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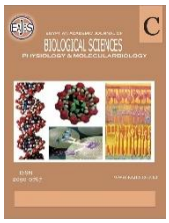

Microwave-Induced Enhancement of Antimicrobial and Antioxidant Activities in Six Traditional Medicinal Plant Extracts

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ABSTRACT

Background: The growing threat of antimicrobial resistance (AMR) and oxidative stress-related diseases underscores the need for sustainable therapeutic alternatives. This study evaluates the antimicrobial and antioxidant properties of six medicinal plant extracts and examines the enhancement of their bioactivities through microwave-assisted treatment. **Methods:** Aqueous extracts were prepared from *Punica granatum*, *Juglans regia*, *Matricaria chamomilla*, *Glycyrrhiza glabra*, *Pterocarpus santalinus*, and *Albizia lebbeck* (commonly known as shrein). After extraction, samples were irradiated using microwave energy (1–5 minutes at 800 W). Antimicrobial activity was assessed against *Escherichia coli* and *Staphylococcus aureus* using the agar well diffusion method. Antioxidant activity was measured using the DPPH radical scavenging assay at 25, 50, and 100 µL extract concentrations. Statistical analysis included one-way ANOVA and factorial regression. **Results**: Microwave irradiation significantly enhanced both antimicrobial and antioxidant activities across all extracts. For instance, chamomile's zone of inhibition against *E. coli* increased from 11 mm to 14 mm, while pomegranate's DPPH inhibition improved from 72.00% to 93.00%. *Pterocarpus santalinus*, initially inactive, displayed a 30.00% antioxidant gain post-irradiation. Factorial regression confirmed that both microwave exposure time and extract concentration were significant predictors of antioxidant response ($p < 0.05$). **Conclusions**: Microwave irradiation provides a green, scalable method to boost the bioactivity of plant-based therapeutics. These findings support the potential of microwave-assisted phytochemicals in combating AMR and oxidative stress-related conditions.

INTRODUCTION

The escalating global crisis of antimicrobial resistance (AMR) has driven renewed interest in alternative therapeutic approaches, particularly those derived from natural sources (Gupta and Sharma, 2022). Plant-based therapies have garnered substantial interest for their varied range of bioactive chemicals, including flavonoids, phenolics, terpenoids, and alkaloids, which have notable antibacterial and antioxidant effects (Mosaddad *et al.,* 2023). These organically sourced substances not only bypass the increasing constraints of synthetic medications, such as resistance and adverse effects, but also conform to modern requirements for biocompatible and environmentally sustainable healthcare solutions (Rishton, 2008). The processes driving the antimicrobial effects of plant extracts are complex and include the rupture of microbial membranes, blockage of essential enzyme pathways, and interference with microbial genetic material (Mosaddad *et al.,* 2023).

Simultaneously, their antioxidant qualities are essential for neutralizing reactive oxygen species (ROS), therefore alleviating oxidative stress associated with chronic illnesses such as cancer, cardiovascular disease, and diabetes (Nithya *et al.,* 2023). The efficacy of these plant extracts is considerably influenced by factors such as plant origin, phytochemical content, and extraction process, all of which may markedly affect biological activity and therapeutic results (Bastos *et al.,* 2025; Sharma *et al.,* 2025).

Numerous kinds of medicinal plants have distinguished themselves due to their significant bioactivities (Rather *et al.,* 2015). *Punica granatum* (pomegranate peel) is abundant in punicalagins and ellagitannins and is recognized for its antibacterial and radical-scavenging properties. Likewise, *Juglans regia* (walnut husk) has elevated concentrations of phenolic acids and juglone, substances recognized for their antibacterial and antioxidant properties (Rashki *et al.,* 2025). *Matricaria chamomilla* (chamomile) provides apigenin and bisabolol, flavonoids that contribute to microbial suppression and antioxidant protection. The therapeutic benefit of chamomile extract is attributed to its anti-inflammatory and relaxing qualities, which may reduce oesophageal irritation and facilitate mucosal repair (Srivastava *et al.,* 2010). *Glycyrrhiza glabra* (mulethi) is valued for its glycyrrhizin and isoflavonoids, often used in the treatment of respiratory and gastrointestinal disorders. Additionally, it has been shown to enhance mucus production and provide a protective barrier against acid damage (Pastorino *et al.,* 2018). *Pterocarpus santalinus* (red sandalwood) and shrein are little explored in scientific literature; yet, both exhibit ethnopharmacological importance and are noted for their anti-inflammatory and antibacterial properties, presumably attributable to their phenolic constituents (Dahat *et al.,* 2021; Mou *et al.,* 2025).

Recent breakthroughs have investigated microwave irradiation as a sustainable, non-thermal processing technique to enhance the extraction and therapeutic efficacy of these chemicals (Rather *et al.,* 2020). Microwave therapy, being a kind of electromagnetic radiation, facilitates phytochemical release by compromising cell wall integrity, therefore enhancing solubility, extraction yield, and biological activity (Nisca *et al.,* 2022; Zin *et al.,* 2020). Prior research indicates that microwave irradiation may significantly enhance polyphenol content and antioxidant capability in plant matrices, including green tea and turmeric (Yaman *et al.,* 2025). Nonetheless, the use of this approach on medicinal plants like pomegranate peel, walnut husk, chamomile, and others is still comparatively underexamined. Moreover, little research has systematically investigated the comparative impacts of microwave treatment on the antibacterial and antioxidant efficacy of these extracts, with even fewer investigations clarifying the biochemical or structural processes that contribute to these improvements.

Despite growing interest in plant-derived bioactivity and green extraction methods, few studies have systematically compared microwave-induced enhancement across multiple ethnomedicinal species using unified experimental designs. Prior research has typically focused on single plant models or lacking in the factorial statistical modeling. This study addresses these gaps by (i) applying microwave irradiation as a post-extraction enhancement strategy across six taxonomically diverse medicinal plants, (ii) evaluating dual bioactivities (antimicrobial and antioxidant) in comparison to control (5 µg ciprofloxacin), and (iii) employing factorial regression to model phytochemical behavior. In doing so, we provide a comprehensive and predictive framework for optimizing microwave-assisted phytotherapy, expanding the scope of bio-enhancement in natural product research.

