



Article

Investigation into the Antioxidant Properties of *Grewia tenax* and its Influence on Bacterial Organisms

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Abstract: *Grewia tenax* (GT) belongs to the Tiliaceae family and is referred to locally in various Arab regions as Qadeem or Wild Red, while its scientific nomenclature is *Qreno Tinx*. This species exhibits a high concentration of antioxidants. The primary objective of this research was to isolate phenolic compounds present in *Grewia tenax* (GT) utilizing absolute methanol; the antioxidant activity was evaluated through two methodologies: Folin-Ciocalteu indexes (FCI), which assess total phenolic compounds (TPC) with gallic acid serving as a standard reference, and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, where ascorbic acid was employed as a benchmark. The findings indicate that the FCI demonstrates that the phenolic compounds extracted from (GT) range from 1076.5 to 4841.5mg GAE/g of extract, where (GAE) signifies gallic acid equivalent. It was noted that the phenolic compounds possess a substantial capability to transform the stable free radical DPPH into diphenylpicrylhydrazine, characterized by a yellow hue, with the optimal IC₅₀ value recorded at 4379.43µg/ml. The antioxidant capacity, compared to ascorbic acid, was found to be 4088.93 µg/ml, representing the highest effectiveness at a concentration of 5000 mg/ml of the extract, while the lowest efficacy was observed at a concentration of 1000 mg/ml. The extract was evaluated against several bacterial strains, including *Staphylococcus aureus*, *Streptococcus* spp., *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., and *Proteus* spp. The results indicate that varying concentrations of the extract significantly inhibited the proliferation of the selected pathogenic microorganisms. The investigation disclosed that *Grewia tenax* (GT) contains substantial quantities of phenolic compounds, which were identified as the principal contributors to its antioxidant properties, and the extract exhibited low to moderate antibacterial activity. Therefore, it can be inferred that *Grewia tenax* (GT) holds the potential for the development of compounds that may facilitate the creation of novel and more efficacious anticancer therapeutics.

Key words: *Grewia tenax*, Antioxidant, Solvent extraction, TPC, DPPH, antibacterial activity.

1. Introduction

Over 50,000 plant species are utilized worldwide within the pharmaceutical and cosmeceutical sectors. Research conducted in nations such as India, Kenya, South Africa (**Koley *et al.*, 2020**), and Namibia (**Ahmed *et al.*, 2018**) has elucidated the role of medicinal plants in the management of pneumonia and other respiratory ailments, including fever, cough, asthma, fatigue, and the common cold. Within the realm of ethnomedicine, medicinal plants are typically administered to alleviate the symptoms associated with various diseases (**Shukla *et al.*, 2016**).

Medicinal plants constitute fundamental components of traditional medicinal practices across nearly all cultural contexts. Various anatomical parts of plants, including bulbs, leaves, roots, barks, peels, seeds, and flowers, are employed for therapeutic applications. The World Health Organization reports that approximately 80% of the populace in developed nations relies on traditional medicine, which encompasses bioactive compounds sourced from medicinal plants (**Heinrich *et al.*, 2021**). The plant kingdom has been established as an extensive reservoir of biologically active compounds exhibiting a diverse array of chemical structures and properties conducive to disease prevention.

The *Grewia* genus is classified within the Teliaceae family (**Kanmaz *et al.*, 2020**). *Grewia tenax* (Forssk.) Fiori (GT), commonly referred to as Guddaim or Gangeti, is a highly valued plant species found in Kachchh as well as across various regions of Africa and Southeast Asia (**Jebin *et al.*, 2019**). The leaves of *Grewia tenax* are employed as a therapeutic agent for the management of numerous ailments, including jaundice and hepatic disorders (**Dev *et al.*, 2019**). Furthermore, the leaves of *Grewia tenax* demonstrate efficacy in combating infections such as tonsillitis, trachoma, and inflammation (**Hadidi *et al.*, 2022**).

Grewia tenax (GT) is a deciduous tropical shrub or arboreal species that proliferates spontaneously in diverse semi-arid and sub-humid regions of Africa and Asia. Due to the substantial nutritional and health benefits associated with GT, it is deemed a promising candidate for domestication and commercialization as an emergent crop (**Gichuru *et al.*, 2025**). Moreover, the authors noted that GT possesses significant concentrations of potassium, calcium, zinc, magnesium, iron, and vitamins, which yield quantities of 20–30 times the recommended daily intake for these nutrients. The GT is abundant in both essential and non-essential amino acids, in addition to bioactive compounds such as sterols, polyphenols, and flavonoids (**Haile *et al.*, 2020**). The extract derived from GT demonstrated antioxidant, hematological, histopathological, hepato-protective, and lipid-lowering properties in a carbon tetrachloride-induced hepatotoxicity model in murine subjects (**Ouarhacha *et al.*, 2020**). The fruits are utilized for the creation of nutritious juice, preserves, and syrups (**Aadesariya *et al.*, 2017**). Notwithstanding the elevated nutritional quality and bioactive attributes of GT, its utilization as a stabilizing agent within food products remains limited.

