



**International Journal of Biology, Pharmacy
and Allied Sciences (IJBPAS)**
'A Bridge Between Laboratory and Reader'

www.ijbpas.com

COMPARISON OF AQUEOUS AND ISOPROPANOL EXTRACTS OF PHYTOCHEMICALS AND ANTIOXIDANT ACTIVITY OF SPENT TEA LEAVES

DUTTA S^{1*}, KAR S², GHOSH A³ AND MUKHERJEE P⁴

- 1: Faculty, Department of Dietetics and Applied Nutrition, Amity University, Kolkata, West Bengal, India-700135
- 2: Assistant Professor, Department of Dietetics and Applied Nutrition, Amity University, Kolkata, West Bengal, India-700135
- 3: Student, Department of Dietetics and Applied Nutrition, Amity University, Kolkata, West Bengal, India-700135
- 4: Student, Department of Dietetics and Applied Nutrition, Amity University, Kolkata, West Bengal, India-700135

*Corresponding Author: Mr. Suchandra Dutta: E Mail: sdhsc_rs@caluniv.ac.in

ORCID ID: 0000-0002-8706-2700

Received 15th March 2023; Revised 8th July 2023; Accepted 23rd Oct. 2023; Available online 1st July 2024

<https://doi.org/10.31032/IJBPAS/2024/13.7.8170>

ABSTRACT

Tea (*Camellia sinensis*) has numerous health-beneficial effects such as antioxidative, anti-inflammatory, and anticarcinogenic properties. Tea has secured its place in the daily diet of nearly 90% population of the world. The present study focuses on comparing the effect of solvent (aqueous and isopropanol) as well as the effect of consecutive dips (up to three times) on the antioxidant profile during the preparation of extracts of Darjeeling tea. Antioxidant activity of aqueous and solvent extracts have been compared based on TPC, TFC, DPPH and TAC which shows the subsequent decrease in the antioxidant content of consecutive dips of aqueous extracts of tea in terms of TPC ranging from 111.39 mg GAE/g to 12.15 mg GAE/g and TAC ranging 937.26 mg AAE/g to 374.55 mg AAE/g of first and third extract respectively. However, contrasting natures have been observed in the solvent extract in terms of TPC ranging from 11.43 mg GAE/g to 30.8 mg GAE/g and TAC ranging from 248.21 mg AAE/g to 287.61 mg AAE/g of the first and third extract respectively. The findings may offer insightful

information on the potential amount of antioxidants remaining in used tea leaves which can be further used as food preservatives, skincare purposes and natural dyes.

Keywords: spent tea leaves, tea extracts, antioxidant, polyphenol, flavonoids

1. INTRODUCTION

Camellia sinensis (Tea) belongs to the *Theaceae* family which is generally found in tropical and subtropical climates. Several types of tea are prepared from the leaves of *Camellia sinensis* considered as a very popular non-alcoholic drink after water. The origins of tea were traced back to ancient China, around 2737 BC [1]. Based on the classification, and processing of tea leaves; they are black (fermented), green (non-fermented), and oolong (semi-fermented). These major differences in tea classification depend on the production, and different processes of drying or fermentation that determine the chemical composition of tea [2]. Consumption of tea is a pleasant

emotional activity that is linked to feelings of enjoyment, joy, and relaxation which was frequently connected to happy memories and experiences [3]. The strong aroma and super refreshing power of *Camellia sinensis* and its health benefits were analysed later. When the British East India Company began cultivating tea in India during the colonial era, *Camellia sinensis* was introduced to the nation. India does a huge business with the import and export of tea which is a big economic contribution to the nation [4]. Darjeeling black tea is mainly preferred for its unique qualities of rare fragrance, whereas Assam tea is famous for its colour [5].



Figure 1: *Camellia sinensis* (Tea)

Camellia sinensis has powerful antioxidants owing mainly to the presence of the Flavanols, epicatechin gallate (ECG), Polyphenols (PP), Epigallocatechin gallate (EGCG), Tannins (TN), epigallocatechin (EGC) and epicatechin (EC) [6]. These compounds show physiological effects by

acting as free radical scavengers [7]. They usually have potential health benefits of these compounds, such as their antioxidant, anti-inflammatory, and anticancer properties also studied their impact on cell proliferation, apoptosis, angiogenesis, and metastasis [8].