MATERIALS AND METHODS

Materials:

The following plant materials were selected for this study based on their

traditional medicinal use and known phytochemical richness: pomegranate peel (*Punica granatum*), walnut husk (*Juglans regia*), chamomile flowers (*Matricaria chamomilla*), mulethi root (*Glycyrrhiza glabra*), shrein leaves (*Albizia lebbeck*), and red sandalwood bark (*Pterocarpus santalinus*). All plant samples were procured from authenticated herbal markets in Pakistan. Plant identity was confirmed through taxonomic authentication using morphological characteristics and regional herbarium references. All laboratory-based research and analyses were conducted at the Laboratory Medicine Department, Faculty of Applied Medical Sciences, Al Baha University, Saudi Arabia. Voucher specimens were cataloged and retained at the department for future reference. Reagents and chemicals used in the study included: 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich), methanol (analytical grade), distilled water, mueller-Hinton Agar (MHA) (HiMedia) for antimicrobial testing, and ciprofloxacin (5 µg) discs as the positive control for antimicrobial assays. Microbial strains used were *Escherichia coli* (MG1655) and *Staphylococcus aureus* (ATCC25923), obtained from the Laboratory Medicine Department, Al-Baha University, Saudi Arabia and maintained on nutrient agar slants at 4 °C prior to experimentation.

Methods:

Preparation of Plant Extracts*:*

All plant materials were first cleansed to eliminate dust and debris, then air-dried in the shade for 7–10 days at ambient temperature (25–30 °C) to maintain their phytochemical integrity. After complete desiccation, the plant components were pulverized using a sterile mechanical grinder and preserved in sealed containers at ambient temperature until extraction. Aqueous decoction was used for extraction because to its environmentally friendly characteristics and significance in traditional medicine as per previously well-developed methods (Gong *et al.,* 2020). Briefly, 10 g of dried plant materials were boiled at simmering point (93-95 ºC) in 300 ml of distilled water (liquor ratio of 30:1) for 60 minutes at neutral pH conditions, then cooled to room temperature (25 ºC). The mixture was then filtered using muslin cloth and Whatman No. 1 filter paper to yield clear aqueous extract. The filtrates were preserved in sterile glass containers at 4 °C and used for antibacterial and antioxidant tests within 48 hr to maintain the stability of active chemicals.

Microwave Treatment of Plant Extracts*:*

To enhance the antimicrobial and antioxidant activity of the plant extracts, microwave irradiation was applied post-extraction process. Aqueous extracts of each plant were subjected to microwave exposure using a domestic microwave oven (Samsung ME731K, 800 W) under controlled conditions. For each treatment, 5 mL of the freshly prepared extract was placed in a borosilicate glass beaker, loosely covered to avoid evaporation losses, and exposed to microwaves for durations of 1, 2, 3, 4, and 5 minutes, respectively. The microwave power was maintained at 100% (800 W) for all treatments (Kamran *et al.,* 2025). After exposure, the irradiated extracts were cooled to room temperature and then stored at 4 °C until further use. Untreated extracts served as controls for comparative analysis. This treatment aimed to investigate the effect of microwave energy on the transformation, or activation of phytochemicals, and its subsequent influence on antimicrobial and antioxidant properties.

Antimicrobial Activity Assay:

The antimicrobial efficacy of untreated and microwave-irradiated plant extracts was assessed against *E. coli* (MG1655) and *S. aureus* (ATCC25923) utilizing the agar well diffusion method, a standard and extensively utilized technique in phytochemical bioactivity research (Jamshidi *et al.,* 2014; Upadhyaya *et al.,* 2022). This method offers a comparative evaluation of bioactivity based on the width of inhibition zones generated by plant extracts. Briefly, the bacterial strains were sub-cultured in nutrient broth and incubated at 37°C for 24 hours (Gowda *et al.,* 2022). Sterile Mueller-Hinton Agar (MHA) plates were evenly injected with

bacterial solutions using sterile cotton brushes. Each well with 6 mm diameter was filled with 100 µL of either untreated or microwave-processed plant extract. Ciprofloxacin discs (5 µg) functioned as the positive control to confirm antimicrobial sensitivity, whilst distilled water acted as the negative control (Eshghi *et al.,* 2018; Momeni *et al.,* 2021). The plates were incubated at 37 °C for 24 hours. Following incubation, zones of inhibition (ZOI) were quantified in millimetres (mm). Each experiment was performed in triplicate, and the data were documented as mean ZOI ± standard deviation to guarantee repeatability and statistical validity.

Antioxidant Activity Assay:

A validated and widely used method for assessing free radical scavenging property in the phytochemical studies, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was used to evaluate the antioxidant potential of both untreated and microwave-irradiated plant extracts (Naidoo *et al.,* 2024; Nguyen *et al.,* 2017). Briefly, aliquots of 25 µL, 50 µL, and 100 µL of each plant extract were separately mixed with 1 mL of DPPH solution in test tubes. The mixtures were vigorously shaken and incubated in the dark at room temperature for 30 minutes. After incubation, the absorbance of the solutions was measured at 517 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800) (Rather *et al.,* 2017). The percentage of DPPH radical scavenging activity was calculated using the formula:

$$Scavenging\ activity\ (\%) = \left(\frac{A_0 - A_s}{A_0}\right) X\ 100$$

Where: A_0 is the absorbance of the DPPH control (without extract) and A_S is the absorbance in the presence of the plant extract. All assays were performed in triplicates, and results were expressed as mean ± standard deviation.

