concentration of crude extract was standardised to final concentration of 0.1 mg/ml by dilution of 1part desiccated extract in 10 parts of DMSO. These extracts were then filtered through 0.45-μm membrane filter before analysis.

The agar well diffusion assay was performed in Mueller Hinton agar media (Tryptone: 17.5 gm, Starch: 1.5 gm, Beef Extract: 2.0 gm, Agar: 18.0 gm, Distilled water: 1000 ml, pH: 7.0). A sterilized metallic cylinder of 4 mm diameter was employed to make wells. The wells were loaded with 50μl of extract (0.1 mg/ml). The plates were incubated at 36 °C for 18 h. While 20 % (v/v) DMSO was used as -ive control, 100 μg/ml Ampicillin (Gibco, UK) was used as +ive control. The antimicrobial activity was calculated by measuring the zone of inhibition around the wells against the examined bacteria and was expressed in mm (Hedge et al. 2005).

**Partial characterization of optimized bio-constituents.**

GC/MS was employed to analyse the active ingredients present in the optimized extract. GC-MS analysis was employed to study the active constituents present in acetone extract of farm waste using Shimadzu QP2010Plus with Thermal Desorption System TD 20. The sample was presented into glass injector working in split mode; carrier gas was He 99.9995% pure with a Linear Velocity Pressure: 81.7 kPa. Rtx-5 MS fused silica Capillary column (30 meters X 0.25 mm. i.d. X 0.25 um film Thickness). The following temperature: Column Oven Temp: 80.0 °C, Injection Temp: 270.00 °C were used. The constituents were identified using Commercial libraries (Prabhukumar et al. 2015).

**Statistical analysis**

Data were stated as mean ± standard deviation. All of the data were conducted by means of a completely randomized block design with at least three replicates. The data analysis was done using a two-way analysis of variance (ANOVA) and the significant differences were calculated (p < 0.05) by means of least significant difference (LSD).

**Results**

The maximum radical scavenging activity was found in all the extracts of FW. This was followed by the extracts of SBTW. The only extract of ITW that had shown the radical scavenging or antioxidant activity was ethanol extract with 1.16 ± 0.25% of this activity. Among the different extracts of FW, the acetone extract showed the highest antioxidant activity of 72.09 ± 0.35% followed by aqueous extract (61.63 ± 0.32%), aqueous ethanol extract (53.49 ± 0.25%), and ethanol extract with 43.02 ± 0.15% radical scavenging activity. The order of radical scavenging activity of several extracts of SBTW is as following: aqueous extract (59.30% ± 0.354%) ˃ aqueous ethanol extract (44.19 ± 0.35%) ˃ ethanol extract (39.53 ± 0.14%) ˃ acetone extract (24.42 ± 0.48%) (Table 1). The acetone extract of FW showed the highest antioxidant activity of 72.09 ± 0.35% and could be considered as the potential source of polyphenols (figure 1).

**Table 1 – Radical Scavenging activity (%) of various extracts of farm waste, Industrial tea waste and Spent black tea waste**

|  |  |  |  |
| --- | --- | --- | --- |
| Extracts | Radical Scavenging activity (%) | | |
| Farm waste | Industrial tea waste | Spent black tea waste |
| Aqueous | 61.63 ± 0.32 | 0 | 59.3 ± 0.354 |
| Aqueous ethanol (60:40) | 53.49 ± 0.25 | 0 | 44.19 ± 0.35 |
| Ethanol | 43.02 ± 0.15 | 1.16 ± 0.25 | 39.53 ± 0.14 |
| Acetone | **72.09** ± 0.35 | 0 | 24.42 ± 0.48 |

**Figure 1 –** **Pictorial presentation of** **Radical Scavenging activity (%) of various extracts of farm waste, Industrial tea waste and Spent black tea waste**

Flavonoid and phenol were detected in all the extracts of FW. Carbohydrates were detected in all the extracts except ethanol extract. Saponin was detected only in aqueous ethanol and aqueous extracts. Quinones and Glycosides were detected only in aqueous and ethanol extract. Tannins were found in all three extracts except acetone extracts. Terpenoids and sterols were found in all extracts except aqueous extract. Coumarins was detected only in ethanol extract. Amino acid, proteins and resins were not detected in any extract of FW. Gums and mucilage were detected in acetone and aqueous ethanol extracts (Table 2).

Flavonoids and phenols were not detected in any extracts of ITW. Carbohydrates were detected only in acetone extract.Saponins were only detected in aqueous ethanol extracts.Quinones were detected in acetone and aqueous extract.Tannins were detected in all extracts except acetone extract.Terpenoids and sterols were detected only in acetone extract.Coumarins were detected in ethanol extract of ITW. Amino acid, proteins and resins were not detected in any extract of ITW. Gums and mucilage were detected only in aqueous extracts (Table 2).

Flavonoids and phenols were detected in all extracts except in the ethanol extracts of SBTW. Carbohydrates were only detected in aqueous extract. Saponins were detected in aqueous ethanol and aqueous extract.Quinones were detected in acetone and aqueous extracts.Glycosides and tannins were detected in all extracts except in acetone extract.Terpenoids, sterols, resins, were not detected in any extracts. Alkaloids were detected in aqueous ethanol extract.Coumarin was detected only in ethanol extracts. Amino acid, proteins were detected only in aqueous ethanol extracts of SBTW. Gums and mucilage were detected in aqueous ethanol and aqueous extracts (Table 2).