Current research has indicated that there exists no universally accepted methodology for the quantitative and precise evaluation of antioxidant activity on a global scale; consequently, the antioxidant activity of plants is measured utilizing a variety of techniques. These techniques vary significantly concerning their assay principles and experimental conditions. Most of these methodologies are predicated on the examination of a reaction wherein a free radical is generated and the extent to which this reaction is inhibited by the concentration of the compound or sample that is the subject of antioxidant capacity measurement (**Ashurst *et al.*, 2020**). The impact of the antioxidant activity of the samples varies primarily due to the characteristics of the solvent employed and, to a greater extent, the methodologies utilized for investigation. The presence of numerous antioxidant constituents within the plant sample complicates the quantification of each antioxidant component individually. Consequently, in numerous investigations, multiple intermediate extractions are employed to guarantee optimal extraction of the available antioxidants (**Alburyhi *et al.*, 2025**).

A plethora of methodologies exists for the extraction of antioxidants from botanical origins and various food substances. The techniques commonly utilized include shaking, high-speed homogenization, maceration, and stirring. Nevertheless, a comprehensive empirical assessment of these methodologies remains to be documented; however, certain studies have identified the constraints associated with these techniques, which encompass suboptimal product quality, safety hazards, and

protracted extraction times (**Hmamou et al., 2022**). Recent progressions in the field have culminated in the establishment of novel extraction methodologies, encompassing microwave-assisted extraction, ultrasonic-assisted extraction, and enzyme-assisted supercritical fluid extraction techniques, which are expressly designed for the isolation of antioxidants derived from botanical sources (**Shatri, 2021**). The evaluation of antioxidant capacities of natural food components or biological systems is conducted through the application of various methodologies, which produce disparate results. In the realm of assessing antioxidant activity *in vitro*, the employment of free radicals is commonly observed, notably 2, 2-diphenyl-1-picrylhydrazyl (DPPH). In relation to a reference standard of Trolox (a water-soluble derivative of vitamin E), the free radical is generated within an aqueous environment. The measurement of the reduction of the yellow-blue DPPH radical via the action of hydrogen-donating antioxidants is accomplished by the decrease of the long-wave absorption spectrum associated with the radical (**Coin, 2023**). This methodology is conventionally referred to as the Trolox equivalent antioxidant capacity assay. It is characterized by its rapid execution and applicability within both aqueous and organic solvent matrices across a broad spectrum of pH values ranging from 5 to 7 (**Alburyhi et al., 2025**).

Chemically, *Grewia tenax* constitutes a heterogeneous assemblage of macromolecules exhibiting varied dimensions and compositions, predominantly consisting of carbohydrates and proteins. It is characterized by a high concentration of non-viscous soluble fibers that possess significant dietary benefits, in addition to containing essential minerals such as potassium, magnesium, and calcium (**Hmamou et al., 2022**). Recent investigations have underscored the antioxidant properties of *Grewia tenax*, its nephroprotective effects, as well as other physiological impacts (**Yadav, et al., 2024**), along with its beneficial implications in the management of various degenerative conditions such as renal failure (**Hamami et al., 2024**), cardiovascular disorders and gastrointestinal ailments (**Ashurst et al., 2020**). *Grewia tenax* has been historically employed for the treatment of diarrhoea, dysentery, and diabetes (**Lfitat et al., 2021**). Its applications extend to the management of diarrhoea, dysentery, and diabetes, as well as addressing dry cough associated with amoebic dysentery, serving as a tonic, antiasthmatic, analgesic, and in the treatment of lesions within the oral cavity. Moreover, additional benefits have been documented. Consequently, *Grewia tenax* holds significant promise for a multitude of advantages within the medical, food, and pharmaceutical sectors. Despite the challenges associated with data extraction concerning antioxidant activities through specialized extraction methodologies of *Grewia tenax* and its derivatives, *Grewia tenax* has the potential to serve as a valuable source of components for functional foods and related applications. (**Mehmood et al., 2020**) Should *Grewia tenax* be recognized as a competitive source of phytonutrients, such knowledge or data would be of paramount importance.