Statistical Analysis:

All experimental results were presented as mean ± standard deviation (SD), based on triplicate measurements for each of the five treatment durations. Comparative statistical analyses between untreated and microwave-treated samples were performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to identify statistically significant differences among groups based on time duration. A p-value of < 0.05 was considered to indicate statistical significance. The factorial regression models were applied to the DPPH inhibition data across different irradiation durations (1–5 minutes) for each plant extract and concentration level (25, 50, and 100 µL), to evaluate the relationship patterns and quantify the rate of increase in antioxidant activity per minute. The goodness-of-fit of each model was assessed using the coefficient of determination (R^2), with values greater than 0.98 indicating an excellent model fit for most plant extracts.

RESULTS AND DISCUSSION

Antimicrobial Activity of Different Plant Extracts:

The antimicrobial activity of the six selected plant extracts including pomegranate peel, walnut husk, chamomile, mulethi, shrein, and red sandal bark was assessed using the disc diffusion method (Zone of inhibition) against two model bacterial strains: *E. coli* and *S. aureus*. The results demonstrated variable zones of inhibition (ZOI), suggesting that the phytochemical composition of each extract distinctly influenced bacterial susceptibility (Table 1 and Fig. 1).

Table 1: Zone of inhibition (mm) of different extracts against *E. coli* and *S. aureus*

Plant extracts	Zone of inhibition (mm)	
	E. coli (MG1655)	***S. aureus* (ATCC25923)**
Pomegranate peel	8 ± 0.345	8 ± 0.119
Walnut husk	9 ± 0.278	10 ± 0.339
Shrein	9 ± 0.122	8 ± 0.089
Mulethi	8 ± 0.189	9 ± 0.249
Chemomile	11 ± 0.249	10 ± 0.213
Red Sandalwood bark	-	8 ± 0.079

Chamomile exhibited the highest antimicrobial activity, particularly against *E. coli* (11 ± 0.249 mm) and *S. aureus* (10 ± 0.213 mm). This corresponds with prior research that associates chamomile's antibacterial properties with its high flavonoid content, including apigenin and bisabolol, which acts on the bacterial cell membranes and several metabolic pathways (Mosaddad *et al.,* 2023). Walnut husk exhibited substantial efficacy, particularly against *S. aureus* with zone of inhibition of 10 ± 0.339 mm. This result correlates with the previous findings on the antimicrobial properties of ellagic acid and juglone which are the major phytoconstituents of walnut husk (Bukhari *et al.,* 2017). They work by inhibiting DNA replication and induce oxidative stress in microbial cells (Mosaddad *et al.,* 2023). Pomegranate peel showed moderate activity of 8 mm zone of inhibition which is in consistent with the presence of punicalagins, tannins, and flavonoids that exert bacteriostatic effects by complexing with bacterial proteins and enzymes (Dogara *et al.,* 2024). The zone of inhibition values align with existing research on pomegranate's efficacy in destroying *S. aureus* biofilms and suppressing *E. coli* proliferation (Celiksoy *et al.,* 2021). Mulethi with an established therapeutic property performed modestly against *E. coli* and *S. aureus* with a zone of inhibition of 8 and 9 mm, respectively. The bioactivity is mostly attributed to glycyrrhizin and liquiritin, known for their ability to break microbial membranes and impede energy metabolism (Chen *et al.,* 2024; Qin *et al.,* 2022). Shrein, though comparatively less studied in the context of microwave-enhanced phytotherapy, demonstrated antimicrobial activity comparable to pomegranate and mulethi, with zones of inhibition measuring 9 ± 0.122 mm against *E. coli* and 8 ± 0.089 mm against *S. aureus*. Its bioactivity is attributed to reported constituents such as flavonoids, saponins, and alkaloids, which have been shown to exert antimicrobial effects by disrupting bacterial membranes and inhibiting protein synthesis (Ghani *et al.,* 2014). Prior studies support its potential as a broad-spectrum antimicrobial agent, though variability in extraction methods and phytochemical profiles may influence potency. Red sandal bark was the least effective with no measurable inhibition against *E. coli* and only a marginal effect on *S. aureus* (8 ± 0.079 mm). This may reflect the lower bioavailability or potency of its lignans and phenolic constituents in aqueous extraction, compared to alcohol-based extracts reported in the literature (Ooko, 2014). In contrast, the antibiotic control ciprofloxacin exhibited a significantly larger ZOI (25 ± 0.289 mm), affirming its clinical potency and serving as a benchmark for plant extract efficacy.

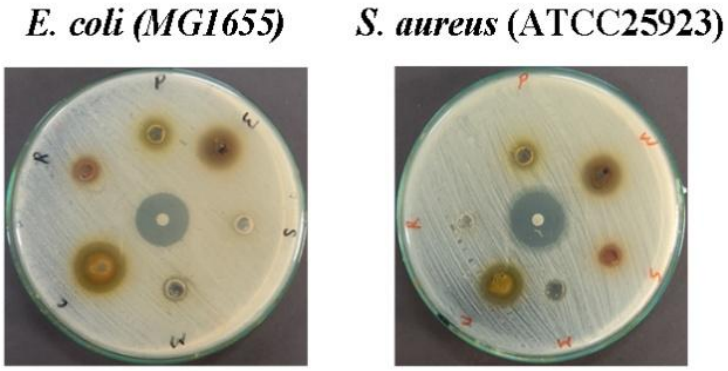

Fig. 1: Antimicrobial activity (zone of inhibition) against *E. coli* and *S. aureus.*

