

## Nutraceutical Attributes of Different Aerial Parts of *Commiphora wightii*

Ali Raza Kashif<sup>1</sup>, Saima Naz<sup>2\*</sup>, Muhammad Usama Younas<sup>2</sup>, Adil Usman<sup>2</sup>, Muhammad Usman Tariq<sup>2</sup>, Sohail Yaseen<sup>2</sup>, Muhammad Rehan<sup>2</sup>, Nadeem Shakeel<sup>2</sup>

<sup>1</sup>Department of Chemistry, International Islamic University, Islamabad, Pakistan.

<sup>2</sup>Department of Chemistry, Division of Science and Technology, University of Education, Lahore, Pakistan.

\*Corresponding Author email: saima.naz@ue.edu.pk

### Abstract

This study aims to evaluate the extract potential of leaf, bark, and fruit of *Commiphora wightii* using various solvents and extraction methods. The crude concentrated extracts (CCEs) obtained from the fruit, leaves, and bark of *C. wightii* exhibited yields ranging from 14.08-10.63, 8.98-6.17, and 7.74-5.04 mg/g of dry weight, respectively. These CCEs showed significant amounts of total phenolic and flavonoid content ranging from 2.84-4.71 GAE (mg/g) and 1.97-3.61 CE (mg/g) in fruit extracts, 2.57-4.13 GAE (mg/g) and 1.93-3.28 CE (mg/g) in leaf extracts, 2.37-3.97 GAE (mg/g) and 1.81-3.01 CE (mg/g) in bark extracts. Additionally, these extracts demonstrated strong inhibitory effects (72-91%) on the peroxidation process in the linoleic system. Antibacterial assays revealed that *Bacillus pumilus* was the most sensitive microorganism, with inhibition zones ranging from 2-16 mm. *Fusarium oxysporum* and *Aspergillus niger* showed varying sensitivity, with minimum inhibitory concentration where values indicating effective antifungal activity, particularly in the fruit extracts. This study highlights the potential of *C. wightii* as a natural resource of antioxidants and antimicrobial agents, with the fruit extracts demonstrating the most pronounced bioactivity. These findings offer appreciated perceptions of using *C. wightii* to develop natural drug products with health benefits.

**Keywords:** *Commiphora wightii*, Phytochemicals, Antioxidant activity, Antimicrobial activity, Bioactive compounds.

### Highlight:

- A significant amount of total phenolic content was reported from both extraction methods.
- Different solvent extracts of *Commiphora wightii* showed high-potential biological activities.
- Aqueous ethanol (80%) exhibited the highest efficacy in extracting phytochemicals.
- Among others, sonication appeared as the most potent green extraction technique.

### 1.0. Introduction

A molecule or molecular ion generated through regular cell metabolism, particularly during redox reactions involving oxygen consumption, is known as a free radical. Due to their inherent instability, free radicals tend to extract electrons from other molecules to attain stability (Chhikara et al., 2021). Reactive oxygen species (ROS) arise from oxidation reactions and can cause cellular damage through chain reactions (Naz et al., 2023). An imbalance in the generation of oxidative species can lead to the unnecessary production of free radicals in the body (Kashif et al., 2024; Unsal, 2018).

An antioxidant is a substance that hinders or counteracts oxidative impairment induced by free radicals in the body (Kashif et al., 2024). Antioxidants neutralize these harmful molecules, reducing their capacity to damage cells and tissues. They help balance generating and eliminating free radicals, critical in defensive cells from oxidative stress and the consequential damage associated with various diseases and the aging process (Munteanu & Apetrei, 2021). Antioxidants are two types: i) synthetic and ii) natural. Synthetic antioxidants can be toxic and expensive, so natural antioxidants are frequently used due to their low toxicity and abundant reserves of pharmaceutical compounds (Carocho & Ferreira, 2013; Chhikara et al., 2021). Phytochemicals are compounds found in plants that have protection properties. These compounds contribute to the antioxidant capabilities et al. of plants, with notable examples including phenols, flavonoids, flavones, anthocyanin, isoflavones, coumarins, iso-catechins, and catechins (Naz, 2023; Nwozo et al., 2023). Fruits and vegetables are essential for enhancing the body's well-being and serve as tools in preventing diseases such as cancer, heart problems, and eye issues (Jamshidi-Kia et al., 2020). Pakistan has a rich array of medicinally valued plants that indigenous people have used in various systems of medicine to treat an extensive range of ailments (Alamgeer et al., 2018).

Pakistan exhibits regional climate differences, geographical diversity, and traditional zones, contributing to its unique characteristics. Approximately 6000 plants have been documented in Kashmir and Pakistan for their medicinal use (Tufail et al., 2020). Improper disposal of these plants can be hazardous, as they produce harmful gases like carbon dioxide and methane. Insufficient incineration of these residues results in high volumes of pollutants such as furans, acid gases, and other hazardous products, leading to severe environmental and health challenges. Given these issues, it is essential to investigate proper utilization techniques for these food residues (Abu Qdais et al., 2019).

*Commiphora wightii* (Guggul), a flowering plant from the Burseraceae family, is widely recognized for its medicinal properties (Sarkar, 2017). It has a rich composition of phytoconstituents, including steroids, diterpenoids, long-chain

aliphatic tetrols, triterpenoids, aliphatic esters, carbohydrates, ferulates, and lignans (Uzma et al., 2022). *C. wightii* has been extensively used in traditional herbal medicine. These bioactive compounds contribute to various pharmacological effects, such as wound healing, analgesic, antibacterial and anti-inflammatory activities (Sharma et al., 2021; Uzma et al., 2020). Phytochemicals are significant in all plants due to their antioxidant and antimicrobial properties. Specific plants may contain substantial quantities of these phytochemicals, which can be verified through various chemical analyses. This study reports the antioxidant and antimicrobial potential of extracts of *Commiphora wightii* (leaf, bark, and fruit) prepared in different solvents to validate their role in natural drug discovery and as a novel source of natural antioxidants in food, nutraceuticals, and pharmaceuticals.

## 2.0. Materials and Methods

### 2.1. Chemicals and Reagents

Aluminum chloride, Folin-Ciocalteu reagent, ferrous chloride, trichloroacetic acid, sodium carbonate (Anhydrous), ammonium thiocyanate, gallic acid, linoleic acid, sodium nitrite, ascorbic acid, ferric chloride, potassium ferricyanate, DPPH (2,2-diphenyl-1-picrylhydrazyl) and BHT (butylated hydroxytoluene) was purchased from Sigma Chemicals. Additional analytical-grade reagents, including methanol, ethanol, chloroform, and *n*-hexane, were purchased from the Merck Chemical Company (Darmstadt, Germany). Some chemicals were also purchased from Oxoid Ltd. (Hampshire, UK) to prepare culture media for antimicrobial tests.