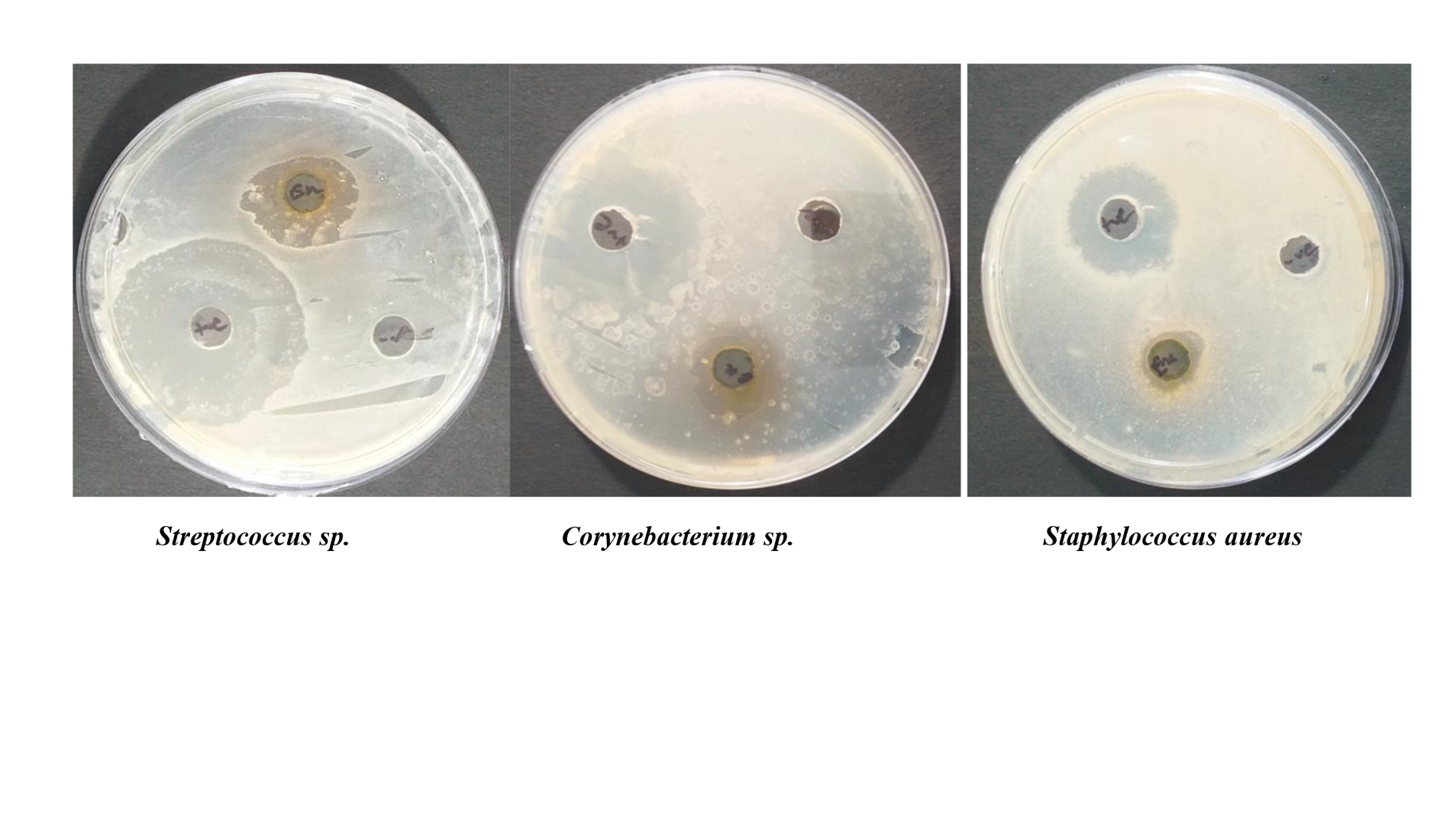
**Table 2 – Bioactive components present in various extracts of farm waste, Industrial tea waste and Spent black tea waste**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **S.NO** | **Name of Tests** | **Farm Waste Extracts** | | | | **Industrial Tea Waste Extract** | | | | **Spent black tea waste Extracts** | | | |
| **Ac** | **Aq-eth** | **Aq** | **Eth** | **Ac** | **Aq-eth** | **Aq** | **Eth** | **Ac** | **Aq-eth** | **Aq** | **Eth** |
| **1(a)** | **Alkaloid (Mayer's test)** | **+** | **+** | **-** | **-** | **-** | **-** | **-** | **-** | **-** | **+** | **-** | **-** |
| **1(b)** | **Alkaloid (Wagner test)** | **+** | **-** | **-** | **-** | **+** | **-** | **-** | **-** | **-** | **+** | **-** | **+** |
| **2(a)** | **Flavonoid (Alkaline reagent test)** | **-** | **+** | **+** | **-** | **-** | **-** | **-** | **-** | **+** | **+** | **+** | **-** |
| **2(b)** | **Flavonoid (ferric chloride test)** | **+** | **+** | **+** | **+** | **-** | **-** | **-** | **-** | **+** | **+** | **+** | **-** |
| **3** | **Phenol (Aqueous ferric chloride test)** | **+** | **+** | **+** | **+** | **-** | **-** | **-** | **-** | **+** | **+** | **+** | **-** |
| **4** | **Glycosides (Keller kiliani test)** | **-** | **-** | **+** | **+** | **-** | **-** | **-** | **-** | **-** | **+** | **+** | **+** |
| **5(a)** | **Saponin (Honeycomb test)** | **-** | **+** | **+** | **-** | **-** | **+** | **-** | **-** | **-** | **+** | **+** | **-** |