The aqueous plant extracts exposed to microwave radiation for 1–5 minutes, showed increased antibacterial activity, especially against *E. coli*, with plant-specific reactions that varied in strength and durability over time (Table 2 and Fig. 2). The microwave-induced structural changes of phytochemicals including enhanced solubility, breakdown of complicated structures, or increased bioactive ingredient accessibility are responsible for this improvement (Nisca *et al.,* 2022; Upadhyaya *et al.,* 2022). Among all the plant extracts, chamomile displayed the most pronounced antibacterial effect with the zone of inhibition of 14 mm in comparison to 11 mm (un-irradiated) after 4 minutes of microwave exposure accounting for 27.27% increase in antibacterial activity. This increase is likely due to the activation of phytoconstituents such as apigenin, bisabolol, and other flavonoids, which disrupt bacterial membranes (Nisca *et al.,* 2022). Walnut husk also showed progressive enhancement, from 9 to 11 mm at 5 minutes (Mosaddad *et al.,* 2023). Mulethi demonstrated a maximum efficiency at 2–3 minutes (10 mm), then declining to 8 mm at 4–5 minutes, indicating a threshold beyond which heat breakdown of glycyrrhizin and saponins may transpire, reducing efficacy. This biphasic tendency corroborates the findings of Shen *et al.* (2023), who observed that microwave treatment exceeding recommended durations may result in phytochemical breakdown instead of augmentation (Shen *et al.* 2023). Red sandal (un-irradiated) ineffective against *E. coli*, exhibited an enhanced zone of inhibition of up to 11 mm at 3 minutes, indicating the activation of antimicrobial compounds such as santalins and flavonoids due to microwave-induced structural disruption (Upadhyaya *et al.,* 2022).

Table 2: Effect of microwave radiation on the zone of inhibition on *E. coli* (MG1655)

Samples	Zone of inhibition (mm) after microwave radiation treatment					P value
	1 min	2 min	3 min	4 min	5 min	
Pomegranate peel	8 ± 0.339	9 ± 0.189	9 ± 0.112	9 ± 0.119	9 ± 0.222	0.000
Walnut husk	9 ± 0.239	10 ± 0.229	10 ± 0.234	10 ± 0.349	11 ± 0.311	0.000
Shrein	-	-	-	-	-	-
Mulethi	9 ± 0.281	10 ± 0.249	10 ± 0.233	8 ± 0.284	8 ± 0.221	0.000
Chemomile	11 ± 0.284	12 ± 0.244	13 ± 0.239	14 ± 0.329	12 ± 0.234	0.000
Red Sandalwood bark	8 ± 0.259	9 ± 0.221	11 ± 0.349	9 ± 0.233	8 ± 0.215	0.000

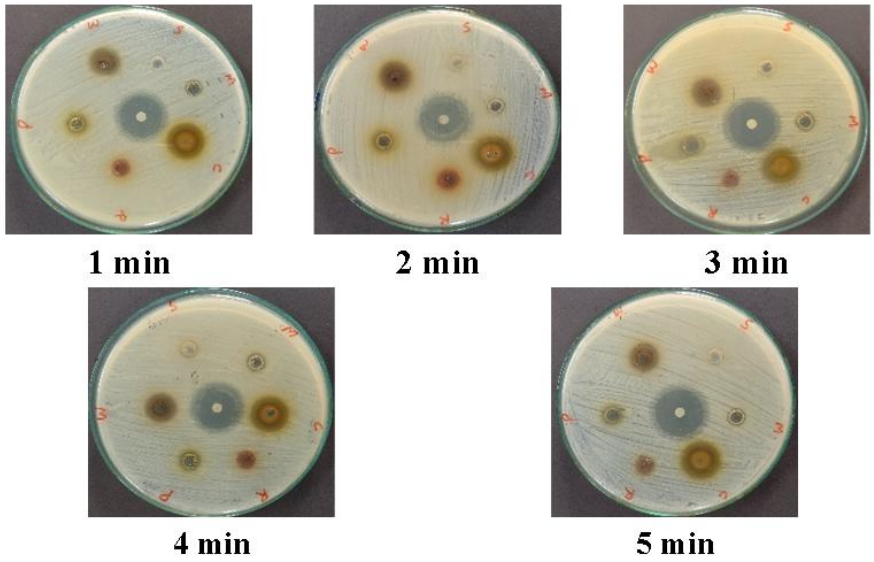

Fig. 2: Antimicrobial activity after microwave irradiation (*E. coli*: MG1655).

Unlike *E. coli*, most of the extracts exhibited negligible alterations in antibacterial efficacy against *S. aureus* following microwave treatment, signifying species-specific reactions (Table 3 and Fig. 3). This may be due to the distinct cell wall architecture of gram-positive *S. aureus*, which might exhibit reduced susceptibility to specific microwave-induced chemical alterations (Woo *et al.,* 2000). Walnut husk and mulethi showed elevated activity throughout all durations (11 and 9 mm, respectively), although red sandal bark demonstrated a slight increase from 8 to 9 mm at the 5-minute mark. The consistent performance indicates that their antibacterial compounds may already exist in accessible forms that are not influenced by microwave-enhanced release mechanisms (Matini and Naghib, 2025). Chamomile remained steady (10 mm), further supporting the notion of a saturation point in active compound bioavailability for *S. aureus*. In the present context, the microwave radiations induce the structural modification or transformation of polyphenols and flavonoids, increasing their solubility and reactivity (Ahmed *et al.,* 2024; Bodea *et al.,* 2022). Additionally, thermal degradation leads in the reduction in the activity as observed in mulethi post 3 minutes.