### 2.2. Sample collection and its pretreatment

Selected parts of the wild *Commiphora wightii* plant were collected from the Thar Desert, Punjab, Pakistan. A taxonomist from the Department of Botany, Arid Agriculture University, Islamabad, Pakistan, assisted in confirming and identifying the collected samples. The samples were chopped into small pieces, air-dried, and stored in plastic bags at -4°C.

### 2.3. Extraction of crude concentrated extracts

Selected parts (leaf, fruit, and bark) of *C. wightii* were washed with tap water to remove associated debris. The clean samples were shade-dried and then ground with an electric grinder to achieve a powder (80-mesh). The fruit samples containing a significant amount of oil were subjected to extraction using a Soxhlet apparatus with *n*-hexane as the solvent. The ground samples (10 g) were extracted using 100 mL of various solvents, including aqueous ethanol, absolute ethanol, aqueous methanol, absolute methanol, and distilled water, through two extraction methods: sonication (50°C for 40 min) and maceration (15 h at 25°C).

The recovered extracts were filtered thrice through Whatman No.1 filter paper to remove insoluble residues. The resulting filtrates were combined into a new glass vial. Excess solvent from the crude extracts was removed using a rotary evaporator (STRIKE 100 Steroglass, Italy) at 50°C to obtain crude concentrated extracts (CCEs). The collected CCEs were stored at -4°C for further analysis (Ahmad et al., 2022).

## 2.4. In vitro Antioxidant Activity

### 2.4.1. Estimation of total phenolic content

The total phenolic content in *C. wightii* extracts was analyzed using the method described by Azadpour et al. (2015). Briefly, 1 mg/mL of CCEs were diluted with 7.5 mL deionized water, and a mixture containing 1.5 mL of sodium bicarbonate and 0.5 mL of Folin-Ciocalteu reagent was added and then incubated in a water bath at 40°C for 20 minutes. The absorbance of the final solution was measured at 755 nm using a UV-VIS spectrophotometer (Hitachi U-2001, model 121-0032). A similar treatment was applied to gallic acid standards with 10-100 ppm concentrations. The results were expressed as gallic acid equivalents GAE (mg/g) of dry weight of *C. wightii* samples. All selected plant samples were analyzed in triplicate.

### 2.4.2. Estimation of total flavonoid content

A method described by Kashif et al. (2024) was employed to quantify the total flavonoid content in specific plant extract samples. Briefly, 5.0 mL of deionized water and 100 mg/mL CCEs were dissolved in 0.3 mL of 5% sodium nitrate in a flask, and the mixture was incubated at room temperature for 10 minutes. Then, the solution was reacted with 2.0 mL of 1.0 M sodium hydroxide and 0.6 mL of 10% aluminum chloride. The absorbance of the final mixture was recorded at 510 nm. Similarly, catechin standards (10-100 ppm) were prepared. The total flavonoid content was expressed as catechin equivalents (CE, mg/g) of dry weight using a catechin calibration curve. All selected plant samples were analyzed in triplicate.

### 2.4.3. Inhibition of linoleic acid peroxidation system

Lipid peroxidation was measured by the percentage inhibition in linoleic acid using the method of Yildirim et al. (2001), with slight modifications. A sample solution was prepared by mixing 65 µL of linoleic acid with 5.0 mL sodium phosphate buffer and 2.5 mg of CCEs in 5.0 mL of 75% ethanol. Afterward, 2.5 mL of deionized water was added, and the mixture was incubated at 40°C for 15 minutes. The extent of oxidation was determined using the method described by Yen et al. (2000). 0.1 mL of sample solution of ferrous chloride (20 mM in 3.5% HCl), 0.1 mL of 30% ammonium thiocyanate, and 5.0 mL of 75% ethanol were mixed. The absorbance of this solution at 500 nm was used to determine percentage inhibition

using Equation 1.

$$I(\%) = \left[ \frac{A_c - A_s}{A_c} \right] \times 100 \quad (1)$$

Where control (treatment without CCE) and sample absorbance values are represented as  $A_c$  and  $A_s$  at 3 weeks, respectively and  $I$  (%) percentage inhibition. Positive controls included butylated hydroxytoluene and ascorbic acid at 200 ppm.

#### 2.4.4. 2, 2-diphenyl-2-picrylhydrazyl radical scavenging analysis

The analysis was carried out with slight modification using the methodology described by Tepe et al. (2005) to estimate the  $IC_{50}$  value of the CCEs recovered from the green extract of the plant sample. Each concentration (0.5 to 3.0 mg/mL) was added to a methanolic solution of DPPH (3 mL; 0.004%) and the mixture was incubated at ambient temperature for 30 minutes. The absorbance of each solution was measured at 517 nm and percentage inhibition was calculated using Equation 2 relative to the blank.

$$I(\%) = 100 \times (A_{\text{blank}} / A_{\text{sample}}) \quad (2)$$

Where  $I$  (%) is the percentage inhibition

#### 2.4.5. Reducing power of CCEs

Antioxidant activity was evaluated by measuring the reducing power of CCEs using the methods described by Ahmad et al. (2022). Briefly, 5.0 mL of 1.0% potassium ferricyanide, 5.0 mL of 0.2 M sodium phosphate buffer (pH 6.6), and CCEs (2.5–10 mg) were combined and incubated for 30 minutes at 50°C. The resulting solution was then mixed with 5.0 mL of 10% trichloroacetic acid, and the mixture was centrifuged (980 g; 10 minutes) using a centrifuge (CHM-17; Kokusen Denki, Tokyo, Japan). 2.5 mL of the recovered supernatant was mixed with 0.5 mL of 0.1% ferric chloride and 2.5 mL of distilled water. The absorbance of the final reaction mixture was recorded at 700 nm using a spectrophotometer (Hitachi U-2001).

### 2.5. In vitro Antimicrobial Study

#### 2.5.1. Microorganisms assay

The solvent extracts obtained from the aerial parts of *C. wightii* were individually tested against *Escherichia coli* (gram-negative bacteria), *Bacillus pumilus* (gram-positive bacteria), *Fusarium oxysporum* and *Aspergillus niger* (fungal strains). The pathogenic microorganisms were sourced from the Department of Biological Sciences, International Islamic University, Islamabad, Pakistan. Bacterial and fungal strains were cultivated on nutrient agar and potato dextrose agar (Oxoid, UK) at 37°C and 30°C, respectively.