Camellia sinensis aids with weight loss by boosting metabolism and suppressing hunger [9]. When paired with a healthy diet and exercise, green tea has been demonstrated to be useful in encouraging weight loss [10]. By avoiding DNA damage and slowing the development of cancer cells, the antioxidants in tea can aid in the prevention of cancer [11]. Excessive tea consumption can have negative health effects on the body, including iron deficiency, anxiety, insomnia, dehydration, and gastrointestinal problems (GI). To minimize bad effects, it is advisable to drink tea cautiously and to avoid ingesting large amounts of it when you are hungry [12].

Antioxidants that are present in abundant amounts in tea aid in defending the body against the harm done by free radicals, which can be a factor in a number of health issues, including cancer, heart disease, and aging [13]. Regular tea consumption has been related to better heart health. According to studies, bioactive compounds present in tea can lower the risk of heart disease by increasing blood flow and decreasing inflammation [14].

The main objective of this study is to compare the antioxidant activity in the aqueous and solvent extracts of spent tea leaves. The study involves in vitro assays to find out the total phenolic content, total flavonoid content, and antioxidant activity of the extract. It may offer the future scope

of utilization of spent tea leaves as a potent source for extracting natural antioxidants and using them as a valuable preservative for food uses.

2. MATERIALS AND METHODS

2.1 Collection of the sample:

Darjeeling black tea leaves were collected from the local market of Kolkata, West Bengal, India.

2.2 Preparation of the Tea extracts:

2.2.1 Preparation of aqueous extract

Distilled water (100ml) was taken in a beaker and heated (100 °C), then poured into the beaker containing 1.27g tea leaves and left for 15 mins for extraction. The tea leaves were then sieved out and poured into the beaker [15]. The same procedure was used to make consecutive 2 more extracts. Later on, these extracts were poured into 3 different glass jars, labelled accordingly and stored in the refrigerator at 4 °C.

2.2.2 Preparation of solvent extract

Isopropanol (100ml) was added and heated slightly (45-50 °C), then poured into the beaker containing 1.27g tea leaves and left for 15 mins for extraction. The tea leaves were then sieved out and poured into the beaker [16]. The same procedure was used to make consecutive 2 more extracts. Later on, these extracts were poured into 3 different glass jars, labelled accordingly and stored in the refrigerator at 4 °C.

2.3 Chemicals Used:

All the chemicals and solvents used in the experiment were of good analytical grade.

2.4 Quantitative Estimation of Physicochemical Properties:

2.4.1 Total Moisture Content: Moisture content was determined by using AOAC Standard [17]. Fresh samples were weighed, evenly spread on a tray, and dried at $55^{\circ}\text{C} \pm 5^{\circ}\text{C}$ for 5 h. Then the sample was cooled at room temperature and the weight was checked. The sample was dried again in the hot air oven for 30 min then the weight was checked after cooling. The same procedure was repeated until the difference between two consecutive readings came to less than 1 milligram. Total moisture content was calculated by using the given formula.

$$\text{Moisture \% by weight} = \frac{\text{initial weight of sample} - \text{final weight of sample}}{\text{initial weight of sample}} \times 100 \quad (1)$$

2.4.2 Total Ash Content: Ash content was determined by using AOAC Standard [17]. Previously dried in the hot air oven, samples were weighed in a crucible and heated at $600^{\circ}\text{C} \pm 10^{\circ}\text{C}$ for 5 h till grey ash results. Cooled in the desiccator and weighed. Total ash content was calculated by using the given formula.

$$\text{Ash \% by weight} = \frac{\text{final weight of sample}}{\text{initial weight of sample}} \times 100 \quad (2)$$

2.6 Quantitative Analysis of Phytochemicals:

2.6.1 Total Phenolic content-The method was slightly modified to determine the total phenolic content [18]. 9.5 ml of distilled water was added to 50 μl of the sample.

Folin-Ciocalteu's reagent was then added, reaching a final volume of 0.5 ml. Then, 2.5 ml of 20% sodium carbonate (Na_2CO_3) solution was added. In a vortex mixer, the test tube's contents were mixed. A UV-Visible Spectrophotometer (Labtronics, Model: LT-2201) was used to measure the absorbance at 725 nm after incubating the sample for 40 minutes at room temperature and in the dark. Gallic acid was employed in a variety of concentrations as the calibration curve's standard under the same parameters. The total phenolic content per gram of dry material was calculated as mg of Gallic acid equivalents (GAE/g).