Table 3: Effect of microwave radiation on the zone of inhibition on *S. aureus* (ATCC25923)

Samples	Zone of inhibition (mm) after microwave radiation treatment					p value
	1 min	2 min	3 min	4 min	5 min	
Pomegranate peel	8 ± 0.079	8 ± 0.079	8 ± 0.079	8 ± 0.079	8 ± 0.079	>0.05
Walnut husk	11 ± 0.198	11 ± 0.198	11 ± 0.198	11 ± 0.198	11 ± 0.198	>0.05
Shrein	8 ± 0.079	8 ± 0.079	8 ± 0.079	-	-	>0.05
Mulethi	9 ± 0.099	9 ± 0.099	9 ± 0.099	9 ± 0.099	9 ± 0.099	>0.05
Chemomile	10 ± 0.118	10 ± 0.118	10 ± 0.118	10 ± 0.118	10 ± 0.118	>0.05
Red Sandalwood bark	8 ± 0.079	8 ± 0.079	8 ± 0.079	8 ± 0.079	9 ± 0.189	>0.05

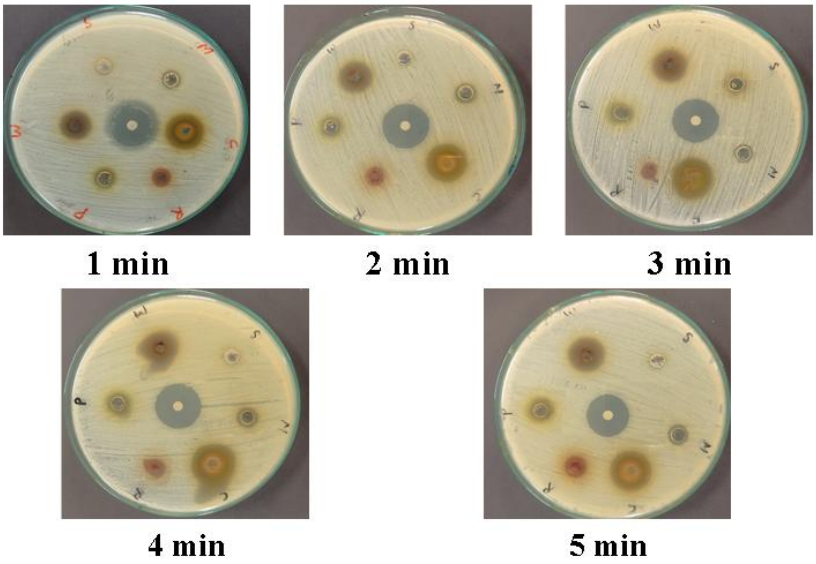

Fig. 3: Antimicrobial activity after microwave irradiation (*S. aureus*: ATCC25923).

Antioxidant Activity:

The antioxidant potential of six plant extracts including pomegranate, walnut, shrein, mulethi, chamomile, and red sandalwood were quantitatively evaluated using the DPPH radical scavenging assay at three concentrations: 25, 50, and 100 µL in 1 mL of methanolic DPPH solution (Fig. 4). Pomegranate peel extract demonstrated the highest antioxidant activity (72.00% at 100 µL), consistent with prior research identifying punicalagins and ellagic acid as effective radical scavengers. These polyphenols exhibit significant reactivity with DPPH radicals owing to their many hydroxyl groups (SUN *et al.,* 2017). Walnut husk showed a significant activity (69.00% at 100 µL), possibly attributable to the constituents juglone and gallic acid derivatives, which also exhibit substantial ferric reducing antioxidant power (FRAP) and DPPH inhibition (Żurek *et al.,* 2022). These chemicals have been demonstrated to be released in significant quantities via aqueous extraction procedures. Among the less effective extracts, shrein exhibited the lowest antioxidant capacity (61.00% at 100 µL) due to the less polyphenolic or flavonoid content or these compounds exist in bound forms (Shahidi and Hossain, 2023). Mulethi recognized for its glycyrrhizin and liquiritin content, exhibited moderate activity (62.00%), consistent with prior research associating its antioxidant properties with triterpenoid saponins and chalcones (Ahmed *et al.,* 2021). Chamomile exhibited moderate inhibition (64.00%) attributed to apigenin and luteolin glycosides, which have electron-donating characteristics (Seelinger *et al.,* 2008). Red sandalwood while generally not considered a strong antioxidant in aqueous extracts, reached 66.00% activity at 100 µL, possibly due to santalin A/B compounds with known phenolic radical scavenging behavior. The sequence of actions at 100 µL is as follows: Pomegranate, Walnut, Red Sandal, Chamomile, Mulethi, and Shrein. This trend highlights the importance of water solubility and chemical polarity in DPPH scavenging activity.

