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Antioxidant and Antimicrobial Activities of Some plant Powders

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ABSTRACT

The increasing concerns regarding the safety of synthetic antioxidants and their potential health risks have encouraged the search for natural alternatives derived from plant sources. This study aimed to evaluate the antioxidant and antimicrobial activities of three plant extracts, namely*(Melissa officinalis)* lemon balm leaves, pomegranate (*Punica granatum*) peel of fruits, and stinging nettle (*Urtica dioica*) leaves, as potential natural substitutes for synthetic antioxidants in food and pharmaceutical applications.

Plant materials were extracted using organic solvents assisted by ultrasound technology to enhance extraction efficiency and maximize the recovery of bioactive compounds. The extraction process yielded extracts rich in phenolic compounds, flavonoids compounds and other secondary metabolites known for their biological activities.The total phenolic and total flavonoid contents of the three powdered samples were determined , and the individual bioactive compounds were subsequently separated and characterized by HPLC apparatus.

The antioxidant capacity was evaluated using standard in-vitro assays, and antioxidant activity was additionally assessed using the DPPH radical scavenging method to determine the ability of the extracts to neutralize free radicals. The results demonstrated strong antioxidant activity attributed to the high phenolic content. Pomegranate peel extract exhibited the highest antioxidant activity, followed by stinging nettle extract then lemon balm extract.

Antimicrobial activity also evaluated against selected pathogenic microorganisms. All extracts showed clear inhibitory effects, with pomegranate peel extract demonstrating the highest antimicrobial activity, followed by stinging nettle and lemon balm extracts. This biological activity is associated with phytochemicals such as phenolic acids, tannins, and flavonoids, which contribute to microbial cell disruption and reduction of oxidative stress.

Conclusion : Plant extracts represent promising natural alternatives to synthetic antioxidants and preservatives due to their dual antioxidant and antimicrobial properties. Their potential applications include food preservation, and pharmaceutical formulations.

Keywords: Natural antioxidants, plant extracts, antimicrobial activity, phenolic compounds .

INTRODUCTION

An antioxidant is any chemical that delays or stops the oxidation of an oxidizable substrate when present at low concentrations than those of that substrate (Halliwell 1995)

Synthetic antioxidants including butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tert- butylhydroquinone (TBHQ) are frequently utilized In the food industry . Antioxidants stabilize the nutritional value, flavor, and color of food, ensuring its continued freshness. Synthetic phenolic antioxidants are mostly employed to stabilize fast foods and frying fats, which have become more popular with consumers in recent years. (Björkhem et al, 1991 Plat et al., 2014).

Natural sources of food additives are prefer to use to overcome the problem of ingesting artificial antimicrobials and antioxidants (Nikoo et al.,2018). For example , pomegranate peels also exhibit strong antioxidant activity (Negi et al., 2003) .Gallotannins, ellagitannins, anthocyanins, hydroxycinnamic acids, and hydroxybenzoic acids are found in pomegranate peel (Akhter et al.,2015 and khrchoufi ., 2018). Pomegranate peel extract (PPE) exhibits antibacterial and antioxidant activities against a variety of harmful microbes (Akhter et al.,2015 and Yang., 2016 alexander et al .,2019).

Also,*Melissa officinalis* include 32 plant spices with extremely high phenolic content. Dias et al. (2012), and (Sofowora et al., 2013).

The, leaves of *Urtica dioica (stinging nettle*) are rich in minerals (Ca, K, Fe), pigments (chlorophyll a and b, carotenoids, lutein, beta-carotene), proteins, carbs, fatty acids (palmititic and linoleic acid), and other trace elements. Nettle leaves are also rich in Isoleucine, leucine, valine, phenylalanine, threonine, methionine, lysine, and Tryptophan. The plant's roots and extracts are rich in a variety of phytochemicals, such as tannins and terpenoids, organic acids, and phenolic compounds. These compounds give the plant diuretic, anti-diabetic, antioxidant, anti-inflammatory, antiviral, anti-bacterial, and antifungalactivites, (Singh et al.,2012)

The objectives of the present study work are :

1-Extraction and puirification of some antioxidants and antimicrobial compounds from natural plants .,leaves *of Melissa officinalis*,peels of pomegranate fruits and leaves of *stinging nettle*.

2- To examine the potential effect of the resultant extracts as antioxidants and antimicrobial compounds

3- To study the effect of applying of the obtained extracts on keeping quality of dairy fats and related products.

2 - MATERIALS AND METHODS :

2.1. Chemicals and reagents.DPPH (2,2 Diphenyl -1 –picryl hydrazyl) , methanol , folin-ciocalteu reagent ,sodium carbonate ,$NaNO_3$, NaOH , $AlCl_3$,Gentamycin , fluconazole ,agar medium ,galic acid , and trifluoro acetica acid were purchased from Sigma Chemicals Co. (sT. Louis, USA).

2.2. plant materials

(Lemon balm leaves , pomegranate peels , stinging nettle leaves) . purchased from at a Zaqaziq local market Egypt .

2.3 Chemical composition analysis of dried powders:

The moisture ,crude protein , fat , and ash contents of pomegranate peels , stinging nettle and lemon balm leaves powders were determined using standard methods as described AOAC (2016) .

2.4 extraction of natural antioxidants :

Ethanol with a volume of 130 ml was used as an organic solvent for the extraction. The classical extraction was performed in such a way that the prepared samples were left at room temperature for 24 h with occasional stirring. After the designated extraction time, the samples were filtered through Whatman filter paper(NO.1) and used for further analysis.