Research investigations regarding the extracts of *Grewia tenax* have demonstrated notable anti-pneumonic efficacy against prevalent bacteria responsible for pneumococcal infections, including *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Haemophilus influenzae*, and *Staphylococcus aureus*. Effective therapeutic modalities have been formulated to eliminate prevalent microbial infections. Notwithstanding these proactive strategies, the continually escalating frequency of microbial infections, typically instigated by parasites, fungi, viruses, and bacteria, presents a significant concern. A multitude of prevalent bacterial infections is attributable to organisms such as *Escherichia coli*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* (**Ouarhacha et al., 2020**). Furthermore, bacterial infections are responsible for a range of chronic illnesses and contribute substantially to global mortality rates (**Ashurst et al., 2020**). Consequently, the identification and development of antibiotics are regarded as a pivotal advancement within the medical field.

It has also been documented in the literature. The predominant bioactive constituents of *Grewia tenax* encompass phenolic acids, flavonoids, terpenoids, lignans, tannins, quinones, coumarins, and alkaloids (**Shatri, 2021**).

The antimicrobial efficacy of the ethanolic extracts derived from *Grewia tenax* (GT) has been observed against various pathogens, including *St. pneumoniae*, *S. aureus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *C. albicans*.

Grewia tenax (GT), is not predominantly acknowledged for its substantial antimicrobial properties; nonetheless, it has been employed as a quintessential material for the environmentally friendly synthesis of nanoparticles and nano-fiber scaffolds, which exhibit applications in infection management due to their extensive antimicrobial efficacy (Cao *et al.*, 2020; Lfitat *et al.*, 2021). Owing to attributes such as biodegradability, non-toxicity, non-mutagenicity, natural abundance, enhanced resistance to microbial assaults, and extended shelf life, GT serve as optimal materials for the nanosynthesis of microcapsules encapsulating herbal extracts (Mehmood *et al.*, 2020).

In this study, we focused on calculating the total content of the phenolic compounds in the methanol extract of the *Grewia tenax* (GT), as well as identifying antioxidant by way 2, 2-diphenyl-1-picrylhydrazyl (DPPH). The antibacterial concentrations were evaluated against six distinct bacterial species. Extracts were investigated for their potential utility as sources of antioxidants as well as antibacterial agents that may be employed to mitigate food spoilage.

2. Materials and Methods

2.1. *Grewia tenax* (GT) Samples

Grewia tenax (GT) was obtained from a local market in Libya. Following the removal of impurities and sand, the samples were subjected to a random selection process to ensure homogeneity among the nodules of GT. Subsequently, the material was ground into a fine mechanical powder using a testing sieve from Fisher Company, adhering to USA standards, with a mesh size of 1.40 mm. The resultant powder was then securely stored in airtight polyethylene bags within a desiccator.

2.2. Chemicals and equipment used

In this study, analytical-grade methanol (95 %, PSPARK Chemical) was used. The DPPH free radical scavenging assay employed 2, 2-diphenyl-1-picrylhydrazyl and ascorbic acid (both from BHD Chemical). For the determination of total phenolic content, sodium carbonate and Folin–Ciocalteu reagent (Chem King) were used. Absorbance measurements were carried out using an Italy 40 40 spectrophotometer and a Biotek ELISA reader (Germany).

2.3. Extraction method

The authentication of *Grewia tenax* (GT) was conducted by Professor A.M. Amletan, who serves as the Head of the Botany Department at Misurata University, Libya, ensuring its unequivocal identification. The cold extraction technique, specifically soaking, was employed, wherein the sample was immersed in a beaker containing a minimal volume of solvent adequate to submerge it (Shatri, 2021). A quantity of 5 grams of the extract was measured utilizing (Glossaries DHAUS) and subsequently immersed in 100 milliliters of cold methanol; the selection of methanol was predicated on its efficacy as a polar solvent for the dissolution of phenolic compounds present in GT. It was observed that polar extracts exhibited superior free radical scavenging capabilities in comparison to their less polar counterparts. The container was subsequently positioned on a magnetic stirrer (Model: RT 15P; Serial: 2 930 700). Following this, the samples were allowed to rotate for a duration of 24 hours. Additionally, the clarified suspension was filtered through a Sartorius PTFE 0.45 µm membrane filter. During filtration, the beaker was wrapped in aluminum foil to prevent spillage and minimize light exposure. The resulting filtrate was stored at ambient temperature until the solvent had completely evaporated. The extraction yield was expressed as a percentage of the initial powder mass. The precipitate was refrigerated until further analysis.