| **5(b)** | **Saponin (Foam test)** | **-** | **+** | **+** | **-** | **-** | **+** | **-** | **-** | **-** | **+** | **-** | **-** |
| **6** | **Quinone (Conc. HCl test)** | **-** | **-** | **+** | **+** | **+** | **-** | **+** | **-** | **+** | **-** | **+** | **-** |
| **7** | **Resin (Turbidity method)** | **-** | **-** | **-** | **-** | **-** | **-** | **-** | **-** | **-** | **-** | **-** | **-** |
| **8(a)** | **Carbohydrate (Molisch test)** | **+** | **+** | **+** | **-** | **+** | **-** | **-** | **-** | **-** | **-** | **-** | **-** |
| **8(b)** | **Carbohydrate (Fehling's test)** | **+** | **+** | **+** | **-** | **-** | **-** | **-** | **-** | **-** | **-** | **+** | **-** |
| **9** | **Tannin (Braymer's test)** | **-** | **+** | **+** | **+** | **-** | **+** | **+** | **+** | **-** | **+** | **+** | **+** |
| **10** | **Terpenoids (Salkowski's test)** | **+** | **+** | **-** | **+** | **+** | **-** | **-** | **-** | **-** | **-** | **-** | **-** |
| **11** | **Sterols (Salkowski's test)** | **+** | **+** | **-** | **+** | **+** | **-** | **-** | **-** | **-** | **-** | **-** | **-** |
| **12** | **Coumarins (Alkaline test)** | **-** | **-** | **-** | **+** | **-** | **-** | **-** | **+** | **-** | **-** | **-** | **+** |
| **13** | **Amino acids & Proteins (Ninhydrin test)** | **-** | **-** | **-** | **-** | **-** | **-** | **-** | **-** | **-** | **+** | **-** | **-** |
| **14** | **Gums & Mucilage (Precipitation test)** | **+** | **+** | **-** | **-** | **-** | **-** | **+** | **-** | **-** | **+** | **+** | **-** |