2.6.2 Total flavonoid content - The flavonoid content was assessed using aluminium chloride with slight modification [18]. 0.3 ml of 5% sodium nitrite (NaNO_2) was added after mixing 100 μl of the sample with 4 ml of distilled water, and the mixture was left to stand for 5 minutes. Following the addition of 0.3 ml of 10% aluminum chloride (AlCl_3), the mixture was allowed to stand for an additional 6 minutes before receiving 1 ml of 1(M) sodium hydroxide (NaOH). A little amount of distilled water was added, and the final volume was adjusted to 10 ml. The absorbance was measured using a UV-Visible Spectrophotometer (Labtronics, Model: LT-2201) at 510 nm wavelength. Quercetin was used in different concentrations as the standard for the standard curve. The number

of flavonoids was determined as mg quercetin equivalents (mg QE/g) per gram of the dried sample.

2.7 Determination of In Vitro Antioxidant Activity:

2.7.1 DPPH radical scavenging assay -

The antioxidant activity assay was measured with some modification by using g 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH) [19]. The ethanolic solution of DPPH was prepared by adding 0.002 g of DPPH in 50 ml of ethanol. 0.5 ml of the sample was mixed with 2.5 ml of DPPH solution and kept in the dark at room temperature for 20 minutes. The absorbance of the mixture was measured at 517 nm in a UV-Visible spectrophotometer (Labtronics, Model: LT-2201). DPPH radical scavenging activity was calculated by the given formula.

$$\% \text{ of inhibition activity} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100 \quad (3)$$

2.7.2 Total antioxidant capacity (Phosphomolybdenum assay) -

The assessment of antioxidant properties of tea extracts was done by using the Phosphomolybdenum method of with a few modifications [19]. To prepare the molybdate reagent solution, 4.704 ml of sulphuric acid (H₂SO₄), 0.392 g of ammonium molybdate, and 0.288 g of sodium phosphate were mixed, and distilled water was added to make up the volume to 80 ml. 300 µl sample was mixed with 3 ml of molybdate reagent in a glass test tube.

The test tubes were sealed with cotton and were placed in incubation in the water bath for 90 minutes at 90°C. At room temperature, samples were cooled down, and the absorbance at 695 nm was determined. Ascorbic acid was used in different concentrations as the standard for the standard curve. Phosphomolybdenum assay was expressed as mg of ascorbic acid equivalents (AAE) per gram of dry sample (mg AAE/g).

2.5 Statistical Analysis:

All the data were calculated as mean ± SD by the SPSS application.

3. RESULTS AND DISCUSSION

3.1 Quantitative estimation of physicochemical properties of Tea (*Camellia sinensis*)

This includes the analysis of Moisture content and Ash content of *Camellia sinensis* which is 4.97% and 34.69% respectively as mentioned in (Table 2).

The moisture content is 4.97 % which is low as the taken sample was already dried during its processing. *Camellia sinensis* plants have an extraordinary capacity for absorbing and storing nutrients from the soil. Hence the ash level is 34.69 % which the tea plant develops, its root system absorbs minerals and transfers them to the leaves.

3.2 Quantitative estimation of phytochemicals and antioxidant activity of spent tea leaves (Table 3)

Table 2: Moisture and Ash Content of Tea (*Camellia sinensis*)

Sample	Moisture (%)	Ash (%)
<i>Camellia sinensis</i> (Tea) leaves	4.97±0.20	34.69±0.17

Note: Data are expressed as mean ± SD (n=3)

Table 3: Quantitative estimation of phytochemicals and antioxidant activity of aqueous and isopropanol extract of spent tea leaves

Antioxidant property	Extract	Total phenolic content (mg GAE/g of dried spent tea leaves)	Flavonoid content (mg QE/g of dried spent tea leaves)	DPPH activity (% of inhibition/ g of dried spent tea leaves)	Total antioxidant capacity (mg AA/g of dried spent tea leaves)
Aqueous extract	1 st	111.29± 0.57	703.43±0.82	94.23± 0.57	937.16±0.71
	2 nd	44.13±1.52	404.24±1.55	96.11±0.87	636.03±2.08
	3 rd	12.38±1.16	258.56±2.50	90.29±0.81	374.23±2.64
Solvent extract	1 st	11.43 ± 0.93	723.72 ± 2.82	93.31±0.51	248.12 ± 1.84
	2 nd	3.59 ± 1.02	707.87 ± 1.26	78.35±0.67	225.54 ± 1.09
	3 rd	30.81 ± 0.87	749.22 ± 1.04	87.24±0.42	287.59 ± 2.42

Note: Data are expressed as mean ± SD (n=3)