The UAE treatment in the ultrasonic bath (Bandelin RK 103H, Germany) was carried out by placing the samples in beakers in an ultrasonic bath with a frequency of 35 kHz and a nominal maximum power of 140 W, with varying treatment times of 10, 15, and 30 min.by national research center.

2.5 Determination of the total phenolic content

Total phenolic content of the extracts were evaluated by a colorimetric method utilizing Folin-Ciocalteu reagent according to (Leon-fernandezAL et al; 2017).

2.6 Determination of total flavonoid content (TFC)

Modified AlCl3 calorimetric method as described by **(Sembiring ; et al; 2018)** .and the results were expressed as microgram of quercetin equivalent.

2.7 identification of phenolic and flavonoid compounds using HPLC was carried out according to the method describing by (kuntic,et al., 2007).

2.8 Evaluation of antioxidant activity by DPPH radical scavenging method

Free radical scavenging activity of different extracts of plant powders were measured by 1, 1-diphenyl-2-picryl hydrazyl (DPPH). As described by (Gonzalez et al., 2016)

2.9 Antimicrobial activity (discassay)

Clear zone discassay methods wereused toevaluate the antimicrobial effect as determind using clsi media by (Magaldi et al, 2004., Espinel-lngrroff et al, 2011)

RESULTS AND DISCUSSION

Table (1) illustrates the chemical composition of lemon balm, pomegranate, and stinging nettle leaf powders, revealing clear variations among the studied plants in terms of their nutritional constituents. These differences can be attributed to botanical characteristics, metabolic activity, and mineral uptake capacity of each plant.

The moisture content ranged from 8.47% to 13.25%, with pomegranate peel powder exhibiting the highest moisture level. Higher moisture content may affect storage stability and susceptibility to microbial growth, whereas the relatively lower moisture values observed in lemon balm powder and stinging nettle suggest better shelf stability.

Stinging nettle leaf powder demonstrated the highest protein content (24.9%), indicating its potential as a valuable plant-based protein source. This elevated protein level may also contribute to its reported bioactive and functional properties, as *Urtica dioica* leaves have been widely described as nutritionally rich, particularly in proteins, minerals, and dietary fiber (Adhikari et al., 2015). Lemon balm showed a moderate protein content (14.8%), which is consistent with previous reports describing *Melissa officinalis* leaves as possessing a balanced nutritional profile alongside biologically active compounds (Atanasova et al., 2023). while pomegranate peel powder contained comparatively low protein (4.20%),were in line with the findings of Naseem et al.,(2012).

Regarding lipid content, lemon balm recorded the highest fat content (7.4%), followed by stinging nettle (5.43%), whereas pomegranate peel showed the lowest fat content (2.2%). The relatively higher lipid content of lemon balm may be associated with its aromatic nature and the presence of lipophilic bioactive constituents, which has been previously reported for Melissa officinalis (Atanasova et al., 2023).

Fiber content varied notably among the samples, with lemon balm exhibiting the highest fiber level (16.11%), closely followed by stinging nettle leaves powder (15.72%). High fiber content enhances the functional value of plant powders, particularly in food applications related to gut health and antioxidant activity. The high fiber content of stinging nettle powder further supports its functional and nutritional importance, as previously documented (Adhikari et al., 2015). Pomegranate leaf powder contained the lowest fiber content (9.72%) (Naseem et al., 2012).

Ash content, which reflects the total mineral composition, was markedly higher in stinging nettle powder (21.45%) compared to lemon balm (13.30%) and pomegranate leaves (3.98%). This finding confirms the strong mineral accumulation capacity of stinging nettle leaves, which have been reported as a rich source of essential macro- and micro-elements (Adhikari et al., 2015).

Overall, the results highlight stinging nettle and lemon balm leaf powders as promising sources of nutrients and functional components, are in agreement with previously published data by (Adhikari et al., 2015; Atanasova et al., 2023). In contrast, pomegranate peels powder, despite its lower macronutrient content, may still possess significant bioactive properties that are not fully reflected by proximate chemical analysis alone (Naseem et al., 2012).

Table (1) : chemical composition of plant powders

Plant powder	Component %				
	Moisture	Protein	Fat	Fibers	Ash
Lemon balm (*Melissa officinalis)*	8.47	14.8	7.4	16.11	13.30
Pomegranate peels *(Punica granatum)*	13.25	4.20	2.2	9.72	3.98
Stinging nettle *(Urtica dioica L)*	9.11	24.9	5.43	15.72	21.45

The results presented in the Table(2) demonstrate clear variations in total phenolic content (TPC) and total flavonoid content (TFC) among the evaluated plant leaf powders, reflecting distinct phytochemical profiles associated with botanical differences.

Pomegranate (*Punica granatum L.):*

Pomegranate leaves exhibited the highest total phenolic content, with a mean value of 150.64 mg GAE/g, indicating a significantly richer phenolic profile compared to lemon balm and stinging nettle powders. The low standard deviation (0.0703) and low standard error (0.229) reflect high consistency and reliability of the measurements. This elevated phenolic concentration suggests a strong potential antioxidant capacity, as phenolic compounds are well known for their radical scavenging properties. These observations are consistent with previous studies reporting the high phenolic content of pomegranate tissues and their associated bioactivities (Tomas-Barberan et al., 2001; Ismail et al., 2012).

Regarding total flavonoid content, pomegranate dried peels recorded a moderate value (55.58 mg QuE/g), indicating that while pomegranate is particularly rich in total phenolics, flavonoids represent a substantial, though not dominant, fraction of its phenolic pool.

