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Extraction of Bioactive Compounds from Different Plants and Evaluation of Their Biological Effects

This study investigates the chemical composition, optical properties, and antibacterial potential of Beetroot, Cinnamon, and Pomegranate extracts using water and alcohol as solvents. X-ray fluorescence (XRF), UV-Visible absorption spectroscopy, Fourier-transform infrared (FTIR) analysis, and bandgap determination were employed to characterize the elemental and molecular composition of the plant extracts where the bioactive compounds were detected. Additionally, antibacterial activity was assessed against *Escherichia coli* and *Pseudomonas aeruginosa* to evaluate their potential as natural antimicrobial agents. The study highlights the influence of solvent choice, extracted bioactive compounds and the type of the plants on the extraction efficiency of bioactive compounds and the functional properties of the extracts. These findings provide insights into the development of plant-based bioactive compounds and natural antimicrobial formulations, emphasizing the role of extraction techniques in enhancing the utility of phytochemicals for food, pharmaceutical, and biomedical applications.

Keywords: Bioactive materials; UV-visible spectrometry; Beetroot dye; Antibacterial activity
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1. Introduction

Antimicrobial drug resistance among pathogenic bacteria has emerged as a major global health challenge [1]. Among the most concerning resistant organisms are Escherichia coli and Pseudomonas aeruginosa, both of which are responsible for a wide range of opportunistic and hospital-acquired infections that are increasingly difficult to treat due to their resistance mechanisms [2,3]. The search for effective and safe alternatives to conventional antibiotics has therefore increased, with growing interest in plant-derived extracts as natural antimicrobial agents [4]. Plants produce a diverse range of bioactive secondary metabolites, including phenolics, flavonoids, alkaloids, and tannins, many of which exhibit antimicrobial and antioxidant properties [5]. In this context, pomegranate, cinnamon, and beetroot have attracted considerable scientific attention. Pomegranate peel and leaves are particularly rich in phenolic compounds such as ellagic acid, punicalagin, and anthocyanins, which contribute to their strong antimicrobial and antioxidant potential [6,7]. Cinnamon contains significant levels of polyphenols, phenolic acids, and proanthocyanidins that display antimicrobial, antifungal, and anti-inflammatory effects [8,9]. Beetroot is another plant of interest due to its high content of betalains and phenolic compounds that possess antioxidant and antimicrobial activities [10,11]. The extraction solvent plays a critical role in determining the yield and composition of bioactive compounds. Solvent polarity directly influences the solubility and extraction efficiency of different phytochemical groups [12]. Aqueous solvents are effective in extracting polar compounds such as sugars and glycosides, while alcoholic solvents (e.g., ethanol, methanol) are better suited for extracting less polar compounds like flavonoids and phenolic acids [13]. Therefore, comparing extracts obtained using solvents of different polarities allows for a better understanding of how solvent choice affects the recovery and biological activity of plant constituents. This study aims to evaluate the antibacterial activity of aqueous and alcoholic extracts of pomegranate, cinnamon, and beetroot against *E. coli* and *P. aeruginosa*, in order to identify the most effective plant-solvent combination for inhibiting these clinically relevant bacteria. The aim of this study was to investigate how solvent choice (water vs. alcohol) influences the extraction of bioactive compounds from selected plants, with special emphasis on determining whether water-based extracts provide more effective functional group recovery and antibacterial performance compared to alcohol-based extract solutions. Water-based extract solution is considered ideal for developing safe, accessible, and multifunctional health products. While organic solvents often yield broader compound profiles, aqueous solutions are safer for food and pharma applications. The main aim of this study is using the extracted solutions for the antibacterial activity against these bacteria.

2. Extraction Process

The plant materials (beetroot, cinnamon and pomegranate) were dried and milled into a fine powder to increase the surface area and improve extraction. A measured amount of the powder was placed inside a piece of filter paper and then put into the soxhlet extraction chamber. The chosen solvent (ethanol and water) was poured into the round-bottom flask of the soxhlet apparatus. When the flask was gently heated, the solvent boiled and turned into vapor. The vapor rose up to the condenser, where it cooled down and turned back into liquid. This liquid solvent then dripped onto the plant powder in the chamber, dissolving the desired compounds. Once the chamber filled with solvent, the liquid containing the extracted compounds flowed back into the flask. This cycle repeated automatically for 4 to 8 hours to fully extract the compounds. After the extraction was finished, the heat was turned off and the system was allowed to cool. The solvent with the extracted compounds was removed from the flask. Finally, the extract solutions were stored for further use or analysis. Figure (1) demonstrate the extraction procedure for these solutions, the final solution concentration is 0.375 mg/ml.