#### 2.5.2. Disc diffusion method

The antimicrobial activity of the extracts from the tested sample was evaluated using the disc diffusion method (Ahmad et al., 2022). Discs soaked in each solvent extract (100 mg/mL) were placed on agar plates inoculated with pathogenic microorganisms. Amoxicillin and flumequine were used as positive controls, while a negative control was also included. All samples were processed under the same experimental conditions.

The minimum inhibitory concentration (MIC) was determined using the microdilution method described by Aleem et al. (2024). MIC represents the specific concentration of leaf, bark, and fruit extracts required to inhibit the growth of microorganisms completely. Concentrated solvent extracts from the selected samples were diluted in a 5-100 mg/mL range in a 96-well plate. Growth and sterility controls were included under the same conditions. 20  $\mu$ L aliquot of each diluted plant extract was added to 160  $\mu$ L of culture medium nutrient broth (NB) for bacterial strains and Sabouraud dextrose broth (SDB) for fungal strains. Then, 20  $\mu$ L of broth culture ( $5 \times 10^5$  CFU) of each tested microorganism was inoculated into the wells. The 96-well plate was incubated at 37°C for 24 hours for bacterial strains and at 30°C for 48 hours for fungal strains. The formation of a white pellet indicated microorganism growth, and the MIC was estimated as the lowest dilution at which microorganism growth was inhibited.

### 2.6. Statistical analysis

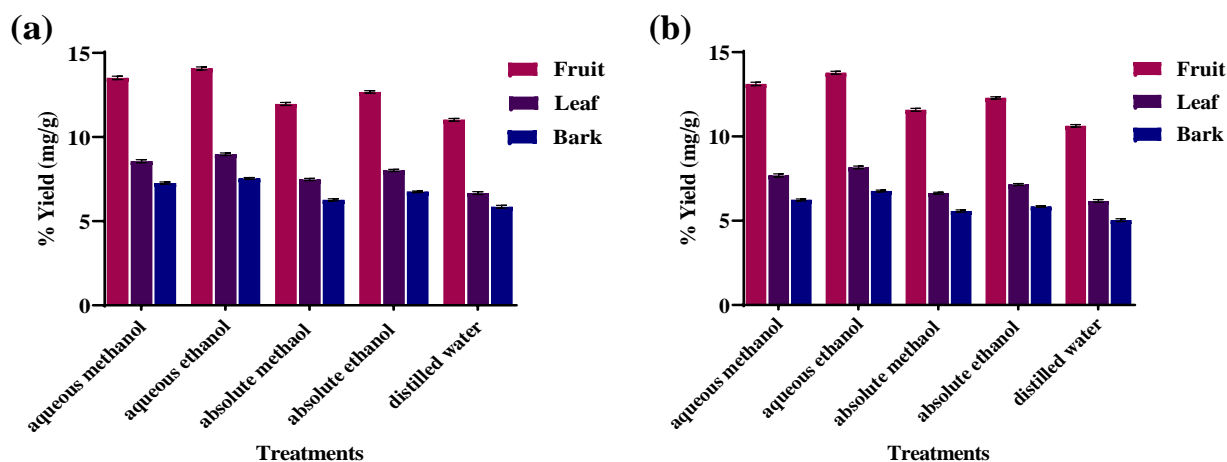
Three different samples of leaf, fruit, and bark of *C. wightii* were assayed. All the experiments were conducted in triplicate unless stated otherwise. Statistical data was analyzed using variance analysis (ANOVA) and STATISTICA 5.5 (Stat Soft Inc, Tulsa, Oklahoma, USA) software. A probability value of  $p \leq 0.05$  denoted the statistically significant difference. All data are presented as mean values  $\pm$  standard deviation (SD) for triplicated determinations.

## 3.0 Results and Discussion

### 3.1. Yield of extracts

The yield (mg/g) of different extracts from selected parts of *C. wightii* is presented in Figure (1). The antioxidant extracts recovered from the fruit, leaf and bark exhibited considerable variation, ranging from 14.08-10.63 mg/g, 8.98-6.17 mg/g and 7.74-5.04 mg/g of dry weight, respectively. The highest yield was recovered using aqueous ethanol with the ultrasonication method, while the lowest yield was obtained with distilled water from the bark of *C. wightii* using the maceration method. This observation can be attributed to the application of ultrasonic waves, which force the cell walls to break and enhance the extraction of phytochemicals, as reported by Motsumi (2020). Aqueous ethanol produced the

maximum yield of fruit, leaves and bark extracts, making it a more effective solvent than others for extracting antioxidant constituents. The present study reveals that the yield differs significantly ( $p < 0.05$ ) depending on the solvent used and the nature of the plant material (Kashif et al., 2024).