Stinging Nettle (*Urtica dioica L.)*

Stinging nettle showed a moderate total phenolic content, with a mean value of 104.87 mg GAE/g, while the relatively higher standard deviation (1.563) may indicate greater natural variability in its phytochemical composition. Previous studies have confirmed that stinging nettle leaves contain considerable phenolic and flavonoid contents, contributing significantly to their antioxidant capacity (Fattahi et al., 2014).

Regarding total flavonoid content, stinging nettle demonstrated the highest value among the studied plants (106.20 mg QuE/g), highlighting it as a major source of flavonoids. Flavonoids are a specific subclass of phenolic compounds widely recognized for their antioxidant, anti-inflammatory, and antimicrobial activities (Fattahi et al., 2014).

Lemon balm (*Melissa officinalis L.)*

Lemon balm recorded the lowest total phenolic content among the evaluated plants, with a mean value of 42.93 mg GAE/g. Despite its comparatively lower phenolic concentration, Melissa officinalis remains a well-

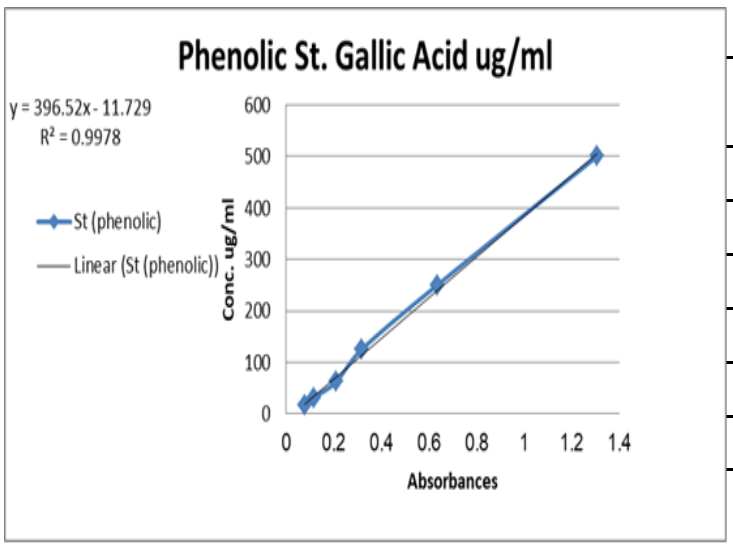
established medicinal plant characterized by the presence of bioactive phenolic compounds that contribute to its antioxidant and antimicrobial properties (Velickovic et al., 2011).

In terms of total flavonoid content, lemon balm exhibited a value of 45.96 mg QuE/g, indicating a moderate contribution to its overall phytochemical profile. Flavonoids have been identified as important contributors to antioxidant activity in medicinal plants, including Melissa officinalis (Tusevski et al., 2014).

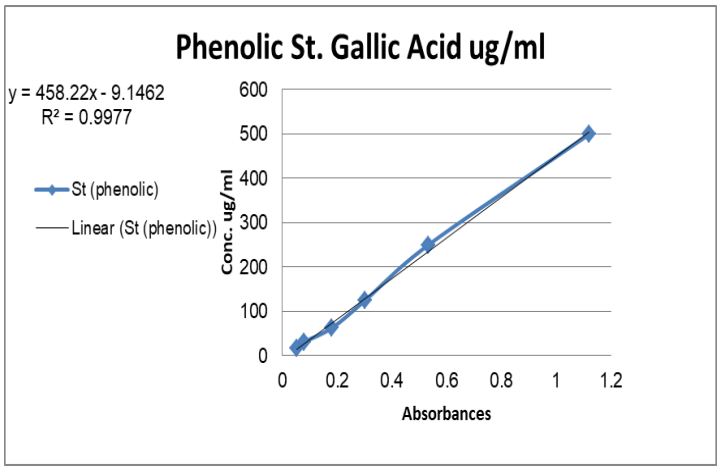
Generally, the results clearly demonstrate that each plant possesses a distinct phytochemical signature. Pomegranate peels are distinguished by their exceptionally high total phenolic content, whereas stinging nettle stands out as a major source of flavonoids. Lemon balm, although lower in concentration, still contributes valuable bioactive compounds. This variability highlights the importance of plant selection based on targeted bioactive constituents and supports the potential application of these plant leaf and peel powders as natural antioxidants in food and nutraceutical formulations .

Table (2): The Total phenolic (mg(GAE)/g) and total flavonoid (mg(QuE)/g) of plant powder

Plant powder	Total phenolic compounds (mg(GAE)/g)		Total flavonoid compounds (mg(QuE)/g)	
(Lemon balm) leaves	Mean	42.93	Mean	45.96
(Melissa officinalis)	Std	0.165	Std	0.246
Leaves	Se	0.054	Se	0.080
Pomegranate peels	Mean	150.64	Mean	55.58
(Punica granatum)	Std	.0703	Std	0.157
	Se	0.229	Se	0.051
Stinging nettle	Mean	104.87	Mean	106.20
(Urtica dioica L)	Std	1.563	Std	0.656
Leaves	Se	0.509	Se	0.214