2.4. Antioxidant assays

2.4.1. Determination of Total Phenolic Content (TPC) Using the Folin–Ciocalteu Method

The total phenolic content (TPC) was quantified using the Folin–Ciocalteu index (FCI) assay, with minor modifications; the employed methodology is in accordance with the protocol specified by (Yadav *et al.*, 2024) An amount of 1 mg of *Grewia tenax* (GT) extract was accurately measured and subsequently dissolved in 1 ml of high-purity methanol. Samples were subsequently diluted with

methanol to create five specific concentrations: 5000, 4000, 3000, 2000, and 1000 µg/ml. For each concentration, 0.1 mL of the sample extract (1 mg/mL) was mixed with 1.5 mL of 10 % (v/v) Folin–Ciocalteu reagent, which was subsequently mixed thoroughly and allowed to equilibrate for a period of 5 minutes. Following this, 1.2 ml (7.5%) of sodium carbonate (Na₂CO₃) (w/v) was incorporated, and the resulting mixture was permitted to rest for 30 minutes in a controlled dark environment. The absorbance was then quantified at a wavelength of 765 nm using a UVD-3500 spectrophotometer after an elapsed time of 2 hours. The findings were subsequently recorded in terms of milligrams of gallic acid equivalent. A calibration curve was constructed using various concentrations of gallic acid. The measured absorbance values were then converted to gallic acid equivalents (GAE) and expressed as milligrams of GAE per gram of dry extract (mg GAE/g extract).

2.4.2 DPPH radical-scavenging activity

The efficacy of the extract in scavenging the stable DPPH free radical was quantitatively assessed (Cao *et al.*, 2020; Lfitat *et al.*, 2021). DPPH was employed to evaluate the proton radical scavenging capability of *Grewia tenax* (GT) extracts, due to its possession of a proton free radical and its distinctive absorbance characteristics at 517 nm. The DPPH compound is characterized as a purple-black solid material. This radical demonstrates stability, and its antioxidant potential is quantified by measuring the IC₅₀ value, which is defined as the concentration of the extract necessary to inhibit 50% of the DPPH radical.

A volume of 0.1 ml from each specified concentration was diligently amalgamated with 2.9 ml of a pre-prepared 0.1 mM DPPH solution. The control group was constituted by the addition of 0.1 ml of the extract in conjunction with 2.9 ml of the DPPH solution. The negative control was established by the integration of 0.1 ml of methanol with 2.9 ml of the DPPH solution, which was afterward subjected to a 30-minute incubation period in a darkened environment. Absorbance measurements were acquired at a wavelength of 517 nm utilizing a spectrophotometer following an elapsed time of 2 hours. The results were documented in terms of milligrams of ascorbic acid equivalent. Methanol functioned as the baseline solution, and the absorbance of the DPPH solution was also evaluated in the absence of the extract, with the I% inhibition rate for the free radical DPPH computed in accordance with the following equation (Shatri, 2021):

Radical scavenging activity (RSA %) = (Control absorbance – Sample absorbance / control absorbance) × 100

2.5. Determination of antibacterial activity of *Grewia tenax* (GT) extracts

Evaluation of Antibacterial Activity

With a few minor modifications, the cup-plate agar diffusion technique was employed to assess the antibacterial efficacy. The agar that had undergone incubation was segregated into two distinct groups, and 20 ml aliquots were subsequently transferred into sterile Petri dishes. The agar was permitted to solidify within the six cups allocated to each group, each possessing a diameter of 10 mm and excised utilizing a sterile corn borer (No. 4). Each of the halves was specifically tailored for one of the experimental compounds. Distinct Petri dishes were established for the reference antibacterial chemotherapeutic agent. Subsequent to the extraction of the agar discs, 0.1 ml aliquots of each extract along with the pure complexes were dispensed into alternating wells utilizing an adjustable volume micro titer pipette, and diffusion was permitted at ambient temperature for a duration of two hours. The plates were subsequently maintained in an upright orientation at a controlled temperature of 37°C for a period of 24 hours. This protocol was replicated for varying concentrations of the complexes alongside the standard antibacterial chemotherapeutic agent. Upon completion of the incubation period, the zones of growth inhibition that emerged were quantified, in addition to the assessment of their average diameter (Osman *et al.*, 2022).

2.6. Statistical analysis

Statistical analyses were performed using Microsoft Excel 2013.

3. Results and Discussion

3.1. Extraction yields

The yield of extraction is contingent upon the solvents utilized, the duration and thermal conditions of the extraction process, as well as the intrinsic chemical characteristics of the sample being analyzed. Given equivalent temporal and thermal parameters, the choice of solvent in conjunction with the chemical properties of the sample emerges as the two preeminent factors influencing extraction efficacy (**Hmamou *et al.*, 2022**). The solvents that have been empirically recommended for effective extraction comprise aqueous blends of methanol, ethanol, and acetone (**Barberis *et al.*, 2020**). In the current investigation, the extraction yield achieved utilizing methanol as the solvent was quantified at a percentage of 4.62%.