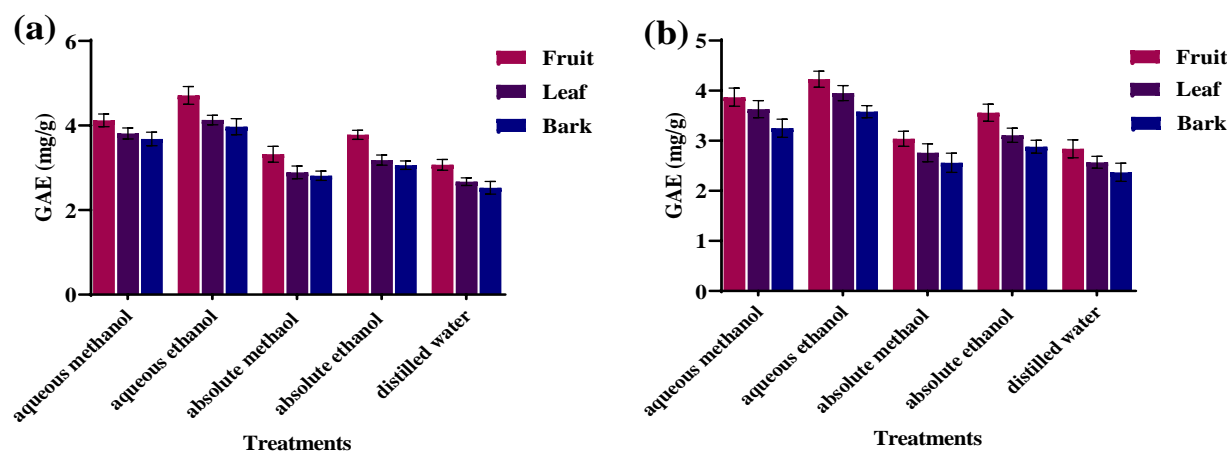


**Figure 1: (a) % Yield using ultrasonication and (b) % yield using maceration method of aerial parts of *C. wightii*.**

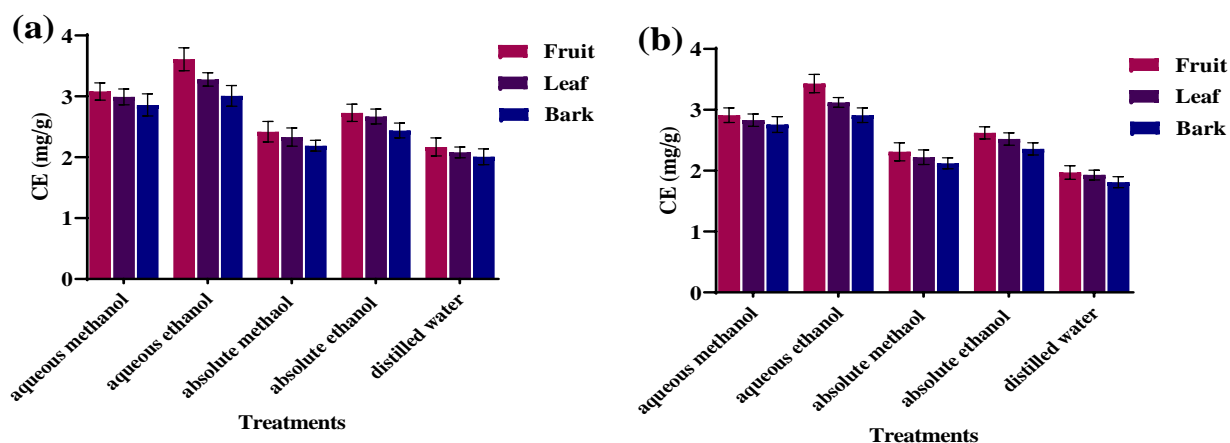
### 3.2. Flavonoid and phenolic content.

Phenolics have gained increased attention in the food sector due to their potential anti-carcinogenic properties and effectiveness in delaying lipid oxidation (Wojdyło et al., 2007). Plant phenolics are widely reported to have strong antioxidant potential (Awika et al., 2003). Some studies have highlighted that total flavonoids and phenols are essential antioxidants in vegetables and fruits (Katalinic et al., 2006). Therefore, identifying new plant sources rich in flavonoids and phenolics is crucial.

The total phenolic and flavonoid content of *C. wightii* leaf, bark, and fruit extracts are shown in Figures (2 & 3). In these solvents and extraction methods, TPC and TFC values for the leaves, bark and fruit ranged from 2.57-4.13 GAE (mg/g) and 1.93-3.28 CE (mg/g), 2.37-3.97 GAE (mg/g) and 1.81-3.01 CE (mg/g), 2.84-4.71 GAE (mg/g) and 1.97-3.61 CE (mg/g), respectively. The highest total phenolic and flavonoid contents were observed in the fruit extract using aqueous ethanol with the ultrasonication method, while the lowest amounts were recovered from the bark extract using distilled water with the maceration method. Analysis of the results also showed highly significant differences in phenolic compound yields among leaves, barks, and fruit, with  $p < 0.05$  for the solvents used. The leaves and fruit contained significantly more phenolics and flavonoids than the bark, with the fruit containing the highest concentrations of both compounds. The variation in the TFC and TPC may be attributed to differences in the solubility of antioxidant compounds in the extraction solvents. Since ethanol has lower toxicity and higher extraction efficiency, it is preferred for extracting antioxidant compounds from plant matrices (Gerken et al., 2007).



**Figure 2: (a) TPC using ultrasonication method and (b) TPC using maceration method of aerial parts of *C. wightii*.**

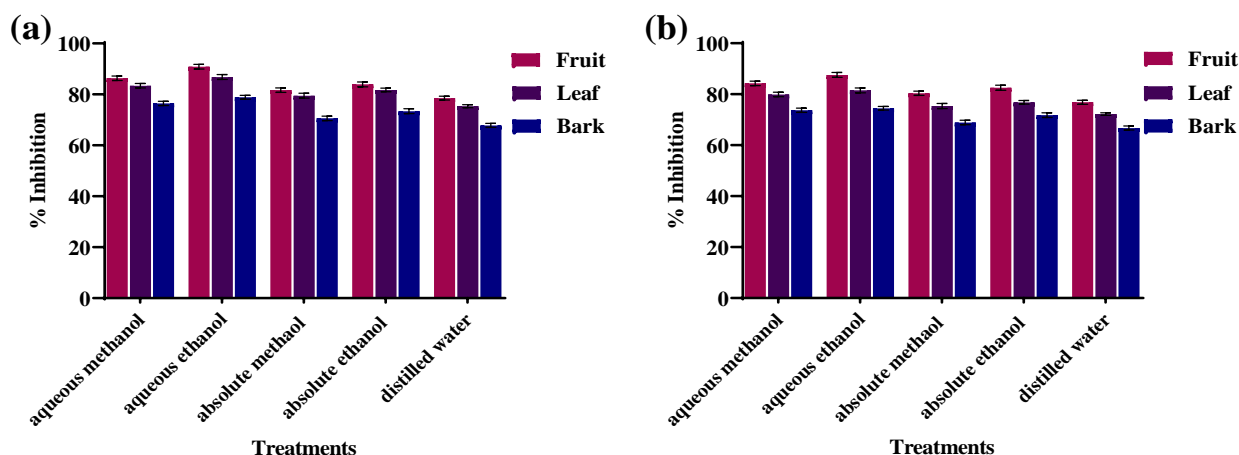


**Figure 3: (a) TFC using ultrasonication method and (b) TFC using maceration method of aerial parts of *C. wightii*.**

### 3.3. Antioxidant activity in the linoleic acid system

Linoleic acid is a polyunsaturated fatty acid that reacts with peroxides, converting Fe (II) to Fe (III). This Fe (III) forms a complex with thiocyanate ions (SCN<sup>-</sup>), and the intensity of this complex can be measured by recording the absorbance at 500 nm using a spectrophotometer. Higher absorbance values indicate greater peroxide formation during reactions, which signifies reduced antioxidant activity (Yen et al., 2000).