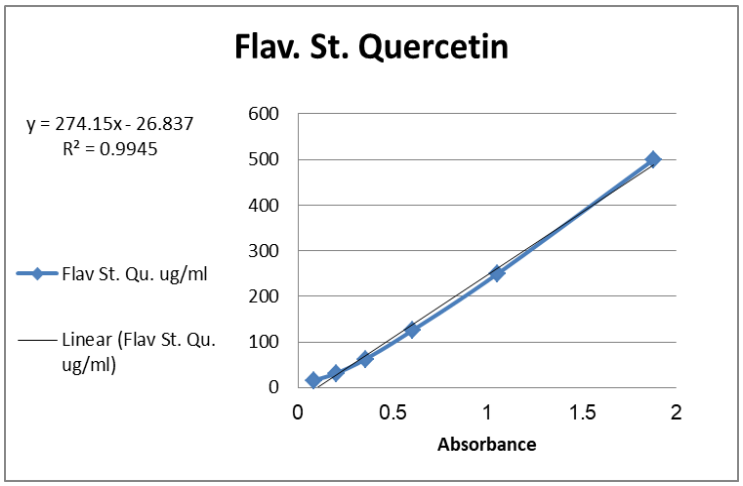


Phenolic St. Gallic Acid ug/ml	Absorbance
500	**1.305**
250	**0.634**
125	**0.317**
62.5	**0.211**
31.25	**0.115**
15.62	**0.078**

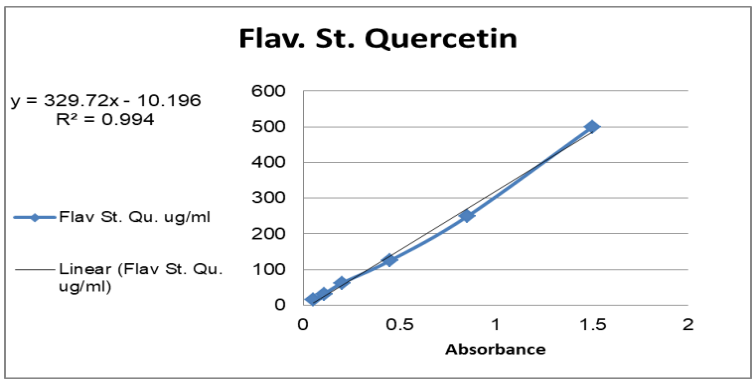


Phenolic St. Gallic Acid ug/ml	Absorbance
500	1.121
250	0.533
125	0.302
62.5	0.18
31.25	0.08
15.62	0.052

Fig (1a) Standard curve of phenolic compounds (results of two replicates) .



Flav St. Qu. ug/ml	Absorbance
500	1.879
250	1.055
125	0.603
62.5	0.357
31.25	0.201
15.62	0.083



Flav St. Qu. ug/ml	Absorbance
500	1.502
250	0.855
125	0.451
62.5	0.204
31.25	0.108
15.62	0.051

Fig (1b) Standard curve of flavonoid compounds (results of two replicates) .

Identification of individual phenolic and flavonoid compounds in plant powders using HPLC chromatographic analysis:

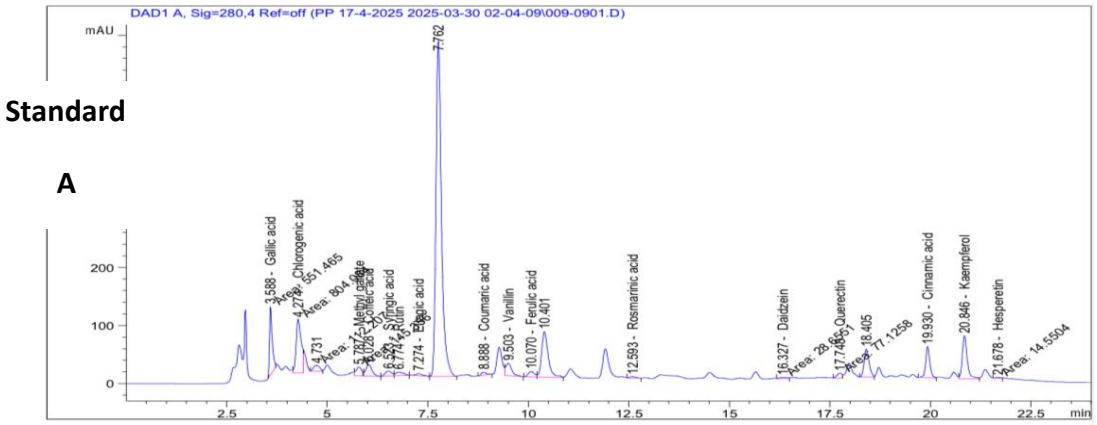
HPLC chromatograms of the phenolic compound of pomegranate peels, stinging nettle and lemon balm in are presented in Table (3) and (Fig. 2) the lemon balm exhibited 11 peaks (Fig. 2B), representing galic acid , chlorogenic acid , methyl gallate ,coffeic acid,syringic acid ,ellagic acid , coumaric acid , vanillin ,ferulic acid ,rosmarinic acid , cinnamic acid . chlorogenic acid and gallic acid recorded the highest levels (112.67 and 39.96 mg /gm extract , respectively). Pomegranate peels (fig . 2C) exhibited 11 peaks , denoting galic acid , chlorogenic acid , methyl gallate ,coffeic acid,syringic acid ,ellagic acid , coumaric acid , vanillin ,ferulic acid ,rosmarinic acid , cinnamic acid . chlorogenic acid and gallic acid