3.2. Total Phenolic Contents (TPC)

The assessment of antioxidant activities was conducted utilizing methanol extraction and quantified as total phenolic content (TPC). The Folin-Ciocalteu reagent methodology was employed, with gallic acid serving as a positive control, indicating a total phenolic content ranging from approximately 1076.5 to 4841.5 mg GAE/g of extract. The findings presented in Table 1 elucidated that the GT extraction contained a substantial concentration of phenolic compounds, which serve to inhibit the advancement of chain oxidation reactions either through the donation of hydrogen atoms or by chelating metal ions. Consequently, these entities act as reductants and antioxidants (**Yadav *et al.*, 2024**); the assays were performed utilizing the complete extract, as this methodology may present superior benefits in comparison to isolated components, owing to the observation that a bioactive singular constituent can demonstrate modifications in its characteristics when engaging with other compounds that are present within the extract (**Nisar, 2021**).

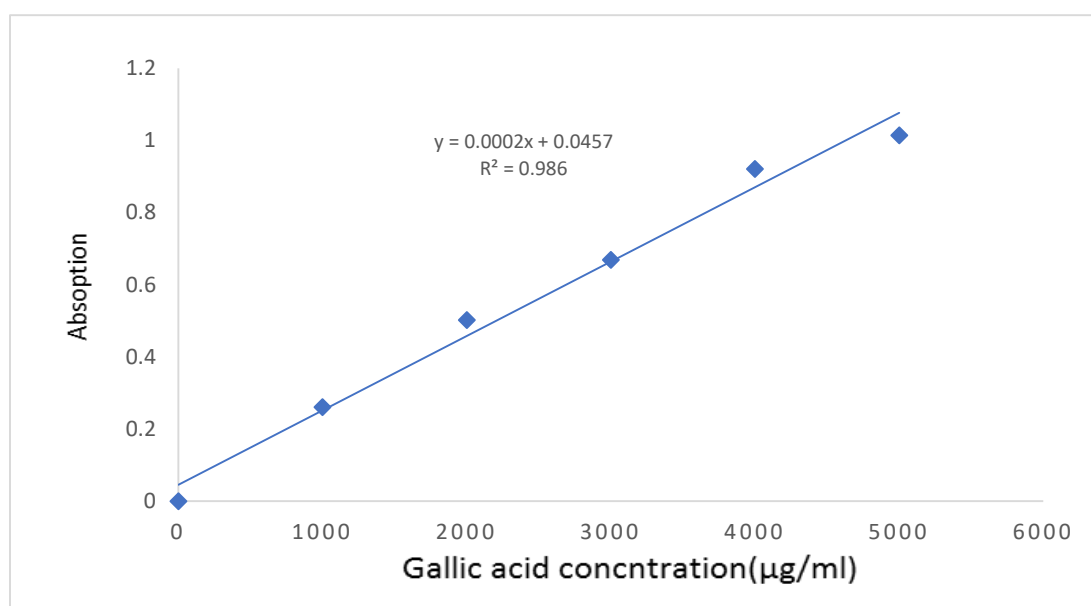
Initially, a calibration curve was established utilizing diverse concentrations of gallic acid (figure 1). Subsequently, extract samples were formulated at varying concentrations (5000, 4000, 3000, 2000, 1000 μ M). At the concentration of 5000 μ M, the samples exhibited significantly elevated values of the total phenolic content (TPC), yielding a value of 4841.5 mg GAE/g of extract. Conversely, the TPC value at the concentration of 1000 μ M for the samples revealed a minimal value of 1076.5 mg GAE/g of extract. The findings suggested that the values of the crude extract displayed a descending trend, with 5000 μ M preceding 1000 μ M, respectively. The results of the investigation provide compelling evidence that phenolic compounds constitute critical constituents of *Grewia tenax* (GT), and specific pharmacological effects may be attributed to the presence of this pivotal element. Through the course of our research, we observed that an increase in the concentration of the extract correlates positively with the quantity of phenolic compounds it encompasses.

The results derived from this investigation demonstrate a significant alignment with a multitude of previously established empirical observations. This indicates that phenolic compounds typically demonstrate enhanced solubility in polar organic solvents as opposed to aqueous environments. Moreover, the results of this investigation align with those documented by other scholars, signifying that among the solvents assessed, the 50% methanol extract produced the highest concentration of total phenolics from *Grewia tenax* (**Hussein *et al.*, 2022**). The empirical evidence presented by the researchers suggests that both ethanol and methanol demonstrate superior efficacy in the extraction of total phenolics from *Grewia tenax* compared to water (**Shatri, 2021**). Moreover, in the evaluation of total phenolic content through the Folin assay, the methanolic crude extract of *Grewia tenax* (GT) samples exhibited an exceptionally elevated phenolic content of approximately (250 $\pm$ 2 mg GAE/g), while the water extract yielded a phenolic content of (192 $\pm$ 2 mg GAE/g) for *Grewia tenax* (GT) (Coin, 2023). This investigation corroborated the health-promoting attributes linked to the presence of phenolic compounds in *Grewia tenax* (GT).