The antioxidant activity of *C. wightii* fruit, leaf, and bark samples was determined by assessing the percentage inhibition of lipid peroxidation Figure (4). The result showed that the percentage of peroxidation inhibition ranged between 72-87% for leaves, 67-79% for bark and 77-91% for fruit. Notably, the aqueous ethanolic extract exhibited a higher percentage inhibition of linoleic acid oxidation than other solvents. This enhanced inhibition is attributed to the maximum amount of phenolic components in the aqueous ethanol extract. The results revealed that fruit extracts demonstrated significantly ( $p < 0.05$ ) higher peroxidation inhibition than leaf and bark extracts, indicating that fruit has superior anti-oxidative activity.



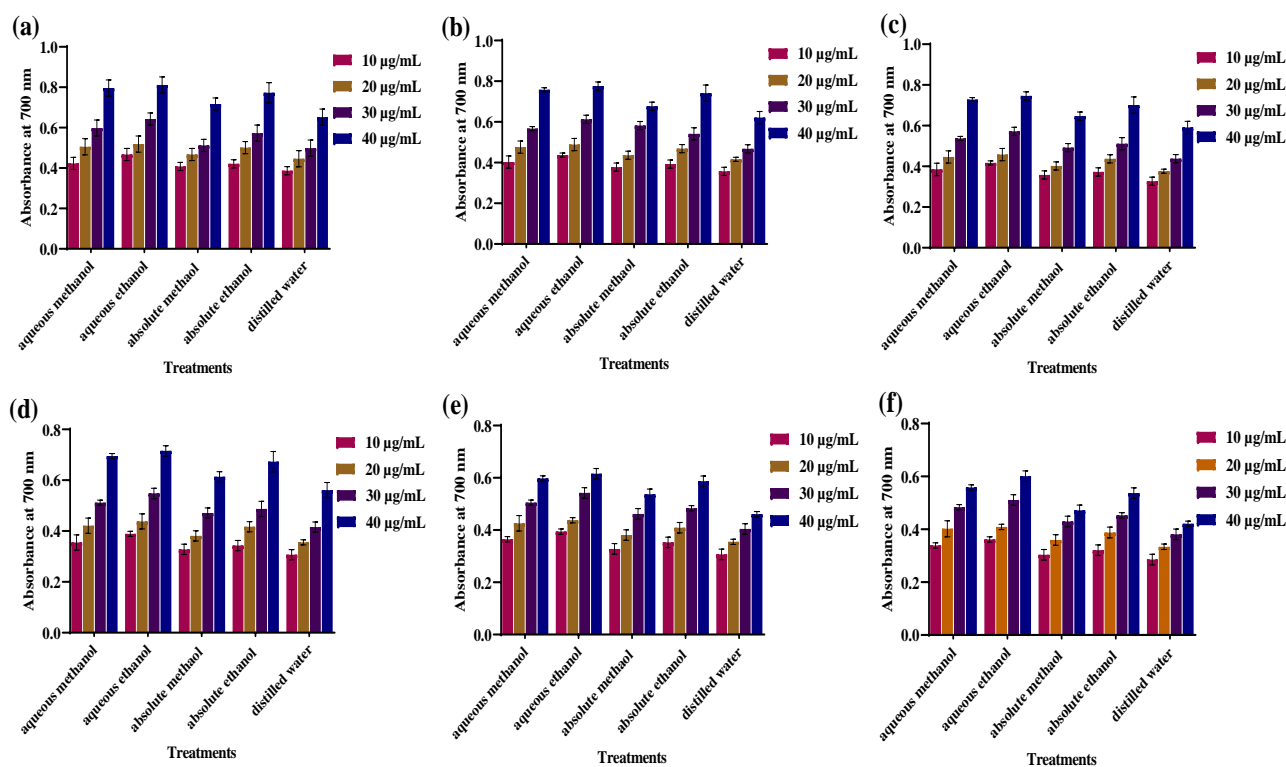
**Figure 4: (a) % Inhibition of linoleic acid using ultrasonication method and (b) % Inhibition of linoleic acid using maceration method of aerial parts of *C. wightii*.**

### 3.4. Reducing power of the extract

Their reducing power can also evaluate the antioxidant potential of plant extracts. This assay measures the reduction of Fe (III) ions to Fe (II) ions, changing colour from yellow to bluish-green. The extent of a colour change indicates the reducing capacity of antioxidant compounds present in the extract. A higher absorbance rate correlates with more intense coloration, thus reflecting more vigorous antioxidant activity (Zou et al., 2004). The relationship between antioxidant activity and reducing power is well established, with higher reducing power corresponding to increased antioxidant activity of bioactive substances (Kordali et al., 2005; Siddhuraju et al., 2002).

The current study evaluated the reducing power of different extracts from *C. wightii*. The results showed a clear trend of increased reducing power with rising extract concentrations (Figure 5). The reducing potential of fruit, bark, and leaf extracts was evaluated at 10 to 40  $\mu\text{g/mL}$  concentrations. Absorbance values for fruit, bark, and leaf extracts ranged from 0.357–0.811, 0.286–0.616, and 0.307–0.746, respectively. The fruit extract obtained through ultrasonication with aqueous ethanol demonstrated the highest absorbance value, indicating the most substantial reducing power, while the bark extract prepared

via maceration with distilled water exhibited the lowest absorbance value. Statistically significant differences ( $p < 0.05$ ) were observed in the reducing power of the different extracts. Unfortunately, comparison with previous studies on the reducing power of *C. wightii* leaf, bark, and fruit extracts was impossible due to the lack of available data.