Where gallic acid and ellagic acid recorded the highest contents (1472.78 and 204.31 mg /gm extract respectively) . eleven peaks standing for galic acid , chlorogenic acid , methyl gallate ,coffeic acid,syringic acid ,ellagic acid , coumaric acid , vanillin ,ferulic acid ,rosmarinic acid , cinnamic acid . chlorogenic acid and gallic acid Appeared in the HPLC chromatogram of stinging nettle (fig. 2D) , where chlorogenic acid and gallic acid scored the highest contents (268.67 and 8.52 mg / gm extract , respectively). The HPLC chromatograms of the flavnoid compounds in the AREA of lemon balm, pomegranate peels and stinging nettle are depicted in (fig 2) . lemon balm (fig . 2B) produced 7 peaks (catechin , rutin ,naringenin ,daidzein ,querectin , kaempferol and hesperetin) where kaempferol and querectin exhibitted the highest levels (64.88 and 9.99 mg / gm extract, respectively . pomegranate peels (fig . 2C) , pinpointed the same 7 peaks (catechin , rutin ,naringenin ,daidzein ,querectin , kaempferol and hesperetin) but naringenin and rutin recorded highest contents (5.93 and 5.49 mg / gm extract respectively. Stinging nettle (fig 2D) conveyed only 7 peaks , where(catechin , rutin ,naringenin ,daidzein ,querectin , kaempferol and hesperetin) rutin and hesperetin achieved the highest concentrations (1.61 and 1.41 mg / gm extract, respectively . as shown in figure (2A) the standard calibration curve was generated using HPLC (Kuntic ,v; et al 2007).

Table (3) : concentration of individual phenolic and flavonoid compounds (µg/ml) in plant powders as determined by HPLC chromatography

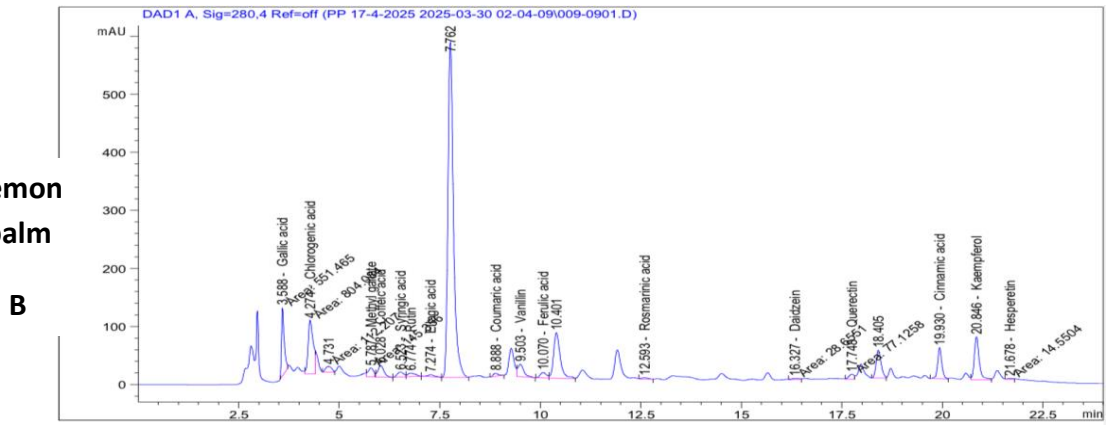
Concentration (µg/ml) of individual phenolic and flavonoid compounds in plant powder				
Component	**Standard**	**Lemon balm** ***(Melissa officinalis L)***	**Pomegranate** ***(Punica granatum)***	**Stinging nettle** ***(Urtica dioica L)***
phenolic compounds				
Gallic acid	20	39.96	1472.78	8.52
Chlorogenic acid	50	112.67	59.34	268.67
Methyl gallate	15	8.24	35.70	1.08
Coffeic acid	20	12.67	64.30	3.82
Syringic acid	20	6.40	0.00	1.18
Ellagic acid	70	5.47	204.31	0.14
Coumaric acid	20	1.73	2.36	0.00
Vanillin	20	7.57	0.00	0.23
Ferulic acid	20	5.96	0.67	1.28
Rosmarinic acid	50	2.87	0.66	1.42
Cinnamic acid	10	8.39	0.13	0.54

Flavonoid compounds				
Catechin	75	0.00	0.00	0.00
Rutin	50	12.19	5.49	1.61
Naringenin	30	0.00	5.93	0.59
Daidzein	20	1.64	0.06	0.23
Querectin	40	9.99	0.16	0.00
Kaempferol	20	64.88	0.48	0.70
Hesperetin	20	0.69	0.93	1.41