Table 1 shows a dose-dependent increase in total phenolic content (TPC), with the highest TPC value observed at 5000 μ g/mL and the lowest at 1000 μ g/mL.

Table (1). Total Phenolic Contents (TPC) and Extract Yield of *Grewia tenax* (GT)

Concentration(($\mu\text{g/ml}$)	Total phenolic Contents (TPC) mg GAE/g of extract	Extract yield (%)
5000	4841.5	4.62%
4000	4376.5	
3000	3116.5	
2000	2281.5	
1000	1076.5	

**Figure (1). Standard gallic acid solution ($\mu\text{g/ml}$)**

3.3. DPPH radical-scavenging activity

Numerous methodologies exist for evaluating the antioxidant activity of both synthetic and natural compounds. Among these, the DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay is a rapid, cost-effective, and widely used method for assessing the antioxidant potential of various natural sources. The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay is extensively employed to assess the free radical scavenging potential of botanical extracts, attributable to its high sensitivity, methodological simplicity, rapid execution, and widespread accessibility in the majority of chemical laboratories. Antioxidants possess the capability to neutralize free radicals through the donation of hydrogen atoms or electrons, which subsequently leads to the reduction of the stable DPPH radical to its non-radical state, resulting in a quantifiable decrease in absorbance at a wavelength of 515 nm. The IC₅₀ value serves as an indicator of the concentration of the sample at which the percentage of inhibition reaches the threshold of 50%. Therefore, IC₅₀ values exhibit an inverse relationship with antioxidant activity; a lower IC₅₀ value signifies an enhancement in the antioxidant effectiveness of the sample being assessed. Table (2) elucidates the DPPH radical scavenging capability of the methanolic extract derived from *Grewia tenax* (GT). The IC₅₀ value of the extract was ascertained to be 4379.43 $\mu\text{g/ml}$ (Figure 2), thereby signifying considerable antioxidant characteristics. This observed efficacy may be ascribed to the presence of phenolic constituents. In contrast, the IC₅₀ value for the standard reference compound, ascorbic acid, was documented at 4088.93 $\mu\text{g/ml}$ (Figure 3).

Previous investigations concerning the antioxidant properties of the *Grewia tenax* extract utilizing various solvents are consistent with the findings of the current study, particularly highlighting that the methanol extract exhibited a pronounced level of antioxidants, with the peak DPPH value being recorded in the methanol fraction (MF) at IC₅₀ values ranging from $14.5 \pm 0.7 \mu\text{g/mL}$ for the twig extracts to $179 \pm 2 \mu\text{g/mL}$ for the root extracts, respectively. In comparison, *Grewia tenax* (GT) demonstrated contrasting results with the acetone fraction (AF) yielding IC₅₀ values of $88.4 \pm 0.9 \mu\text{g/mL}$ and $268 \pm 2 \mu\text{g/mL}$ for the twig and root extracts, respectively (Coin, 2023), indicating no statistically significant differences.

Due to the insufficient data pertaining to the DPPH values of GT extract and its respective fractions, it is posited that the methanolic extract of GT and its fractions may exhibit anti-radical scavenging properties (Hussein *et al.*, 2022). Consequently, there is an urgent necessity for further investigations concerning antioxidant assays in *Grewia tenax* (GT).

Table 2 delineates the dose-dependent augmentation of antioxidant activity, with the maximum proportion of antioxidants recorded at 5000 $\mu\text{g/mL}$ and the minimal proportion noted at 1000 $\mu\text{g/mL}$.

Table (2). DPPH radical scavenging activity of methanolic extract of *Grewia tenax* (GT) and ascorbic acid

Concentration $\mu\text{g/ml}$	DHPP	Control Abs	Sample Abs	RSA% Sample	RSA% Control
5000	0.741	0.302	0.321	56.57	59.16
4000	0.619	0.297	0.329	46.77	51.86
3000	0.425	0.273	0.279	34.31	35.62
2000	0.295	0.219	0.231	21.47	26.61
1000	0.125	0.110	0.108	13.48	12.98
IC ₅₀ ($\mu\text{g/ml}$)				4379.43	4088.93