**Ac – Acetone, Aq-eth – Aqueous ethanol, Aq – Aqueous, Eth – Ethanol**

**(+) Presence (-) Absence**

Acetone extract of FW exhibited the maximum antimicrobial activity against all tested pathogens viz. Streptococcus sp (19.3 ± 0.35 mm), Corynebacterium sp (9.3 ± 0.25 mm) and Staphylococcus aureus (3.3 ± 0.25 mm) (Figure 2).



**Figure 2 - Antimicrobial activity of Acetone extract of Farm waste against all tested pathogens viz. Streptococcus sp, Corynebacterium sp and Staphylococcus aureus.**

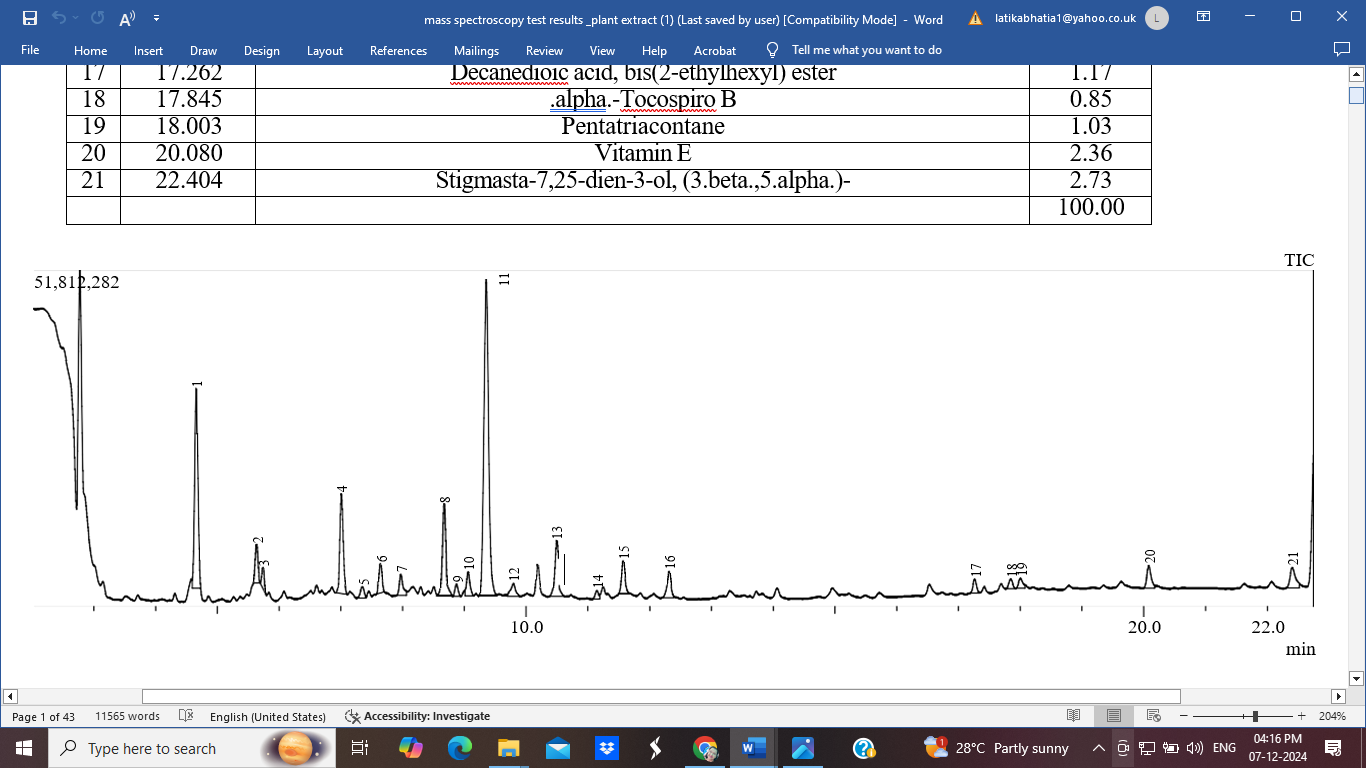
The aqueous ethanol and ethanol extracts of SBTW showed better antimicrobial activity against the Streptococcus sp and S. aureus in comparison to similar extracts of FW and ITW. The antimicrobial activity of acetone extract of SBTW waste against Corynebacterium sp was significant (13.33 ± 0.35 mm) and better in comparison to the same extract of FW (9.33 ± 0.25 mm). Acetone extract of SBTW was also found efficient against Streptococcus sp with zone of inhibition of 13.33 ± 0.35 mm. There was insignificant difference in antimicrobial activity of acetone extract of FW and SBTW against Staphylococcus aureus.

Aqueous extracts of ITW and SBTW dint exhibited antimicrobial activity against any examined bacteria. Similarly, aqueous ethanol extract of FW and ITW dint exhibited antimicrobial activity against Streptococcus sp. and S. aureus. The only extracts of ITW that displayed antimicrobial activity were ethanol and acetone extract that showed the zone of inhibition of 8.6 ± 0.20 mm and 2.6 ± 0.10 mm for Corynebacterium sp and S. aureus respectively. None other extracts of ITW could exhibit antimicrobial activity (Table 3).

**Table 3 – Anti-microbial activity of various extracts of farm waste, Industrial tea waste and Spent black tea waste**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| S.no | **Extract** | **Antimicrobial study against** | **ZONE OF INHIBITION (mm)** | | |
| **Farm Waste** | **Industrial Tea Waste** | **Spent black tea waste** | |
| 1 | Aqueous | *Streptococcus sp.* | 2.5± 0.01 | 0.0 | 0.0 | |
| 2 | Acetone | **19.3** ± 0.35 | 0.0 | **13.33** ± 0.35 | |
| 3 | Aqueous ethanol | 0.0 | 0.0 | 1.5 ± 0.06 | |
| 4 | Ethanol | 6.6 ± 0.32 | 0.0 | 13.0 ± 0.25 | |
| 5 | Aqueous | *Corynebacterium sp.* | 1.6 ± 0.31 | 0.0 | 0.0 | |
| 6 | Acetone | **9.33** ± 0.25 | 0.0 | **13.33** ± 0.35 | |
| 7 | Aqueous ethanol | 4.4 ± 0.30 | 0.0 | 1.6 ± 0.05 | |
| 8 | Ethanol | 12.4 ± 0.34 | **8.6** ± 0.2 | 6.7 ± 0.31 | |
| 9 | Aqueous | *Staphylococcus aureus* | 2.6 ± 0.15 | 0.0 | 0.0 | |
| 10 | Acetone | **3.3** ± 0.25 | **2.6** ± 0.1 | 3.6 ± 0.30 | |
| 11 | Aqueous ethanol | 0.0 | 0.0 | **9.3** ± 0.25 | |
| 12 | Ethanol | 2.6 ± 0.33 | 0.0 | 4.6 ± 0.15 | |