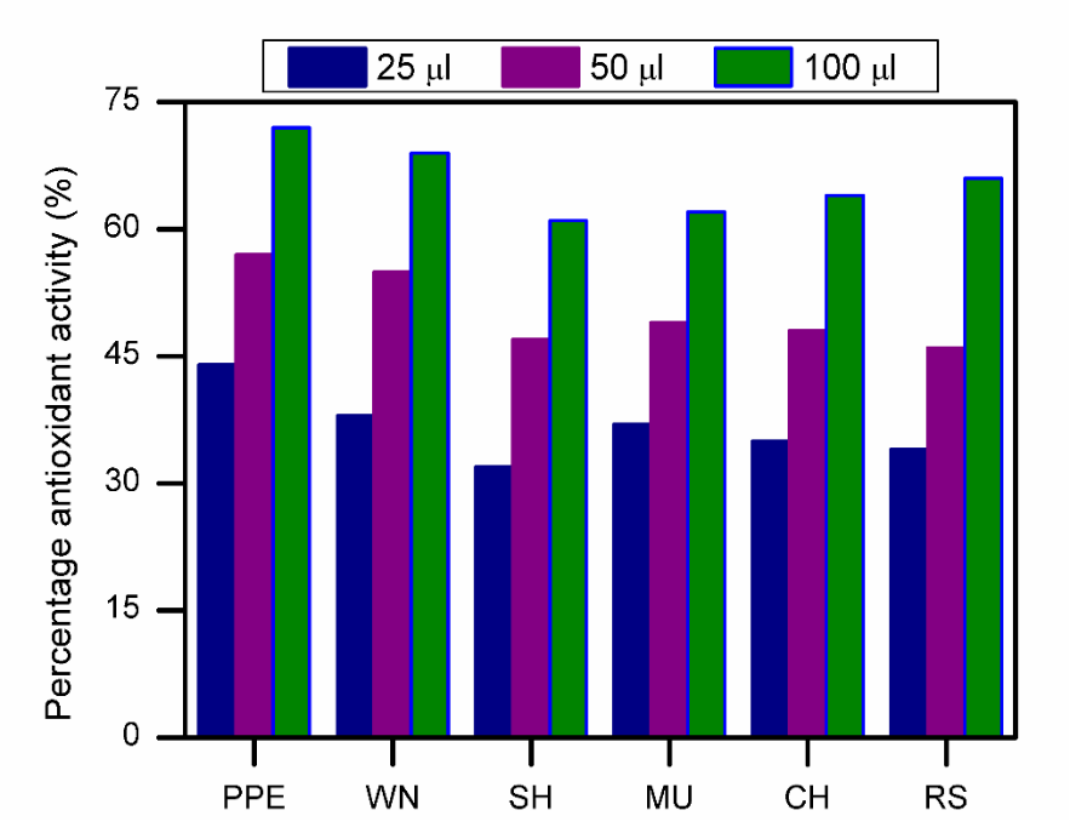

Fig. 4: Antioxidant activity (DPPH scavenging, untreated extracts) at three different concentrations (25, 50, and 100 µL).

Microwave irradiation markedly affected the antioxidant properties of all six plant extracts, with improvements noted at all doses and durations (1–5 minutes). The DPPH radical scavenging activity exhibited a time- and dose-dependent increase, with most samples reaching their peak at approximately 5 minutes of exposure. These improvements align with current literature indicating that microwave energy promotes liberation of bound phenolic compounds and structural alteration of flavonoids, hence augmenting their free radical scavenging capacity (Nisca *et al.,* 2022; Wang *et al.,* 2025). The data presented in Figurs 5-7, delineates the alteration in antioxidant activity from 1 to 5-minute exposure across three concentrations (25, 50, and 100 µL) of plant extract in DPPH methanolic solution. The pomegranate peel extract serves as a potent source of antioxidants, exhibiting a moderate enhancement (15–20%) following irradiation. Similarly, the walnut husk extract showed up to 21% increase in antioxidant activity due to enhanced solubilization of ellagitannins, gallic acid, and juglone, compounds recognized for their hydroxyl-mediated radical scavenging and metal-chelating behavior (Nisca *et al.,* 2022) (Fig. 5).

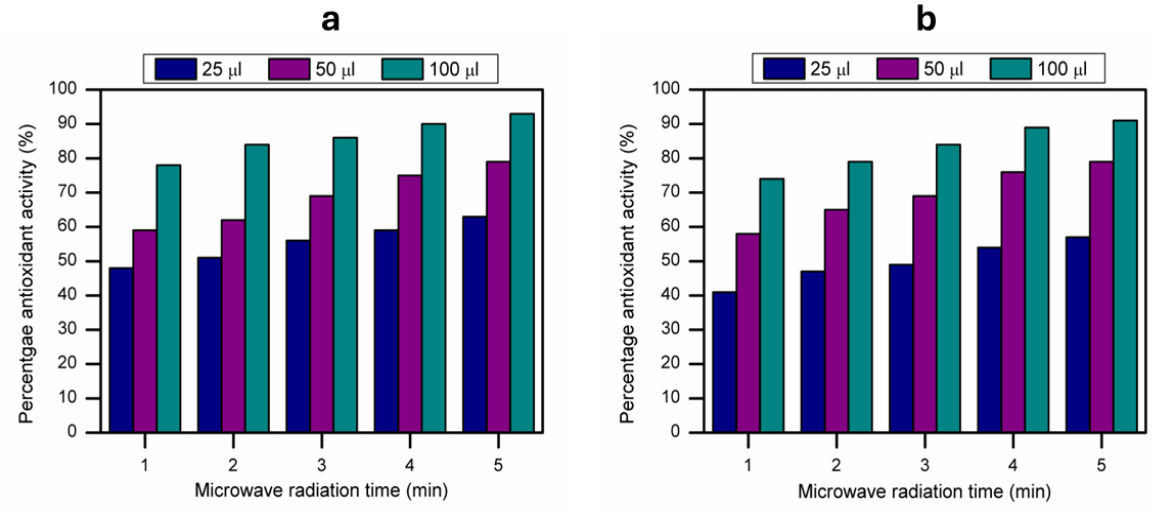

Fig. 5: Antioxidant activity after microwave irradiation (a) pomegranate peel extract and (b) walnut extract.

Nonetheless, shrein extract demonstrated a significant increase (up to +24.00% at 50 µL). This indicates the existence of bound or latent polyphenolics, probably triggered through microwave-assisted hydrolysis. This behavior mimics with other lesser known ethnomedicinal plants studied by Bodea *et al.* (2022) where structural disruption unveiled cryptic antioxidant potential (Bodea *et al.,* 2022). *Glycyrrhiza glabra* displayed consistent enhancement across concentrations (up to +21.00%) (Fig. 6). Its primary antioxidants such as glycyrrhizin, liquiritigenin, and flavonoid glycosides are heat-stable and their antioxidant profile may benefit from partial hydrolysis and release of aglycones during microwave exposure (Zin *et al.,* 2020).