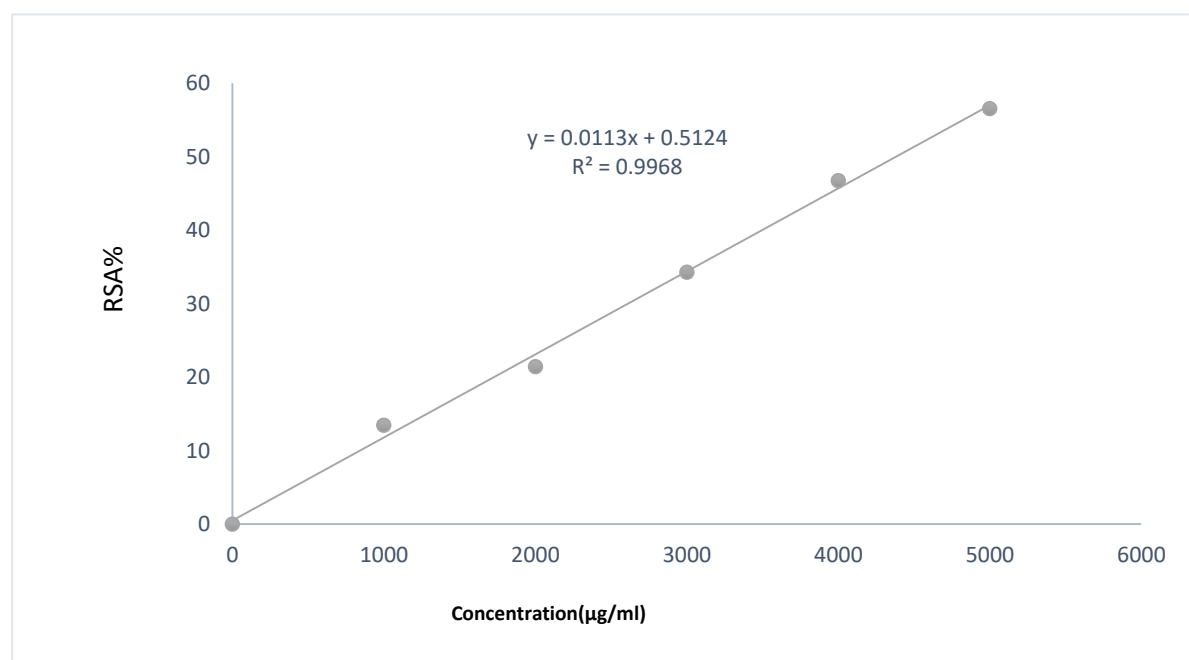


Figure (2). DPPH scavenging activity of methanolic extract of *Grewia tenax* (GT)