As mentioned above, the maximum radical scavenging activity was reported in all the extracts of farm waste and among them, the highest activity was shown by its acetone extract i.e. 72.09%. Moreover, the acetone extract of farm waste was also found to exhibit the maximum antimicrobial activity against all the tested bacteria. It is presumed that the acetone extract of farm waste is a prospective source of bioactive constituents responsible for these anti-oxidant and anti-microbial activity. To further verify the spectrum of these bioactive constituents and to validate these findings, Gas chromatography- mass spectrophotometry was performed. The important components that were detected by this method were 4-Phenylbut-3-ene-1-yne, 1-Tridecene, Biphenyl, 1-Pentadecene, Benzene, (1-butylheptyl), 8-Pentadecanone, Benzene, (1-methyldecyl), 1-Nonadecene, Benzene, (1-methylundecyl), Neophytadiene, Caffeine, Benzene, (1-methyldodecyl), 1-Nonadecene, 4-Oxazolecarboxylic acid, 4,5-dihydro-2-phenyl-, 1-methylethyl ester, Phytol, 1-Heptacosanol, Decanedioic acid, bis(2-ethylhexyl) ester, alpha-Tocospirone B, Pentatriacontane, Vitamin E, Stigmasta-7,25-dien-3-ol, (3.beta.,5.alpha.) (figure 3). Peaks of these compounds are mentioned in the table 4 along with their retention time and area percentage.



**Figure 3 – Chromatogram obtained after GC-MS of acetone extract of farm waste of Camellia sinensis. Identification of peaks 1 to 21 are mentioned in table 4.**

**Table 4 – Bioactive compounds detected in acetone extract of farm waste of Camellia sinensis by Gas chromatography- Mass spectrophotometry**

|  |  |  |  |
| --- | --- | --- | --- |
| **Peak#** | **Retention**  **Time** | **Name of compounds** | **Area%** |
| 1 | 4.657 | 4-Phenylbut-3-ene-1-yne | 15.57 |
| 2 | 5.636 | 1-Tridecene | 2.49 |
| 3 | 5.738 | Biphenyl | 1.19 |
| 4 | 7.001 | 1-Pentadecene | 8.04 |
| 5 | 7.350 | Benzene, (1-butylheptyl)- | 1.03 |
| 6 | 7.638 | 8-Pentadecanone | 2.24 |
| 7 | 7.971 | Benzene, (1-methyldecyl)- | 1.93 |
| 8 | 8.666 | 1-Nonadecene | 7.59 |
| 9 | 8.871 | Benzene, (1-methylundecyl)- | 0.93 |
| 10 | 9.061 | Neophytadiene | 1.98 |
| 11 | 9.350 | Caffeine | 36.52 |
| 12 | 9.794 | Benzene, (1-methyldodecyl)- | 1.59 |
| 13 | 10.489 | 1-Nonadecene | 4.89 |
| 14 | 11.144 | 4-Oxazolecarboxylic acid, 4,5-dihydro-2-phenyl-, 1-methylethyl ester | 0.68 |
| 15 | 11.570 | Phytol | 2.74 |
| 16 | 12.314 | 1-Heptacosanol | 2.45 |
| 17 | 17.262 | Decanedioic acid, bis(2-ethylhexyl) ester | 1.17 |
| 18 | 17.845 | alpha.-Tocospiro B | 0.85 |
| 19 | 18.003 | Pentatriacontane | 1.03 |
| 20 | 20.080 | Vitamin E | 2.36 |
| 21 | 22.404 | Stigmasta-7,25-dien-3-ol, (3.beta.,5.alpha.)- | 2.73 |

**Discussion**

During the tea production, tea waste is generated and it mostly consists of fibrous waste. A small part of this waste is used as raw material for feedstock or as compost. However, a very large part of the waste generated during tea processing is thrown away. Incorrectly incinerated or disposed tea waste causes major environmental problems. These wastes pollute the water, soil, and air. Therefore, sustainability studies have increased in recent years.

In this study, antioxidant activity of various extracts was measured by the process of DPPH scavenging. The DPPH free radical scavenging assay is most common and comprehensively used method for determining the antioxidant activity, as it takes less time in comparison with other methods (Alma et al. 2003).

DPPH is a commercially accessible dark-coloured crystalline powder with static organic nitrogen radical comprising a sole valence electron at one atom of the nitrogen bridge (Eklund et al. 2005). DPPH is comprised of free radical molecules and has two chief functions: it monitors the chemical reactions encompassing radicals and as a standard for the point and intensity of signals of electron paramagnetic resonance Gulcin and Alwasel (2023). Measurement of the rate reduction of the chemical reaction upon DPPH addition determines the radical nature of that reaction. The deep violet colour of DPPH changes to a pale-yellow colour when the hydrogen atom is abstracted from the antioxidant compound. Greater the reduction of DPPH, greater is the antioxidant power in the extracts, which is described as percentage of inhibition. All processes must be operated in dark or dim light (Ismail et al. 2018).