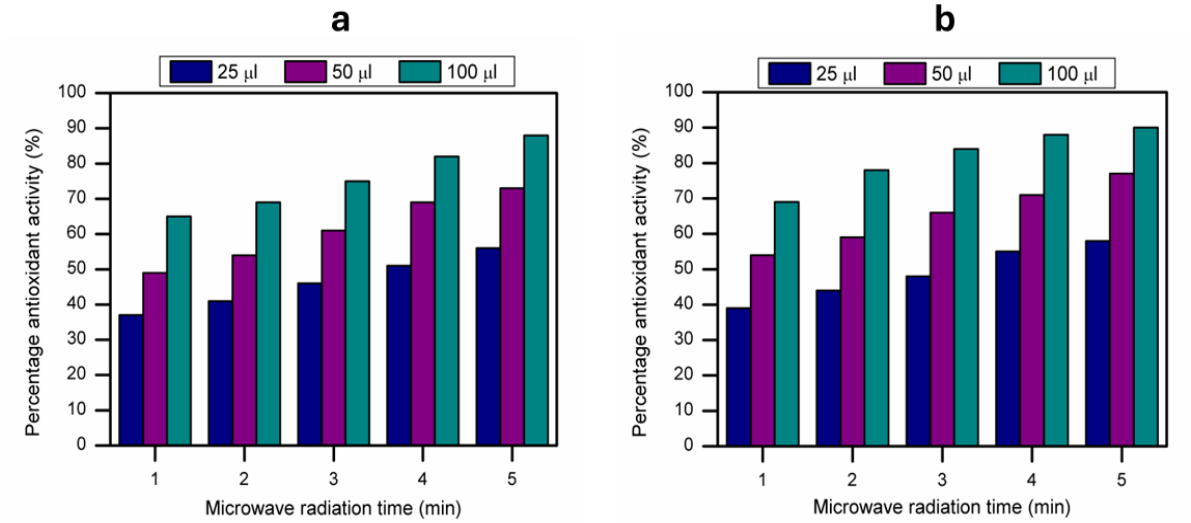

Fig. 6: Antioxidant activity after microwave irradiation (a) shrein and (b) mulethi.

Chamomile showed a +23.00% gain at 50 µL, driven by microwave-facilitated release of apigenin, quercetin, and luteolin glycosides. These flavonoids possess high DPPH scavenging capacity and irradiation likely improved solubility and bioavailability (Upadhyaya *et al.,* 2022). Finally, red sandalwood showed the highest enhancement (+30.00% at 50 µL), likely due to the microwave-induced breakdown of santalin

A/B and phenolic acids, increasing their solubility and antioxidant reactivity (Fig. 7). This result parallels other studies where irradiation of colored wood extracts yielded elevated DPPH and FRAP scores through pigment liberation and structure transformation (Bodea *et al.,* 2022)

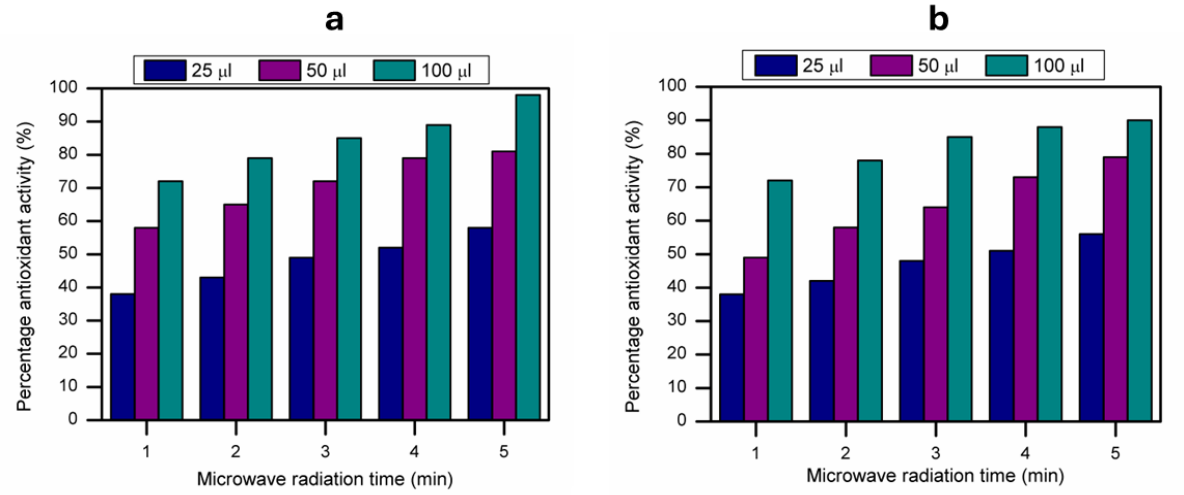

Fig. 7: Antioxidant activity after microwave irradiation (a) Chamomile and (b) Red sandalwood.

Statistical Analysis:

All data were presented as mean ± standard deviation (SD) derived from triplicate measurements, and statistical analyses were performed to assess the impact of microwave irradiation duration and extract concentration on the antibacterial and antioxidant properties of the evaluated plant extracts. The impact of microwave irradiation (1–5 min) on antimicrobial efficacy was evaluated using one-way ANOVA, succeeded by Tukey's HSD post-hoc test for multiple comparisons. Results indicated statistically significant changes ($p < 0.001$) in the zone of inhibition (ZOI) for all tested extracts against *E. coli*, although no significant variation was noted against *S. aureus* across the treatments. Pomegranate and walnut exhibited consistent responses, but chamomile had a notable rise in ZOI from 11 to 14 mm over 4 minutes ($p < 0.001$). Red sandalwood and mulethi demonstrated maximal responses at intermediate intervals, indicating a non-linear augmentation. These variations underscore extract-specific reactions to heat manipulation and indicate that optimal antibacterial efficacy is achieved within a limited temporal window for distinct plants.

To model antioxidant activity, factorial regression analysis was used employing microwave time, extract concentration, and their interaction as predictors of antioxidant activity to more specifically investigate factor contributions. While the interaction term (time × concentration) was non-significant, indicating additive rather than synergistic effects, results revealed that both microwave time and concentration significantly contributed to increased DPPH inhibition across all extracts, confirming great model fits, R^2 values varied from 0.8916 to 0.9805. Red Sandalwood ($\beta = 5.95$) demonstrated the strongest antioxidant enhancing slope while pomegranate showed continuous high-performance over-all irradiation times ($R^2 = 0.9805$) (Table 4). With variability in plant species resulting from variations in phytochemical composition and thermal response behavior, the statistical analyses taken together confirmed that microwave irradiation greatly increases both antimicrobial and antioxidant activity in a time- and dose-dependent manner.