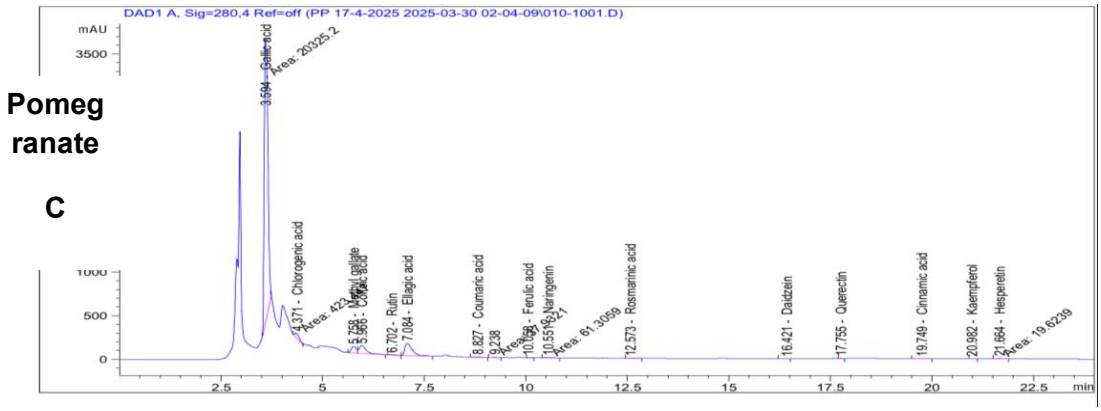
Standard

A



Lemon balm

B



Pomegranate

C

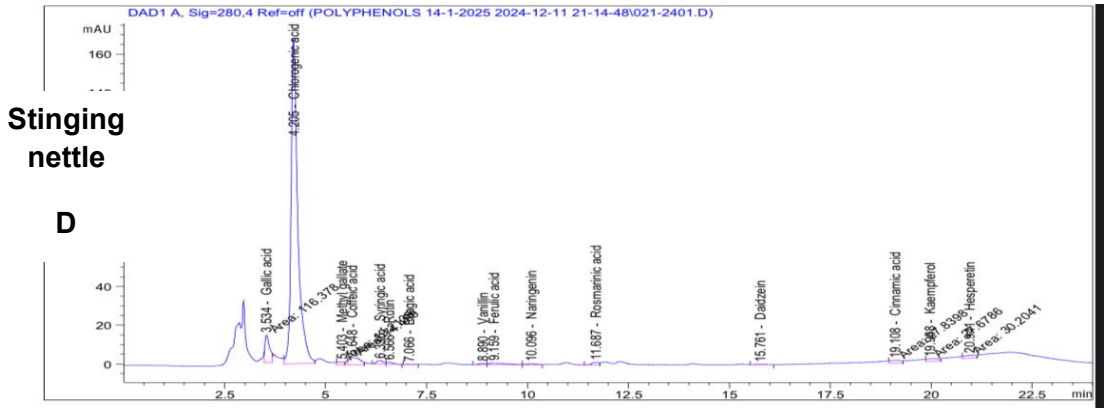


Fig (2): Retention time (min) of individual phenolic and flavonoid compounds of plant powders as determined by HPLC chromatography.

Table (4)Evaluation of antioxidant activity (DPPH) radical scavenging of three plant powders.

The antioxidant potential of three different plant powders (Lemon balm, Pomegranate, and Stinging nettle) was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, with ascorbic acid serving as the positive control. The DPPH assay is a widely accepted and reliable method for determining the free radical scavenging capacity of natural compounds (Brand-Williams et al., 1995). The results, presented as the mean optical density (OD) and the percentage of DPPH radical scavenging activity, are summarized in Table (4).

The data reveals a clear dose-dependent relationship for all DPPH tested samples, wherein the radical scavenging activity increased proportionally with the concentration of the extracts. This indicates that higher concentrations of the plant powders and ascorbic acid were more effective at neutralizing the DPPH free radical.

Ascorbic acid, the standard antioxidant used for comparison, exhibited the highest scavenging activity, reaching 97.8% at a concentration of 1000 µg/ml. Among the plant extracts, (Pomegranate) demonstrated the most potent antioxidant capacity, with a scavenging activity of 95.8% at 1000µg/ml. This finding is consistent with previous studies reporting the high antioxidant activity of pomegranate, attributed to its rich content of phenolic compounds (Elfalleh et al., 2012).

(Stinging nettle) showed a scavenging activity of 94.4% at the same concentration, a result supported by studies highlighting the antioxidant

potential of Lamiaceae species, particularly due to rosmarinic acid and other bioactive components (Albayrak et al., 2013). (Lemon balm) displayed the lowest activity among the extracts, achieving 93.1% scavenging at 1000µg/ml. These results align with previous reports on the nutritional and antioxidant properties of stinging nettle, both in terms of its leaf flour and methanolic extracts (Adhikari et al., 2015; Mhamdia et al., 2022). The comparative analysis indicates that while all three plant powders possess significant antioxidant properties, pomegranate powder is the most effective free radical scavenger. The order of antioxidant potency can be established as: Ascorbic Acid > Pomegranate > Stinging Nettle > Lemon Balm. These findings suggest that the tested plant powders, particularly pomegranate, are rich sources of natural antioxidants. The observed scavenging activity is likely attributable to the presence of bioactive phytochemicals, such as polyphenols and flavonoids, which are known for their ability to donate hydrogen atoms or electrons to neutralize free radicals (Shahidi and Ambigaipalan, 2015)

Table (4) :DPPH free radical scavenging activity of the lemon balm, pomegranate peels and stinging nettle

Ascorbic Acid St.			**Lemon balm**		**Pomegranate**		**Stinging nettle**	
(Conc. µg/ml)	**OD Mean**	**DPPH scavenging**	**OD Mean**	**DPPH scavenging**	**OD Mean**	**DPPH scavenging**	**OD Mean**	**DPPH scavenging**

		%		%		%		%
1000	0.029	97.8	0.090	93.1	0.055	95.8	0.074	94.4
500	0.073	94.4	0.138	89.5	0.104	92.1	0.121	90.8
250	0.099	92.5	0.218	83.4	0.171	87.0	0.201	84.7
125	0.133	89.9	0.306	76.8	0.250	81.0	0.292	77.8
62.5	0.227	82.7	0.424	67.8	0.350	73.4	0.393	70.2
31.25	0.339	74.3	0.538	59.1	0.434	67.1	0.513	61.1
15.625	0.452	65.7	0.641	51.3	0.530	59.8	0.617	53.1
7.8125	0.582	55.8	0.757	42.5	0.620	52.9	0.732	44.4
3.9	0.685	48.0	0.875	33.6	0.731	44.5	0.845	35.9
1.95	0.762	42.1	0.983	25.4	0.837	36.4	0.928	29.5
0	1.317	0.0	1.317	0.0	1.317	0.0	1.317	0.0
IC50 = 3.45 ± 0.08 µg/ml			IC50 =14.93 ±0.12 µg/ml		IC50 =6.22 ±0.13 µg/ml		IC50 =12.10±0.19 µg/ml	

Data in Table (5, photo (1) and fig 3) showed The antimicrobial efficacy of*(Melissa officinalis)* lemon balm , (*Punica granatum*) pomegranate, and *(Urtica dioica)* stinging nettle extracts was evaluated against four reference bacterial strains (*Bacillus subtilis* ATCC 6633*, Staphylococcus aureus* ATCC 6538, *Klebsiella pneumoniae* ATCC 13883, and *Salmonella typhi* ATCC 6539) using the agar diffusion assay, with gentamicin employed as a standard antibiotic control. The results revealed pronounced variations in inhibitory efficacy among the tested plant extracts as well as among the examined bacterial strains.