The factors that influence the DPPH radical scavenging activity are chemical structure of the radical scavenger, solubility of the compound and the Polarity and pH of the reaction medium (Saito et al. 2004). The detected variances in DPPH activity in various forms of extracts may be because of one or more of these factors. The number of hydroxyl groups determines the DPPH scavenging activity of phenolic compounds in extracts of tea leaf (Sroka and Cisowki 2003). The antioxidant activity is also affected by antagonism or synergy or antagonism among the various classes of polyphenols and the radical molecules present in extracts. Therefore, basically, the scavenging ability of DPPH might have depended total flavonoids content and on the amount of total phenolic compound present in the several extracts (Abdeltaif et al. 2018). The acetone extract of FW showed the highest antioxidant activity or stronger scavenging ability of 72.09 ± 0.35% and could be contemplated as the potent source of polyphenols. Flavonoid, phenol and Terpenoids were detected in acetone extract of FW and are assumed to be responsible for this significant activity. Furthermore, a positive correlation between the antioxidant power and total phenolic content indicated that the phenolic compound could be one of the main contributors to the antioxidant capacities of this waste (Zaroug et al. 2014; Sir Elkhatim et al. 2018). The results obtained indicates the substantial amount of phenolic compound are prevalent in the tea wastes due to which they showed high antioxidant activity. Antioxidant parameters between the FW and SBTW were found positively correlated that revels that these wastes had the potential to be an alternate natural resource for the bioactive compounds. These results were in conformity with the statement that was reported by Lee et al. (2008), who stated that the extracts that were rich in flavonoids or phenolic content, exhibited a much higher DPPH scavenging potential in comparison to the other extracts. Furthermore, Romdhane et al. (2017) explored that the range of antioxidant activity of the coffee residues was 64.57% and 52.83%. Likewise, a high antioxidant activities rate of the old black tea leaves and black tea waste was reported by Farhoosh et al. (2007). Ethanol extracts were reported better scavengers of DPPH than ethanol extracts by Siddhuraju and Becker (2003). In contrast, the results of this research disclose that the aqueous extracts of FW and SBTW showed significant antioxidant activity, perhaps the highest in the latter i.e. (59.30% ± 0.354%). The absence of the antioxidant activity of most of the extracts of ITW would have been due to degradation of the natural antioxidants and the development of novel ones in course of tea production (Vignoli et al. 2011).

According to the report of Ghafoor et al. (2011), in comparison with artificial compounds with antioxidant properties, the phenolic compounds obtained from natural resources were found more suitable for applying in food products and were endorsed as natural food additives. Furthermore, according to Dalar et al. (2014), the addition of phenolic in food provides nutritional and health benefits. Wide variation is seen in phenolic content of black tea and it depends on many factors like variety of tea, its geographical origin, harvesting and agricultural practices, harvesting season, processing and analytical approaches. In add-on to these aspects, phenolic content of black tea leaves is also affected by extraction conditions such as extraction solvent used, extraction time, ratio of solvent and tea etc. Tea preparation customs reflects that when water is used extraction solvent brewing times of 30 - 40 min is commonly used for extract preparation. Nevertheless, better yields of phenolics could be attained by employing aqueous ethanol and methanol, and extended extraction times (Astill et al. 2001). The diverse trends in phenolic contents of the extracts can be due to distribution of these compounds within the tea plant and their stability during processing. This results in their presence in the tea waste in variable quantity.

Acetone extract of FW exhibited the highest antimicrobial activity against all tested pathogens viz. Streptococcus sp (19.3 ± 0.35 mm), Corynebacterium sp (9.3 ± 0.25 mm) and Staphylococcus aureus (3.3 ± 0.25 mm). Flavonoid, phenol and Terpenoids were detected in acetone extract of FW and are assumed to be responsible for this significant activity.

The only extracts of ITW that exhibited antimicrobial activity were ethanol and acetone extract that showed the zone of inhibition of 8.6 ± 0.20 mm and 2.6 ± 0.10 mm for Corynebacterium sp and S. aureus respectively. This inhibition is possibly because of the presence of Flavonoids, phenols, Quinones, Tannins, Terpenoids and Coumarins present in these extracts. Flavonoids, Terpenoids, Tannins and phenols are known anti-microbial agents.

The aqueous ethanol and ethanol extracts of SBTW exhibited superb antimicrobial activity against the Streptococcus sp and S. aureus. This may be due to the presence of alkaloids and coumarin present in these extracts respectively. Alkaloids are a class of naturally occurring compounds found in plants, animals and microorganisms. Among the various medicinal properties, the anti-microbial effect is significant along with its anti-inflammatory and anti-cancerous effect (Moyo et al. 2014). The antimicrobial activity of acetone extract of SBTW against Corynebacterium sp and Streptococcus sp was found significant (13.33 ± 0.35 mm). Flavonoids, Quinones and phenols were detected in acetone extract of SBTW. Flavonoids and phenolic acids are plant compounds with various biological roles and potential health benefits (Singh et al. 2020). They are potential antioxidants with potential of neutralizing free radicals and protect against oxidative stress. They are also known anti-microbial that can inhibit bacteria, virus and fungus (Sultana et al. 2007). Quinones are known for their therapeutic applications in cancer treatment, cardiovascular diseases, neurodegenerative diseases (e.g. Parkinson’s and Alzheimer’s) and anti-microbial agents (e.g., lapachol). Tea leaf gums are complex carbohydrates, primarily polysaccharides and glycoproteins that contribute to tea’s smooth, velvety texture. Some gums exhibit antioxidant properties. Mucilages are soluble, gel-like substances extracted from tea leaves that exhibits some anti-inflammatory effect and anti-oxidant activity. Pectin, gum Arabic, mucilinic acid, galactomannans, xylans and arbinogalactans are few types of gums and mucilages found in tea (Thaipong et al. 2006). Sterols were detected in few extracts of TW and ITW. They are well known for their antioxidant activity, anti-inflammatory, anti-diabetic, anti-cancerous effect. Stigmasterol, Ergosterol, Brassicasterol are few types of sterol found in tea (Septyaningsih et al. 2018).