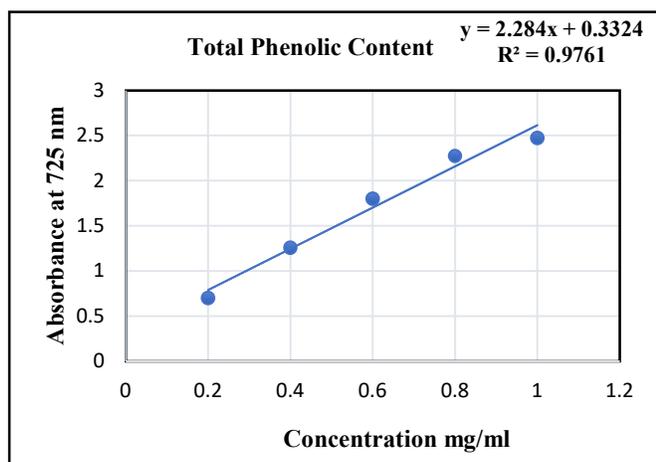


Figure 2: Standard Curve of Total Phenolic Content

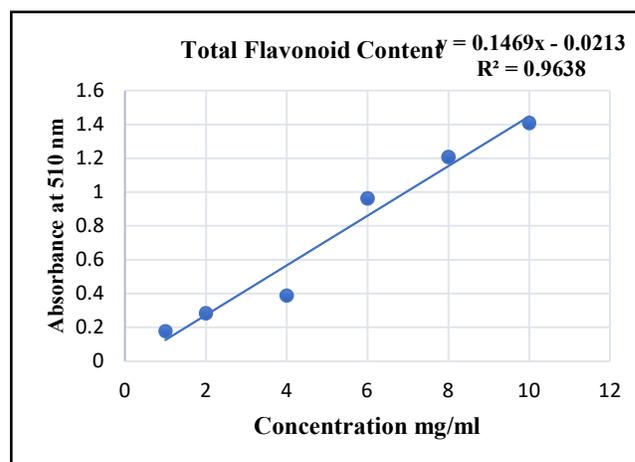


Figure 3: Standard Curve of Total Flavonoid Content

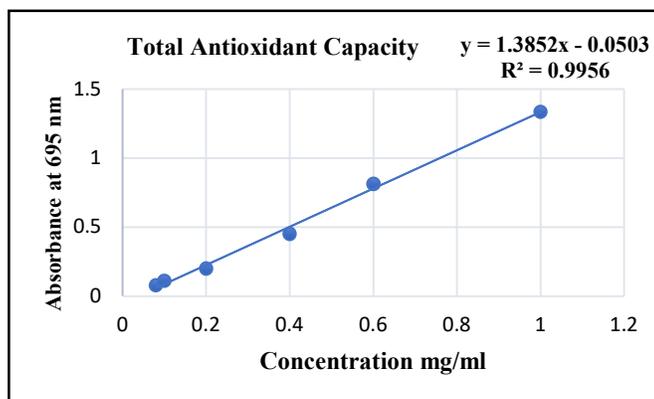


Figure 4: Standard Curve of Ascorbic acid

Phenolic compounds are identified with multiple phenol rings and hydroxyl groups, which have the ability to scavenge and neutralize free radicals [20]. In this study, Total Phenolic Content (TPC) was identified with the help of Folin-Ciocalteu reagent for *Camellia sinensis* (aqueous extract) was gradually decreasing from the 1st dip to 3rd dip of tea 111.29 mg GAE/g >44.13 mg GAE/g >12.38 mg GAE/g as with every dipping gradually phenolic compounds are extracted from the tea leaves into the water, resulting in a reduced concentration of phenolic content in the spent leaves. However, for the isopropanol extract, the phenolic content of initial and final dips of tea 11.43 < 30.81 mg GAE/g is increasing. The gallic acid standard curve equation ($y = 2.284x + 0.3324$, $R^2 = 0.9761$) was used to determine the total phenolic content (Figure 2).

Flavonoids are natural compounds derived from plants having numerous health benefits like anti-inflammatory, anticarcinogenic, antimicrobial and anti-allergic properties [21]. Flavonoids, due to their radical scavenging activity are being considered as potent antioxidant. In this study, the total flavonoid content was calculated by the standard curve equation of quercetin ($y = 0.1469x - 0.0213$, $R^2 = 0.9638$) (Figure 3). The values obtained show the trend that for aqueous extract antioxidant activity decreases with consecutive dips; 1st > 2nd >

3rd dip of tea are 703.43 mg QE/g, 404.24 mg QE/g and 258.56 mg QE/g respectively. In case of isopropanol extract of *Camellia sinensis*, TFC has been found the most for final dip (749.22 mg QE/g) and lowest for second dip (707.87 mg QE/g). The variation between the findings and the other results can be there due to the use of the extraction methods, as the antioxidants extraction generally gets affected by many factors like the extraction method, temperature, and extraction time [22]. Both flavonoids and phenolic compounds have the capability to chelate with these metal ions and help in the reduction of oxidation and increase the shelf life of food [21].