The findings demonstrated that pomegranate extract exhibited the highest antibacterial activity, with inhibition zone diameters ranging from 19 to 22 mm, as shown in photo (1) indicating a broad-spectrum antimicrobial effect against both Gram-positive and Gram-negative bacteria. The most pronounced inhibitory effect was observed against *Klebsiella pneumoniae* (22 ± 0.3 mm), which may be attributed to the high content of phenolic compounds, particularly hydrolysable tannins and polyphenols, known to disrupt bacterial cell membrane integrity, increase membrane permeability, and inhibit key enzymes involved in essential metabolic pathways (Elshafie et al., 2021).

In contrast, stinging nettle extract showed moderate antibacterial activity, with inhibition zones ranging from 13 to 19 mm, exhibiting comparatively greater efficacy against Gram-positive bacteria, especially *Bacillus subtilis* (19 ± 0.2 mm). This observation can be explained by the relatively simpler cell wall structure of Gram-positive bacteria, which facilitates stronger interactions with phenolic acids, organic acids, and bioactive secondary metabolites present in stinging nettle extracts (Kukric et al., 2012)

In contrast, lemon balm extract displayed the lowest antibacterial activity among the evaluated plant extracts, with inhibition zones ranging from 12 to 15 mm. Despite its comparatively limited efficacy, lemon balm extract exerted measurable inhibitory effects against all tested bacterial strains, likely due to the presence of essential oils and phenolic constituents, particularly rosmarinic acid, which may impair bacterial growth by altering membrane permeability and interfering with metabolic processes (Carvalho et al., 2021; Okmen, (2017)

When compared with the standard antibiotic gentamicin, pomegranate extract exhibited comparable inhibitory effects in certain cases, particularly against *Bacillus subtilis* and *Klebsiella pneumoniae,* highlighting its promising potential as a natural source of antimicrobial agents, either as a partial alternative or as an adjuvant

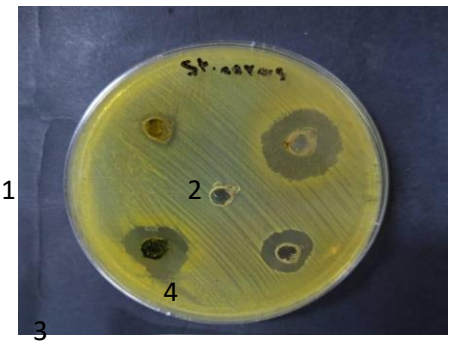
to conventional antibiotics. Furthermore, the relatively low standard deviation values reflect high experimental precision and reproducibility, thereby reinforcing the reliability of the obtained results.

The observed differences in antimicrobial activity among the investigated plant extracts underscore the critical role of phytochemical composition, concentration of bioactive compounds, and structural and physiological differences between Gram-positive and Gram-negative bacteria. Generally, these findings support the potential application of pomegranate, stinging nettle, and lemon balm extracts in pharmaceutical formulations and food preservation systems, within the broader context of developing safe and effective natural alternatives to synthetic antimicrobial agents (Kukric et al., 2012; Okmen, 2017; Carvalho et al., 2021; Elshafie et al., 2021)

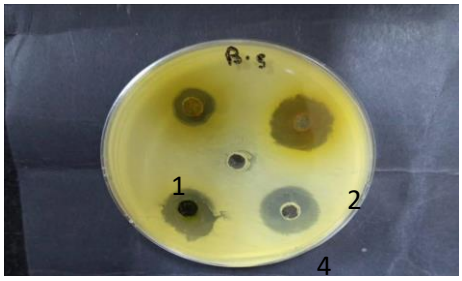
Table (5) : Antimicrobial Activity of Melissa officinalis, Punica granatum, and Urtica dioica Extracts

Microorganism	Inhibition zone (mm)				
	Control Antibiotic		Lemon balm *(Melissa officinalis L)*	Pomegranate *(Punica granatum)*	Stinging nettle *(Urtica dioica L)*
		Results			
Bacillus subtilis (ATCC 6633)	Gentamycin	19±0.4	13±0.2	20±0.4	19±0.2
Staph.aureus (ATCC 6538)	Gentamycin	14±0.6	12±0.7	20±0.5	14±0.1
Klebsiella pneumonia (ATCC13883)	Gentamycin	16±0.6	15±0.3	22±0.3	16±0.3
Salmonella typhi (ATCC 6539)	Gentamycin	13±0.8	14±0.5	19±0.5	13±0.5
Candida albicans (ATCC 10221)	Fluconazole	21±1	13±0.2	18±0.2	21±0.2

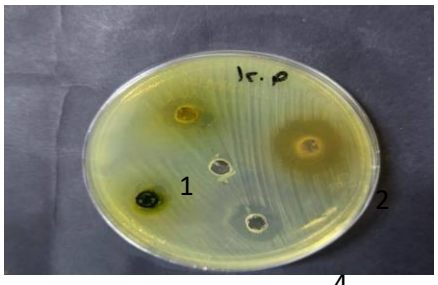
(a)