The effect on bacteria varies corresponding to the solvent. The factors that influence the antimicrobial activity are the composition and content of the phenolics in extracts, and the extraction solvent employed (Bansal et al. 2013). Extracts of black tea were found effective against Staphlocococcus aureus and Bacillus cereus(Friedman et al. 2006; Turkmen et al. 2007).

The bioactive components present in acetone extract of farm waste of Camellia sinensis were determined by gas chromatography – mass spectrophotometry. The important components that were detected by this method were 4-Phenylbut-3-ene-1-yne, 1-Tridecene, Biphenyl, 1-Pentadecene, Benzene, (1-butylheptyl), 8-Pentadecanone, Benzene, (1-methyldecyl), 1-Nonadecene, Benzene, (1-methylundecyl), Neophytadiene, Caffeine, Benzene, (1-methyldodecyl), 1-Nonadecene, 4-Oxazolecarboxylic acid, 4,5-dihydro-2-phenyl-, 1-methylethyl ester, Phytol, 1-Heptacosanol, Decanedioic acid, bis(2-ethylhexyl) ester, alpha-Tocospirone B, Pentatriacontane, Vitamin E, Stigmasta-7,25-dien-3-ol, (3.beta.,5.alpha.). These compounds neutralize the free radicals and reactive oxygen species (ROS) that are responsible for lipid oxidation and food spoilage (Romanini et al. 2023). They are known antioxidants. Moreover, phenolic compounds hold antimicrobial action against pathogenic bacteria, making them effective preservatives for prolonging the shelf life of foods and inhibiting the growth of foodborne pathogens, as well as anticancer properties, making them valuable assets in several industries, incorporating pharmaceuticals, food, and cosmetics (Punzo et al. 2021).

Biphenyl are found to have importance in pharmaceuticals as they are used there as intermediates in the synthesis of numerous pharmaceuticals like anti- histamines, antacids, and anti-inflammatory agents. It displays antioxidant properties that guard against cell damage and oxidative stress (Romanini et al. 2023). Biphenyl also show anti- microbial properties which can aid safeguard against infections and disease. 1- Pentadecene is used in preparing pharmaceuticals like anti-histamines and anti-inflammatory agents. 1- Nonadecene is a linear alpha- olefin with chemicals formula C19H38. Neophytadiene is a diterpene hydrocarbon having antimicrobial and anti-oxidant properties (Almanza-Oliveros et al. 2024). They can be employed to create anti-microbial coating for various surfaces, decreasing the risk of microbial contamination. Neophytadiene can be employed as an anti-oxidant supplement, aiding to protect against oxidative stress and cell damage. They can also be used producing anti-cancer and anti-inflammatory agents. Phytol is a diterpene alcohol found in many plant species. Its properties are same as these described for neophytadiene. 1- Heptacosanol is a long chain aliphatic alcohol with chemical formula C27H56O. It exhibits emollient properties and is used as an excipient in pharmaceutical formulations, providing emollient and anti-microbial properties. Pentatriacontane is a long chain alkane with the chemical formula C35H72. It has biomedical usages, comprising tissue engineering and drug delivery. It is used as an excipient in pharmaceutical formulations, providing lubricant and emollient properties (Punzo et al. 2021). Alpha-tocospirone B is a natural compound prevalent in many plant species including rice-bran and wheat germ. It is recognized for its antioxidant properties, anti-inflammatory effects, neuroprotective effects, cardiovascular health and cancer prevention. 8- Pentadecene is a medium chain triglycerides (MCT) ketone with a 15- carbon chain that exhibits neuroprotective effect and anti-inflammatory effect. It improves cardiovascular health. Neophytadiene is a diterpene hydrocarbon found in various plant species.it also exhibits anti-oxidant activity and anti-microbial properties. It can be thus used to create anti-microbial coating and anti-oxidant supplements (Romanini et al. 2023).

1-Tridecene has been reported to exhibit both antimicrobial & antioxidant properties.  
 1-Tridecene has been shown to display antibacterial activity against several bacteria, including Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa (Ferreira and Santos 2022). 1-Tridecene has also been described to show antifungal activity against many fungi, including Candida albicans and Aspergillus niger. 1-Tridecene has been described to show free radical scavenging activity, which can aid to protect against oxidative stress and cell damage (Punzo et al. 2021). 1-Tridecene has been described to have antioxidant capacity, which can aid to protect against lipid peroxidation and oxidative damage. The precise method of action of 1-Tridecene's antimicrobial and antioxidant properties is not fully understood (Armstrong et al. 2020). Nevertheless, it is thought that the compound's hydrophobic nature and ability to co-operate with biological membranes may contribute to its antimicrobial and antioxidant effects. The antimicrobial and antioxidant properties of 1-Tridecene make it a potential candidate for various applications. 1-Tridecene could be employed as a natural antimicrobial agent in pharmaceutical formulations (Romanini et al. 2023). 1-Tridecene could be employed as a natural antioxidant and antimicrobial agent in cosmetic products. 1-Tridecene could be employed as a natural preservative to extend the shelf life of food products. However, extend research is required to fully search the potential applications and benefits of 1-Tridecene's antimicrobial and antioxidant properties (Almanza-Oliveros et al. 2024).