A higher inhibition percentage indicates a stronger antioxidant capacity, which signifies a greater reduction in the concentration of the free radicals of DPPH. In this study for aqueous extract, the percentage of inhibitions are as follows for 1st dip-94.23%, 2nd dip – 96.11%, 3rd dip 90.29%. Parallely it can be seen that the outcomes of inhibition % for solvent extracts are 1st dip-93.34%, 2nd dip -78.12% and 3rd dip -87.10%. This trend shows that aqueous extracts have greater scavenging capacity than solvent extracts in terms of DPPH radical scavenging activity.

The phosphomolybdate method is one of the popular evaluating tools to estimate total antioxidant capacity of plant extracts. In the presence of extracts, Mo (VI) is reduced to

Mo (V) and forms a green-coloured phosphomolybdenum V complex, which shows maximum absorbance at 695 nm [22]. The total antioxidant capacity was calculated by the standard curve equation of ascorbic acid ($y = 1.3852x - 0.0503$, $R^2 = 0.9956$) (Figure 4). The values obtained show the trend that for aqueous extract antioxidant activity decreases with consecutive dips; the aqueous extract of 1st dip: 937.16 mg AAE/g > 2nd dip: 636.03 mg AAE/g > 3rd dip: 374.23 mg AAE/g, whereas, a contrasting trend has been observed in case of solvent extracts where the value of final dip is more than the initial dip; 1st dip: 248.12 mg AAE/g < 3rd dip: 287.59 mg AAE/g. So, it may be concluded that aqueous extract has overall higher scavenging capacity of free radicals than the solvent extracts.

4. CONCLUSION

Study shows that a significant portion of antioxidants can only be extracted during the initial dip. However, it can be clearly observed from this present study that good quantity of these antioxidant compounds remains in the spent tea leaves even after 3 consecutive dippings. Although the exact antioxidant content may vary depending on factors such as tea type, brewing method, and duration of brewing. Phytochemicals that are isolated from spent tea leaves can act as potent natural antioxidants to be used as a preservative for other food items. Few metal

ions such as iron and copper usually catalyze the oxidative reactions in food, which leads to degradation and spoilage of the food. Both flavonoids and phenolic compounds have the capability to chelate with these metal ions and help in the reduction of oxidation and increase the shelf life of food. Flavonoids can also inhibit the activity of polyphenol oxidase which is the responsible enzyme for the browning of food can be limited to a certain extent which will increase the shelf life of food. So, this study mainly provides the future scope of reutilization of spent tea leaves for extraction of natural antioxidants which can be used in food preservation and skin care essentials.

ACKNOWLEDGEMENT: The support provided by the Department of Dietetics and Applied Nutrition, Amity Institute of Applied Sciences, Amity University Kolkata, is gratefully acknowledged.

5. REFERENCES

- [1] Wang N, A Comparison of Chinese and British Tea Culture, *Asian Culture and History*, 3(2), 2011, 13-18 doi:10.5539/ach.v3n2p13
- [2] Kahn N, Mukhtar H, (2013). Tea and Health: Studies in Humans, *Current Pharmaceutical Design*, 19(34), 2013, 6141-6147.
doi: [10.2174/1381612811319340008](https://doi.org/10.2174/1381612811319340008)