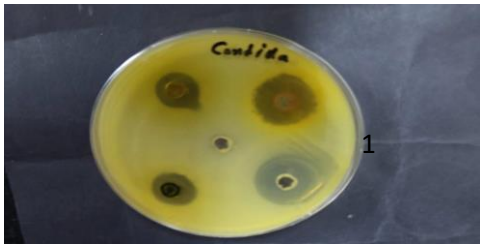
(b)



(c)



(d)



(e)

Photo(1) Zone of inhibition in control and three plant extract samples as shown in (a) *Staph.aureus* , (b) *Salmonella typhi* (c) *Bacillus subtilis* (d) *Klebsiella pneumonia* (e) *C.* (Gonzalez et al., 2016) *andida albicans* . The numbers represent :1- lemon balm extract ; 2- Pomegranate peel extract 3-stinging nettle extract 4- control .

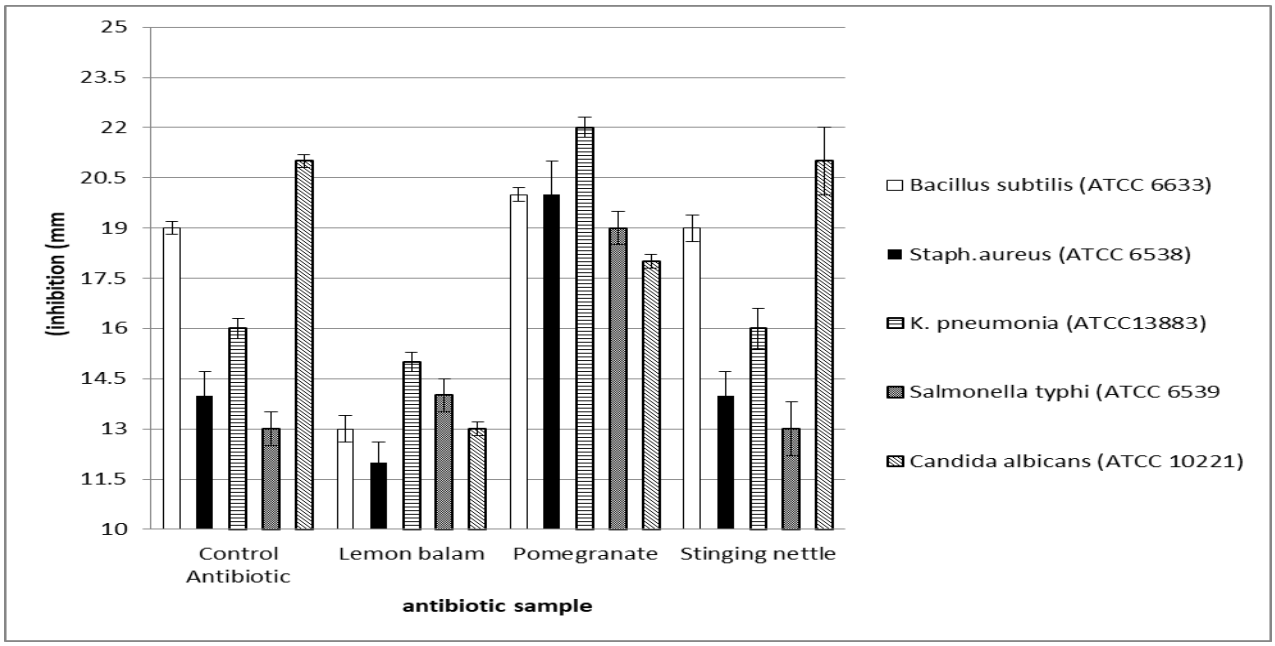


Fig (3) : These graphs represent the antimicrobial activity of the three plant extracts, with the control serving as the standard reference.

Control for Bacteria was Gentamycin and for fungi was fluconazole at concentration of 1.0 mg/ml.

50 mg of the samples were dissolved in 1.0 ml distilled water .

Conclusion:

Based on the comprehensive evaluation of the natural extracts from Melissa officinalis (lemon balm), Punica granatum (pomegranate) peel, and Urtica dioica (stinging nettle), this study unequivocally demonstrates their significant potential as natural alternatives to synthetic antioxidants and antimicrobial agents. The ultrasound-assisted organic solvent extraction method proved effective in yielding extracts rich in bioactive compounds, notably phenolic compounds and flavonoids, which are directly correlated with their observed biological activities.

Specifically, pomegranate peel extract consistently exhibited the highest antioxidant and antimicrobial activities, attributed to its superior phenolic content. Stinging nettle and lemon balm extracts also showed promising activities, albeit to a lesser extent. The potent antioxidant capacity, as evidenced by DPPH radical scavenging, confirms their efficacy in neutralizing free radicals. Furthermore, the plant powders showed an ability for killing some types of pathological microorganisms Results also showed 18 components of phenolic and flavonoid components found in the three powders studied, seven of them are flavonoids and eleven components belong to phenolic compounds , as found by HPLC chromatography. These findings provide a robust scientific basis for the utilization of these plant extracts in various industries. Their application could extend to developing safer food preservatives, enhancing nutraceutical formulations, and contributing to novel pharmaceutical agents, thereby addressing the growing consumer demand for natural and sustainable products. Future research should focus on in vivo studies to validate these effects in biological systems, elucidate the precise mechanisms of action at a molecular level, and optimize extraction and formulation parameters for specific industrial applications. Further fractionation and characterization of the active compounds would also be beneficial to fully harness their therapeutic and protective potentials.

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