Stigmasta-7,25-dien-3β-ol, usually identified as stigmasta, is a plant sterol prevalent in several plant species, including soybeans, corn, and wheat. Stigmasta has been shown to display antimicrobial properties, which can help protect plants against pathogens and pests.  
 Stigmasta has antioxidant properties, which can help safeguard plants against oxidative stress and cell damage (Quazi et al. 2011). Stigmasta has been shown to help reduce cholesterol levels in humans, which can help avoid cardiovascular disease (Punzo et al. 2021). Stigmasta has been demonstrated to exhibit anticancer activities, which can aid avoid the progression and spread of cancer cells. Stigmasta has been reported to have immunomodulatory effects, which can help support the immune system. Stigmasta is used in the producing various pharmaceuticals, including cholesterol-lowering agents and anticancer drugs (Ferreira and Santos 2022).

Vitamin E is a fat-soluble vitamin that plays an important role in various biological processes. Vitamin E is a potent antioxidant that aids to safeguard cells from destruction initiated by free radicals, which can contribute to aging, cancer, and other diseases. Vitamin E supports to preserve the integrity of cell membranes, guarding them from oxidative damage and maintaining cellular function (Yamauch 1997). Vitamin E is engaged in the regulation of the immune system, helping to protect against infections and diseases. Vitamin E neutralizes free radicals, protecting them from causing cellular damage (Romanini et al. 2023).

Decanedioic acid, bis(2-ethylhexyl) ester, also known as di(2-ethylhexyl) decanedioate, is a synthetic compound used in various industrial applications. 4-Phenylbut-3-ene-1-yne is a synthetic organic compound with potential biological significance. The compound has been described to display antimicrobial action against various microorganisms, including bacteria and fungi (Punzo et al. 2021). The cytotoxic activity of 4-phenylbut-3-ene-1-yne makes it a potential candidate for anticancer therapy. The antimicrobial action of the compound suggests its potential use as an antimicrobial agent in various applications, including pharmaceuticals and agriculture. The neuroprotective effects of 4-phenylbut-3-ene-1-yne make it a potential candidate for the treatment of neurodegenerative diseases, such as Alzheimer's disease (Almanza-Oliveros et al. 2024; Jaradat et al. 2021).

4-Oxazolecarboxylic acid is a heterocyclic compound with a breadth of biological activities. 4-Oxazolecarboxylic acid has been shown to display antimicrobial action against various fungi, bacteria, and viruses (Romanini et al. 2023). The compound has been reported to have anticancer properties, inhibiting the growth of cancer cells and inducing apoptosis.  
4-Oxazolecarboxylic acid has anti-inflammatory effects, reducing inflammation and oxidative stress in various disease models (Ferreira and Santos 2022). The compound has neuroprotective effects, protecting against neurodegeneration and improving cognitive utility in animal models. 4-Oxazolecarboxylic acid could be used to cure several inflammatory ailments, like arthritis and asthma. The compound's neuroprotective effects make it a potential candidate for the treatment of neurodegenerative diseases, like Alzheimer's and Parkinson's (Almanza-Oliveros et al. 2024; Canan et al. 2017).

It is significant to emphasize that the bioactive components of acetone extract of farm wastes of Camellia sinensis exhibits antioxidant and antibacterial properties, placing it as an agro-residue with implying potential for usage in the developing innovative functional foods, dietary supplements and cosmetics, associating with the model of circular bioeconomy for its valorization.

**Conclusion**

Phenolic compounds were detected in extracts of FW, ITW and SBTW that indicates the feasibility of using these cost-effective and renewable resources of natural bioactive compounds in the food industries for developing functional foods. Natural antioxidants are present in these wastes. Phytochemicals can be recovered from both the FW and SBTW which may have a substantial prospect as cosmetic constituents, pharmaceuticals and as food additives, for various industries. FW, ITW and SBTW as raw material are of commercial significance for developing value-added products with antioxidant and antimicrobial properties. It is possible to extract alkaloids, flavonoids, phenols, glycosides, saponin, quinone, tannin, terpenoids, sterols, coumarins, proteins, carbohydrates from these tea wastes by applying various solvents in extraction methods. These compounds are significant in terms of nutritional value and biological activity. Aqueous ethanol solvents present cost-effective, non-toxic, eco-friendly alternatives for recovering antibacterial and antioxidant phenolics from these wastes. Optimization of extraction processes would lead to valorize these high-value-added components, thus offering benefits to save money and energy and protect the environment. Further research of these optimizations are needed at industrial level. Multiple bioactive products can be developed from these wastes if caffeine is separated from phenolics. Furthermore, the bioavailability, bioactivity, and toxicology of black tea waste phytochemicals should be thoroughly evaluated in vitro and in vivo studies prior allowing for the reuse of these wastes in food industries.

**Declaration of Competing Interest**

Authors declare they don’t have any competing interest to declare. The authors have no competing interests as defined by Springer, or other interests that might be perceived to influence the results and/or discussion reported in this paper.

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