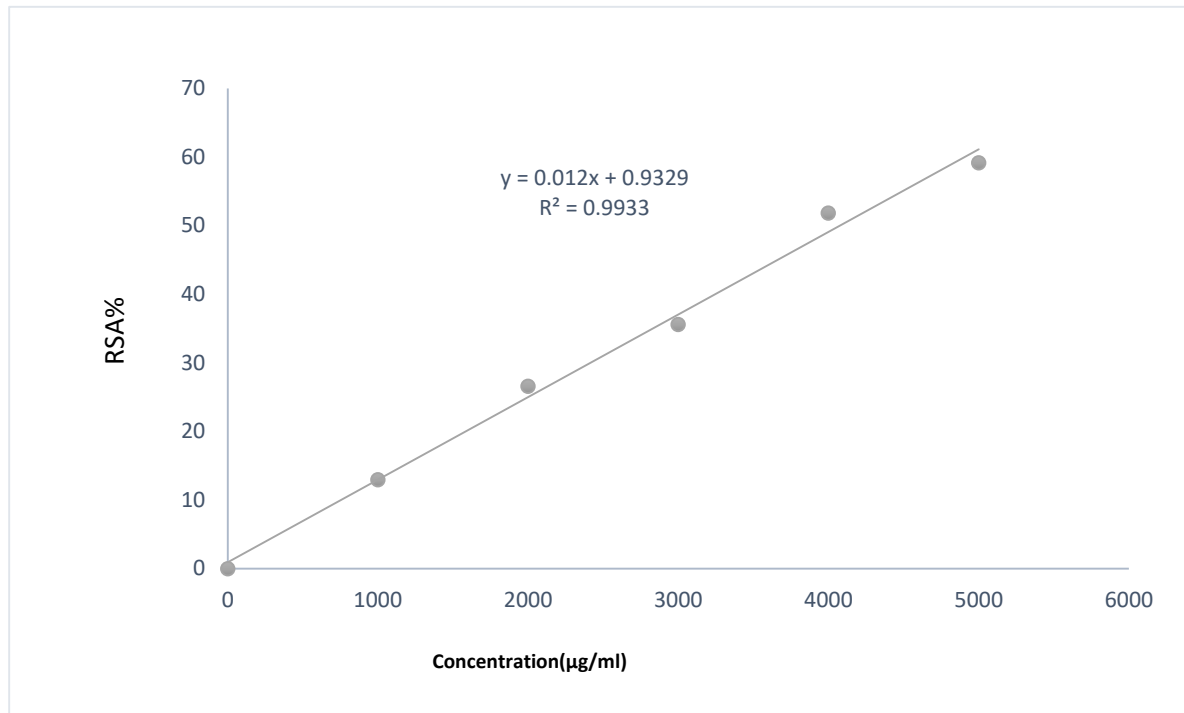


Figure (3). DPPH scavenging activity of ascorbic acid

3.4. Antibacterial Studies

The antibacterial activity of the extract was evaluated against six bacterial species: *Staphylococcus aureus*, *Streptococcus* spp., *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., and *Proteus* spp., using a range of concentrations (5000, 4000, 3000, 2000, and 1000 µg/mL). At a concentration of 1000 µg/ml, the extract demonstrated a favorable impact on two distinct bacterial strains, specifically *Staphylococcus aureus* and *Streptococcus* spp. At a concentration of 2000 µg/ml, it exhibited a beneficial effect on four bacterial types, namely *Staphylococcus aureus*, *Streptococcus* spp, *Escherichia coli*, and *Klebsiella* spp. When tested at a concentration of 3000 µg/ml, the extract revealed a markedly positive effect across all bacterial strains. Regarding concentrations of 4000 and 5000 µg/ml, these levels manifested the most pronounced positive effects on all bacterial types. The findings obtained were in alignment with results reported by researchers in the literature (**Rajput *et al.*, 2023**). Through the course of our investigation, we observed that an increase in the concentration of the extract corresponded to an amplification of its antibacterial efficacy.

Twig extract GT exhibited relatively high activity compared to root extract GT against all test organisms except with *P. aeruginosa* and *R. oryzae*. This observation was attributed to elevated levels of flavonoids in the twig extract (**Coin, 2023**).

From literature, susceptibility test results for stigmasterol (100 µg/mL) showed microbial growth inhibition with 23 mm to 30 mm zone of inhibition against *MRSA*, *S. aureus*, *S. faecalis*, *E. coli*, *P. fluorescens*, *C. albicans*, *C. krusei* (**Yusuf *et al.*, 2018**). The observation made corroborate finding made by (**Rakholiya & Chanda, 2014**) where Gram-negative bacterial strains displayed high susceptibility towards the *M. charantia* extracts (aerial part, peel, pulp and seed) compared to Gram-positive bacteria. *S. aureus* was the most susceptible Gram-positive bacteria.

Table 3 delineates the dose-responsive augmentation of antibacterial efficacy, with the maximum inhibition recorded at 5000 µg/ml and the minimal inhibition identified at 1000 µg/ml.

Table (3). Antibacterial Activities of methanolic extract of *Grewia tenax* (GT)

Concentration µg/ml	<i>Staph Aureus</i>		<i>Streptococcus</i> spp		<i>E. coli</i>		<i>Klebsiella</i> spp		<i>Pseudomonas</i> spp		<i>Protues spp</i>	
	A*	%	A*	%	A*	%	A*	%	A*	%	A*	%
5000	++	50	++	45	+	30	+	35	++	55	+	35
4000	++	45	++	55	+	25	+	30	++	45	+	25
3000	++	40	++	40	+	25	+	20	++	40	+	20
2000	+	30	+	30	+	20	+	25	-	30	-	20
1000	+	25	+	25	-	5	-	5	-	10	-	10

Percentage of Inhibition: Below 20% = (-) low active, 20% – 40% = (+) Active, 40% – 60% = (++) mildly active & 60% – 80% = (+++) moderately active, (80%, up) = (++++ highly active, *Activity

4. Conclusion

Grewia tenax (GT) constitutes a significant medicinal resource that has historically been employed in various therapeutic applications. It shows pharmacological traits akin to those of a distinguished aromatic succulent herb that lives for years. In addition, the (GT) exhibited remarkable antioxidant and antimicrobial characteristics. The present study underscores the prospective utilization of GT extracts as a source of antioxidants. Moreover, it intends to evaluate the total phenolic content present in the (GT) and its antibacterial properties. The phenolic concentration was determined to lie within the range of 1076.5 – 4841.5 mg GAE/g of extract. The assessment of antioxidant activity was performed utilizing the DPPH assay methodology. The IC₅₀ value was established to be 4379.43 µg/ml. In conclusion, the research demonstrated that *Grewia tenax* (GT) encompasses a substantial amount of phenolic compounds, which were identified as the primary contributors to its antioxidant and antibacterial properties.

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