- [3] Lutgendorf P, Making tea in India, Thesis Eleven, 113(1), 2012, 11–31. doi:10.1177/0725513612456896
- [4] Fielder C. H., On the Rise, Progress, and Future Prospects of Tea Cultivation in British India, Journal of the Statistical Society of London, 32(1), 1869, 29.
- [5] Bhutia S, Darjeeling Tea Industry: A Geographical Perspective on Production, Development, Promotion and Prospects. Khoj: An International Peer Reviewed Journal of Geography, 3, 2006, 84-96. Doi: <https://doi.org/10.5958/2455-6963.2016.00009.6>
- [6] Salah N, Miller N J, Paganga G, Tijburg L, Bolwell G P, Riceevans C, Polyphenolic Flavanols as Scavengers of Aqueous Phase Radicals and as Chain-Breaking Antioxidants, Archives of Biochemistry and Biophysics, 322(2), 1995, 339–346. doi:10.1006/abbi.1995.1473
- [7] Liu Z, Bruins M E, de Bruijn, W J C, Vincken J.P, A comparison of the phenolic composition of old and young tea leaves reveals a decrease in flavanols and phenolic acids and an increase in flavonols upon tea leaf maturation, Journal of Food Composition and Analysis, 86,2020, 103385. doi:10.1016/j.jfca.2019.103385
- [8] Farhoosh R, Golmovahhed G A, Khodaparast M H H, Antioxidant activity of various extracts of old tea leaves and black tea wastes (*Camellia sinensis* L.). Food Chemistry, 100(1), 2007, 231–236. doi:10.1016/j.foodchem.2005.09.046
- [9] Zhang H, Qi R, Mine Y. The impact of oolong and black tea polyphenols on human health, Food Bioscience 29, 2019, 55-61. doi:10.1016/j.fbio.2019.03.009
- [10] Graham H N, Green tea composition, consumption, and polyphenol chemistry, Preventive Medicine, 21(3), 1992, 334–350. doi:10.1016/0091-7435(92)90041-f
- [11] Butt M S, Ahmad R S, Sultan M T, Qayyum M M N, Naz A, Green Tea and Anticancer Perspectives: Updates from Last Decade, Critical Reviews in Food Science and Nutrition, 55(6), 2014, 792–805. doi:10.1080/10408398.2012.680205
- [12] Naito Y, Yoshikawa T, Green Tea and Heart Health. Journal of Cardiovascular Pharmacology, 54(5), 2009, 385–390. doi:10.1097/fjc.0b013e3181b6e7a1

- [13] Sharma V K, Bhattacharya A, Kumar A, Sharma H K, Health Benefits of Tea Consumption, Tropical Journal of Pharmaceutical Research, 6(3) 2007. doi:10.4314/tjpr.v6i3.14660
- [14] Samanta S, Potential bioactive components and health promotional benefits of tea (*Camellia sinensis*), 41(1), 2020, 65-93. doi:<https://doi.org/10.1080/07315724.2020.1827082>
- [15] Singh Arora, D Jeet Kaur, G, Kaur H, Antibacterial Activity of Tea and Coffee: Their Extracts and Preparations, International Journal of Food Properties, 12(2), 2009, 286–294. doi:10.1080/10942910701675928
- [16] Hu J, Zhou D, Chen Y, Preparation and Antioxidant Activity of Green Tea Extract Enriched in Epigallocatechin (EGC) and Epigallocatechin Gallate (EGCG), Journal of Agricultural and Food Chemistry, 57(4), 2009, 1349–1353. doi:10.1021/jf803143n
- [17] Horwitz W, Latimer G W, Official Methods of Analysis of AOAC Internationals, 18th edition, AOAC Internationals, Maryland USA, 2005, 76-83
- [18] Sulaiman CT, Balachandran I, Total Phenolics and Total Flavonoids in Selected Indian Medicinal Plants, Indian Journal of Pharmaceutical Sciences, 74(3), 2012, 258-260, doi: [10.4103/0250-474X.106069](https://doi.org/10.4103/0250-474X.106069)
- [19] Chaves N, Santiago A, Alias J C, Quantification of the Antioxidant Activity of Plant Extracts: Analysis of Sensitivity and Hierarchization Based on the Method Used, Antioxidants, 9(1), 2020, 76. doi: 10.3390/antiox9010076
- [20] Csepregi K, Kocsis M, Hideg É, On the spectrophotometric determination of total phenolic and flavonoid contents, Acta Biologica Hungarica, 64(4), 2013, 500–509. doi:10.1556/abiol.64.2013.4.10
- [21] Dutta S, Khaled KL, Phytochemical Investigation and In Vitro Antioxidant Activity of *Syzygium jambos* Fruit and Its Seed, Asian Journal of Pharmaceutical and Clinical Research, 16(2), 2023, 35-40. doi : <http://dx.doi.org/10.22159/ajpcr.2023v16i2.46496>
- [22] Abdeltaif S, Sir Elkhathim K, Hassan A, Estimation of Phenolic and Flavonoid Compounds and Antioxidant Activity of Spent Coffee and Black Tea (Processing) Waste for Potential Recovery and Reuse in Sudan. Recycling, 3(2), 2018, 27. doi:10.3390/recycling3020027