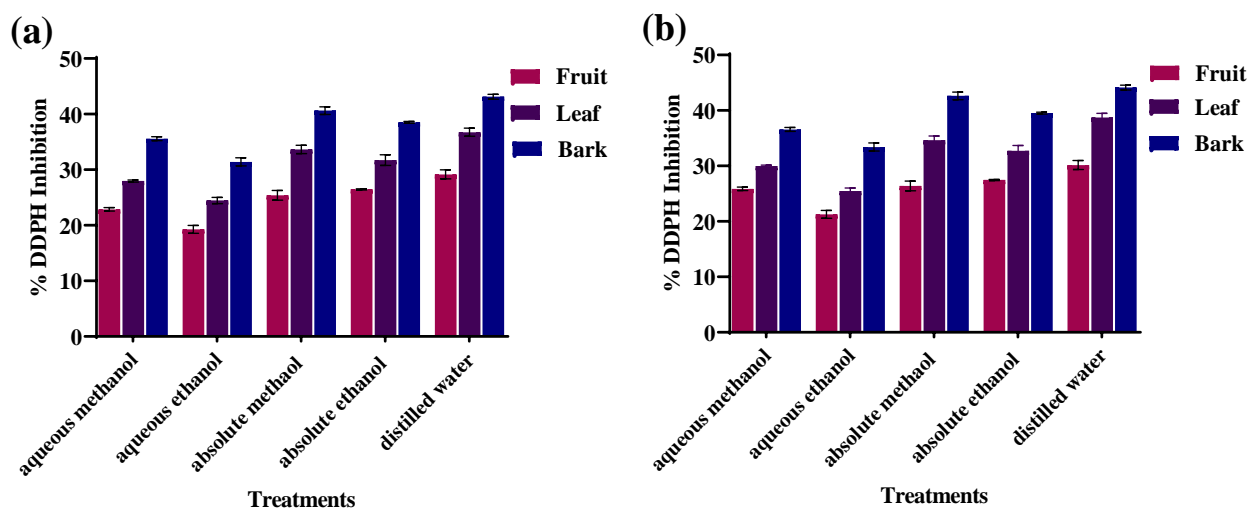


**Figure 5: Reducing power of (a) fruit using ultrasonication method, (b) fruit using maceration method, (c) leaf using ultrasonication method, (d) leaf using maceration method, (e) bark using ultrasonication method and (f) bark using maceration method of *C. wightii*.**

### 3.5. DPPH radical scavenging assay

2,2-diphenyl-1-picrylhydrazyl is a stable organic free radical with a deep violet colour with absorption maxima in the 515-528 nm range. When DPPH accepts a proton from the phenolic compounds present in plant extracts, its colour changes from violet to yellow. This colour shift reflects the activity of the extract. It is generally agreed that the radical scavenging activity determined by DPPH is closely linked to the phenolic content of the plant extract, with a more significant number of hydroxyl groups enhancing antioxidant activity (Sánchez-Moreno, 2002).

The leaf, bark, and fruit extracts demonstrated strong radical scavenging activity with  $IC_{50}$  values ranging from 19.17-31.68  $\mu\text{g/mL}$ , 24.46-36.77  $\mu\text{g/mL}$ , and 31.41-44.59  $\mu\text{g/mL}$ , respectively (Figure 6). The aqueous ethanol extract of leaves exhibited the most effective free radical scavenging activity, with the lowest  $IC_{50}$  value recorded at 19.17  $\mu\text{g/mL}$ . Notably, the ethanolic floral extract displayed significantly superior radical scavenging ability ( $p < 0.05$ ) compared to other solvent extracts. Compared to leaf and bark extracts, the lowest  $IC_{50}$  values observed in the fruit extracts highlight their enhanced ability to neutralize free radicals. However, BHT (synthetic antioxidant) exhibited higher antioxidant activity than all the studied extracts. The scavenging ability of the extracts against DPPH radicals can be attributed to the presence of phenolic compounds, which play a critical role in their antioxidant activity (Siddhuraju et al., 2002).



**Figure 6:** % DPPH Inhibition (a) using ultrasonication method and (b) using maceration method of aerial parts of *C. wightii*.

**3.6. Antimicrobial activity**

The antibacterial activity of *C. wightii* leaf, fruit, and bark extracts was evaluated against various pathogenic microorganisms, as summarized in Tables (1-4). Among the tested bacteria, *Bacillus pumilus* exhibited the highest sensitivity to the extracts, with zones of inhibition ranging from 2- 16 mm when exposed to the leaf, fruit, and bark extracts, and for *Escherichia coli*, the leaf, fruit, and bark extracts demonstrated the lowest minimum inhibitory concentration (MIC) values which ranged from 221-248 µg/mL, 202-240 µg/mL, and 248-278 µg/mL, respectively (Tables 1 & 2). Regarding the fungal strain *Fusarium oxysporum*, the MIC values for the leaf, fruit, and bark extracts ranged from 234-287 µg/mL, 244-291 µg/mL, and 250-294 µg/mL, respectively. The inhibition zones for *F. oxysporum* ranged from 3-9 mm for leaf extracts, 3-7 mm for fruit extracts, and 2-8 mm for bark extracts. In contrast, *Aspergillus niger* showed zones of inhibition ranging from 2-10 mm for leaf extracts, 3-14 mm for fruit extracts, and 2-11 mm for bark extracts, with MIC values between 229-284 µg/mL for leaf extracts, 191-276 µg/mL for fruit extracts and 221-269 µg/mL for bark extracts (Tables 3 and 4). The variability in the chemical composition of the extracts likely accounts for the observed differences in their antibacterial activity. Previous studies have shown that changes in the chemical composition of plant extracts can significantly influence their biological activities. In this study, *E. coli* was found to be less sensitive to the *C. wightii* extracts, displaying lower inhibitory effects compared to findings reported by Singh & Siddiqui (2015).

**Table 1. Antibacterial activity of extracts of *C. wightii* using ultrasonication method.**

Extracting solvents	Bacterial strains	ZOI (mm)			MIC (µg/mL)		
		Bark	Fruit	Leaf	Bark	Fruit	Leaf
Absolute ethanol	<i>Bacillus pumilus</i>	4	5	4	316	277	302
	<i>Escherichia coli</i>	9	12	10	252	211	224
Aqueous ethanol	<i>Bacillus pumilus</i>	6	7	8	270	252	262
	<i>Escherichia coli</i>	11	16	14	248	202	221
Absolute methanol	<i>Bacillus pumilus</i>	4	5	4	312	268	318
	<i>Escherichia coli</i>	7	11	8	275	216	270
Aqueous methanol	<i>Bacillus pumilus</i>	4	6	5	312	249	262
	<i>Escherichia coli</i>	8	13	10	281	199	302
Distilled water	<i>Bacillus pumilus</i>	3	5	4	362	319	345
	<i>Escherichia coli</i>	5	10	6	274	237	245
Positive control (Amoxicillin)	<i>Bacillus pumilus</i>	–	23	–	–	136	–
	<i>Escherichia coli</i>	–	27	–	–	85	–

Values are means of three replicates, analyzed individually.