Table 4: Factorial regression analysis for increase in the antioxidant activity with irradiation time (1-5 minutes).

Extract	R^2	Intercept	Time β	Conc. β	Time × Conc. β
Pomegranate	0.9805	33.75	4.45 *	0.41 **	–0.0037 ns
Walnut	0.9276	29.55	4.35 *	0.42 **	0.0031 ns
Shrein	0.9685	23.90	5.00 **	0.35 **	0.0114 ns
Mulethi	0.9647	25.00	5.20 **	0.42 **	0.0017 ns
Chemomile	0.8916	25.40	5.50 *	0.45 **	–0.0046 ns
Red Sandal	0.9572	20.15	5.95 **	0.48 **	–0.0071 ns

$p < 0.05$ (*), $p < 0.01$ (**), and not significant (ns) if $p \geq 0.05$

Study Limitations and Future Work:

The results of this work highlight the dual therapeutic possibilities of medicinal plant extracts as both antibacterial and antioxidant agents, therefore providing a sustainable, plant-based substitute for synthetic molecules. Particularly in extracts from chamomile, pomegranate peel, and walnut husk, shown activity against *Escherichia coli* and *Staphylococcus aureus* supports their possible use in addressing antimicrobial resistance (AMR), a major and expanding worldwide health risk. Their strong antioxidant action concurrently supports more general uses in control of oxidative stress, a major factor for chronic diseases like cancer, cardiovascular disease, and neurological diseases. Significantly, the increase of these bioactivities by microwave irradiation emphasizes the viability of this method as a green, scalable, and reasonably affordable means of enhancing the functional efficacy of phytochemicals (Elnour *et al.,* 2024; Mali and Kumar, 2023). By means of factorial analysis and regression-based modelling, predictive optimization is further enabled, therefore enabling precision-tuned microwave settings to enhance bioactivity without compromising compound stability. These revelations have practical ramifications in many different fields: in food preservation as natural antibacterial or antioxidant additions; in medicines as adjuncts or substitutes for antibiotics; and in cosmeceuticals, for the design of safer, plant-based products. In line with worldwide efforts to address AMR, lower oxidative disease burden, and support sustainable innovation, this work helps to build environmentally friendly, targeted, and effective natural therapeutics by validating microwave-assisted enhancement and offering a model-driven framework for phytochemical optimization (Laina *et al.,* 2024).

Subsequent research should focus on the isolation and structural elucidation of certain bioactive molecules accountable for the identified antibacterial and antioxidant properties, utilizing sophisticated methodologies such as LC-MS, GC-MS, and NMR spectroscopy. Comprehending the biochemical and structural pathways that facilitate microwave-induced enhancement is essential for optimizing treatment parameters and maintaining compound stability. To enhance therapeutic applicability, antimicrobial activity must be evaluated against a wider array of pathogens, encompassing fungi and multidrug-resistant bacterial strains. High-potency extracts, such as chamomile and walnut husk, should be further advanced into pharmaceutical and cosmeceutical formulations, focusing on delivery mechanisms and stability of shelf life. Furthermore, toxicological profiling both in vitro and in vivo is crucial to validate the safety of these extracts for clinical or commercial use. Exploring potential synergistic interactions between various plant extracts or with traditional antimicrobials may result in improved therapeutic effectiveness. This study establishes a basis for future research that combines green extraction techniques with natural product-oriented medication development, highlighting the transformational potential of microwave technology in functional plant-based therapies.

Conclusion

This research examined the antibacterial and antioxidant characteristics of six traditional medicinal plant extracts such as *Punica granatum*, *Juglans regia*, *Matricaria chamomilla*, *Glycyrrhiza glabra*, *Pterocarpus santalinus*, and *Albizia lebbeck* before and after microwave irradiation Chamomile extract had the greatest antibacterial activity (11 mm ZOI against *E. coli*) and pomegranate peel had the most antioxidant activity (72.00% DPPH inhibition at 100 μL) among the untreated extracts. These results indicate plant-derived chemicals' ethnomedicinal use and therapeutic potential. Microwave radiation (1–5 minutes) increased antibacterial and antioxidant activities in all extracts. Microwave irradiation increased DPPH scavenging by 15.00–30.00% and ZOI expansion, especially in chamomile (14 mm) and walnut husk (11 mm). Even low-activity extracts like shrein and red sandalwood improved after heat modification, revealing phytochemical potential. Results from complementary factorial regression analysis indicate that microwave time and extract concentration independently predict antioxidant activity ($p < 0.05$), with non-significant interaction indicating additive effects. Red sandalwood had the greatest antioxidant enhancement rate ($\beta = 5.95$), whereas pomegranate peel performed well across all test circumstances ($R^2 = 0.9805$). Microwave-induced disruption of plant cell structures, thermal loosening of bound phenolic compounds, and improved solubilization of flavonoids, tannins, and glycosides increase bioactivity. Similar outcomes have been reported for microwave-assisted extraction, a green, energy-efficient, and scalable method for phytochemical yield and activity. The improved extracts might be used in pharmaceutical, nutraceutical, food preservation, and cosmeceutical products. The regression modelling methodology established herein may also forecast microwave parameter optimization in future investigations. This study confirms microwave-assisted enhancement as a transformational natural product chemistry method that combines phytotherapy with extraction technology. The results enable safe, sustainable, and effective plant-derived therapies by providing a platform for mechanistic, formulation-based, and in vivo research.

Declarations:

Ethical Approval: This study did not involve human participants or animals. The research was limited to in vitro laboratory analyses of plant extracts and thus did not require ethical approval.

Informed Consent: Not applicable. The study did not involve human participants, patient data, or biological samples.

Declaration of Generative AI and AI-assisted Technologies in the Writing Process: The author has not used any of the generative AI or AI-assisted tool in the writing of this article.

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