**Table 2. Antibacterial activity of extracts of *C. wightii* using maceration method.**

Extracting solvents	Bacterial strains	ZOI (mm)			MIC ( $\mu\text{g/mL}$ )		
		Bark	Fruit	Leaf	Bark	Fruit	Leaf
Absolute ethanol	<i>Bacillus pumilus</i>	4	4	3	320	281	306
	<i>Escherichia coli</i>	8	10	9	226	215	227
Aqueous ethanol	<i>Bacillus pumilus</i>	5	6	6	274	254	266
	<i>Escherichia coli</i>	9	15	12	251	206	225
Absolute methanol	<i>Bacillus pumilus</i>	2	5	3	316	271	321
	<i>Escherichia coli</i>	5	10	7	278	219	275
Aqueous methanol	<i>Bacillus pumilus</i>	3	5	4	315	254	265
	<i>Escherichia coli</i>	7	10	8	284	203	306
Distilled water	<i>Bacillus pumilus</i>	2	5	3	365	323	349
	<i>Escherichia coli</i>	5	9	5	278	240	248
Positive control (Amoxicillin)	<i>Bacillus pumilus</i>	–	23	–	–	136	–
	<i>Escherichia coli</i>	–	27	–	–	85	–

Values are means of three replicates, analyzed individually.

**Table 3. Antifungal activity of extracts of *C. wightii* using ultrasonication method.**

Extracting solvents	Bacterial strains	ZOI (mm)			MIC ( $\mu\text{g/mL}$ )		
		Bark	Fruit	Leaf	Bark	Fruit	Leaf
Absolute ethanol	<i>Aspergillus niger</i>	7	12	9	248	203	242
	<i>Fusarium oxysporum</i>	3	4	5	268	275	264
Aqueous ethanol	<i>Aspergillus niger</i>	11	14	10	221	191	229
	<i>Fusarium oxysporum</i>	3	5	4	292	271	284
Absolute methanol	<i>Aspergillus niger</i>	6	8	7	260	253	247
	<i>Fusarium oxysporum</i>	4	5	4	271	268	271
Aqueous methanol	<i>Aspergillus niger</i>	8	10	8	252	242	252
	<i>Fusarium oxysporum</i>	8	7	9	250	244	234
Distilled water	<i>Aspergillus niger</i>	3	4	3	267	273	282
	<i>Fusarium oxysporum</i>	3	4	3	285	288	286

Values are means of three replicates, analyzed individually.

**Table 4. Antifungal activity of extracts of *C. wightii* using maceration method.**

Extracting solvents	Bacterial strains	ZOI (mm)			MIC ( $\mu\text{g/mL}$ )		
		Bark	Fruit	Leaf	Bark	Fruit	Leaf
Absolute ethanol	<i>Aspergillus niger</i>	5	10	7	251	206	245



	<i>Fusarium oxysporum</i>	2	3	4	271	278	267
Aqueous ethanol	<i>Aspergillus niger</i>	10	12	9	223	194	231
	<i>Fusarium oxysporum</i>	2	3	3	294	274	287
Absolute methanol	<i>Aspergillus niger</i>	5	6	6	263	255	249
	<i>Fusarium oxysporum</i>	2	4	3	273	271	274
Aqueous methanol	<i>Aspergillus niger</i>	6	8	7	255	245	254
	<i>Fusarium oxysporum</i>	6	5	8	252	247	237
Distilled water	<i>Aspergillus niger</i>	2	3	2	269	276	284
	<i>Fusarium oxysporum</i>	2	3	3	288	291	289

Values are means of three replicates, analyzed individually.

### Conclusion

In conclusion, the extracts from *Commiphora wightii* demonstrated significant antioxidant, antibacterial and antifungal activities, with variations attributed to the solvent types and plant parts used. Aqueous ethanol proved to be the most effective solvent for extracting phenolics and flavonoids, contributing to the high antioxidant potential observed in the fruit extracts. The antibacterial and antifungal assays revealed that *Bacillus pumilus* and *Aspergillus niger* were the most susceptible microorganisms. These findings suggest that *C. wightii* has promising potential for use in developing natural antioxidant and antimicrobial agents, and they highlight its relevance to food and pharmaceutical applications.

### Acknowledgement:

The authors are thankful to the Department of Chemistry, International Islamic University, Islamabad, and the Department of Chemistry, Division of Science and Technology, University of Education, Lahore, Pakistan, for providing the research facilities to conduct this project.

**Conflict of Interest:** It is declared that there is no conflict of interest among authors.

### Reference

- Abu Qdais, H., Saadeh, O., Al-Widyan, M., Al-tal, R., & Abu-Dalo, M. (2019). Environmental sustainability features in large university campuses: Jordan University of Science and Technology (JUST) as a model of green university. *International Journal of Sustainability in Higher Education*, 20(2), 214–228.
- Ahmad, N., Arslan, M., Kashif, A. R., & Abbas, A. (2022). *Nutraceutical and biological attributes of wild Withania coagulans fruits*. 8(3), 106–113.
- Alamgeer, Sharif, A., Asif, H., Younis, W., Riaz, H., Bukhari, I. A., & Assiri, A. M. (2018). Indigenous medicinal plants of Pakistan used to treat skin diseases: A review. *Chinese Medicine (United Kingdom)*, 13(1), 1–26.
- Aleem, A., Ahmad, N., Raza, A., Hameed, Z., Mustafa, M., Usman, A., & Ahmad, F. (2024). *Appraisal of Various Solvent Extracts from Basil Aerial Parts for their Biological Attributes*. 1, 1–13.
- Awika, J. M., Rooney, L. W., Wu, X., Prior, R. L., & Cisneros-Zevallos, L. (2003). Screening Methods to Measure Antioxidant Activity of Sorghum (*Sorghum bicolor*) and Sorghum Products. *Journal of Agricultural and Food Chemistry*, 51(23), 6657–6662.
- Azadpour, M., Rezaei, M., Taati, M., Ghasemi Dehnoo, M., & Ezatpour, B. (2015). Antioxidant, antibacterial, and wound-healing properties of methanolic extract of *Pistacia khinjuk*. *Comparative Clinical Pathology*, 24(2), 379–385.
- Carocho, M., & Ferreira, I. C. F. R. (2013). A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food and Chemical Toxicology*, 51(1), 15–25.
- Chhikara, N., Kaur, A., Mann, S., Garg, M. K., Sofi, S. A., & Panghal, A. (2021). Bioactive compounds, associated health benefits and safety considerations of *Moringa oleifera* L.: an updated review. *Nutrition and Food Science*, 51(2), 255–277.
- Gerken, T., Girard, C. A., Tung, Y. C. L., Webby, C. J., Saudek, V., Hewitson, K. S., Yeo, G. S. H., McDonough, M. A., Cunliffe, S., McNeill, L. A., Galvanovskis, J., Rorsman, P., Robins, P., Prieur, X., Coll, A. P., Ma, M., Jovanovic, Z., Farooqi, I. S., Sedgwick, B., ... Schofield, C. J. (2007). The obesity-associated FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase. *Science*, 318(5855), 1469–1472.
- Jamshidi-Kia, F., Wibowo, J. P., Elachouri, M., Masumi, R., Salehifard-Jouneghani, A., Abolhassanzadeh, Z., & Lorigooini,

- Z. (2020). Battle between plants as antioxidants with free radicals in human body. *Journal of HerbMed Pharmacology*, 9(3), 191–199.
- Kashif, A. R., Batool, K., Rabbani, S. M., Javed, N., Asadullah, M., & Yaseen, S. (2024). Comparative Analysis of Phenolic and Flavonoid Content in Mango Varieties : Evaluating Antioxidant and Antimicrobial Activity Highlight : *J. Adv. Nutri. Sci. Technol*, 4(3–4), 52–65..
- Kashif, A. R., Naz, S., Usman, A., Javed, N., Younas, M. U., & Zahoor, A. (2024). In vitro study of antioxidant and antimicrobial potential of Moringa oleifera leaves as a green food preservative in chicken burger Highlight : *J. Adv. Nutri. Sci. Technol*, 4(1–2), 1–8.
- Katalinic, V., Milos, M., Kulisic, T., & Jukic, M. (2006). Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chemistry*, 94(4), 550–557.
- Kordali, S., Kotan, R., Mavi, A., Cakir, A., Ala, A., & Yildirim, A. (2005). Determination of the chemical composition and antioxidant activity of the essential oil of Artemisia dracunculus and of the antifungal and antibacterial activities of Turkish Artemisia absinthium, A. dracunculus, Artemisia santonicum, and Artemisia spicig. *Journal of Agricultural and Food Chemistry*, 53(24), 9452–9458.
- Motsumi, et al. (2020). *Teedia lucida Root extracts by ultrasonication and maceration techniques: Phytochemical; screening, Antimicrobial and antioxidant potentials*. 2–3.
- Munteanu, I. G., & Apetrei, C. (2021). Analytical methods used in determining antioxidant activity: A review. *International Journal of Molecular Sciences*, 22(7).
- Naz, S., Kashif, A. R., Tawab, A., Rasool, M. Z., Rauf, A., Hussain, S., & Khan, U. (2023). Assessment of the Antidiabetic Properties of Essential Oil from Cannabis sativa. *Journal Advances of Nutrition Science and Technology*, 3(3–4), 78–86.
- Naz, S., Rasheed, M. N., Saeed, J., A., Ghuffar, A., Kashif, A. R., & Waheed, R. (2023). Isolation of Bioactive compounds from the essential oil of Jambolan (Syzygium cumini) and Invitro evaluation of biological potential. *Journal Advances of Nutrition Science and Technology*, 3(1–2), 15–23.
- Nwozo, O. S., Effiong, E. M., Aja, P. M., & Awuchi, C. G. (2023). Antioxidant, phytochemical, and therapeutic properties of medicinal plants: a review. *International Journal of Food Properties*, 26(1), 359–388.
- Sánchez-Moreno, C. (2002). Methods Used to Evaluate the Free Radical Scavenging Activity in Foods and Biological Systems. *Food Science and Technology International*, 8(3), 121–137.
- Sarkar, D. (2017). Synthesis of Plant-Mediated Silver Nanoparticles Using Commiphora Wightii (Guggul) Extract and Study Their Antibacterial Activities Against Few Selected Organisms. *World Journal of Pharmacy and Pharmaceutical Sciences*, March, 1418–1425.
- Sharma, R., Bhat, Z. F., Kumar, A., Kumar, S., Bekhit, A. E. D. A., & Naqvi, Z. (2021). Characterization of Commiphora wightii based bioactive edible film and its efficacy for improving the storage quality of meat products. *Journal of Food Safety*, 41(5).
- Siddhuraju, P., Mohan, P. S., & Becker, K. (2002). Studies on the antioxidant activity of Indian Laburnum (Cassia fistula L.): A preliminary assessment of crude extracts from stem bark, leaves, flowers and fruit pulp. *Food Chemistry*, 79(1), 61–67.
- Singh, B. R. A. J., & Siddiqui, M. Z. (2015). *Antimicrobial activity of Commiphora wightii gum ( Guggul gum ) extract against gram positive and gram negative bacteria*. 1(2), 36–39.
- Tepe, B., Daferera, D., Sokmen, A., Sokmen, M., & Polissiou, M. (2005). Antimicrobial and antioxidant activities of the essential oil and various extracts of Salvia tomentosa Miller (Lamiaceae). *Food Chemistry*, 90(3), 333–340.
- Tufail, M., Hussain, K., Nawaz, K., Bhatti, K. H., Yasin, G., & Ali, S. S. (2020). Ethnobotanical survey of important wild medicinal plants of tehsil Gojra, district toba tek singh, Punjab, Pakistan. *Ethnobotany Research and Applications*, 20, 1–14.
- Unsal, V. (2018). Natural phytotherapeutic antioxidants in the treatment of mercury intoxication-A review. *Advanced Pharmaceutical Bulletin*, 8(3), 365–376.
- Uzma M, Dhanwini R. Prasad, Sunayana N, V. B. R. & S. H. P. (2022). Studies of in vitro antioxidant and anti-inflammatory activities of gold nanoparticles biosynthesised from a medicinal plant, Commiphora wightii. *Materials Technology*, 37(9), 915–925.
- Uzma, M., Sunayana, N., Raghavendra, V. B., Madhu, C. S., Shanmuganathan, R., & Brindhadevi, K. (2020). Biogenic synthesis of gold nanoparticles using Commiphora wightii and their cytotoxic effects on breast cancer cell line (MCF-7). *Process Biochemistry*, 92, 269–276.
- Wojdyło, A., Oszmiański, J., & Czemerys, R. (2007). Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chemistry*, 105(3), 940–949.

- Yen, G. C., Duh, P. Der, & Chuang, D. Y. (2000). Antioxidant activity of anthraquinones and anthrone. *Food Chemistry*, 70(4), 437–441.
- Yildirm, A., OKTAY, M., & BİLALOĞLU, V. (2001). The antioxidant activity of the leaves of *Cydonia vulgaris*. *Turkish Journal of Medical Sciences*, 31(1), 23–27.
- Zou, Y., Lu, Y., & Wei, D. (2004). Antioxidant activity of a flavonoid-rich extract of *Hypericum perforatum* L. in vitro. *Journal of Agricultural and Food Chemistry*, 52(16), 5032–5039.

**Received: August 5th 2024**

**Accepted: September 28th 2024**