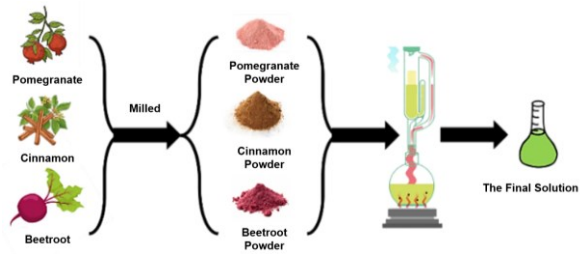

Fig. (1) The extraction procedure of Beetroot, Cinnamon and Pomegranate solutions using water and alcohol, separately

3. Results and Discussions

The elemental analysis of the studied Beetroot, Cinnamon and Pomegranate solution were depicted using X-ray fluorescence (XRF), the elemental traces which were extracted are illustrated in tables (1) and (2); water and alcohol were used as solvents to dissolve these plants and extracted their solution based bioactive compounds. Heavy metal (toxic trace elements) including Cr, As, Mn, Ni, Cu, Zn, Cd and Co extracted from Beetroot, Cinnamon and Pomegranate in both water and Alcohol based solution were observed. However, these essential heavy metals are toxic in some ranges, they have also significant roles in physiological processes of living organisms [14]. Heavy metal pollution in agricultural soils is a major global concern, as these metals can accumulate in crops, posing risks to human and animal health [14].

Heavy metals become toxic to humans when they accumulate in the body as nano-toxic materials, disrupting cellular functions. Toxicity may lead to fatigue, organ damage, nervous system issues, allergies, and in some cases, cancer [23]. The main observation in this test is that the elemental composition for each sample is varied based on the solvent type; some of the observed elements may related to the fertilized soil [24], such as alumina (Al_2O_3), silica (SiO_2) and sulfate (SO_4) with different concentrations. Most heavy metals are recognized as carcinogenic agents [25], while certain metals, such as copper (Cu) and zinc (Zn), play vital roles as enzymatic cofactors in essential intracellular processes and are components of DNA-binding proteins; the majority of heavy metals have been associated with the development of various cancers and other diseases [26]. Furthermore, Arsenic (As), cadmium (Cd), chromium (Cr), and nickel (Ni) are classified as group one carcinogenic heavy metals by the International Agency for Research on Cancer (IARC) [27].

Numerous studies have demonstrated that exposure to these heavy metals can disrupt tumor suppressor gene expression, impair DNA repair mechanisms, and interfere with metabolic enzyme functions through oxidative stress-induced membrane [28]. However, the significance of these elemental traces in the current study can be neglected due to their low concentrations in the extracted solutions. Additionally, research indicates that the health risks associated with heavy metal exposure are closely linked to the source of contamination [29]. Moreover, elements such as CoO and MnO_2 are known as Carcinogen, toxic [22]. The concentrations of the heavy metals mentioned in table (1) are relatively low in both water and alcohol-based solutions. However, other elements show higher concentration such as Fe, P, K, Ca and Ag as shown in table (2). Ag_2O are considered toxic in excess [30], it produced through biological methods offers several benefits, including low synthesis cost, strong antimicrobial activity, minimal cytotoxicity to mammalian cells, and potential applications in pharmacology and biomedicine comparable to nanoparticles (NPs) synthesized by conventional techniques [31]. Like silver nanoparticles, the green synthesis of Ag_2O using therapeutic plant extracts is an effective approach to enhance their antimicrobial properties [23]. The higher concentration of Ag traces is believed to have great impact in the biological effect. Other elemental traces such as iron (Fe) is essential for blood but it considers toxic in excess [32]. This trace exhibits as hematite compound with higher concentration of 502.7 ppm in water-based Beetroot compare to alcohol-based solution and other samples. Usually, Beetroot has a higher concentration of iron than many other vegetables [33]. However, in alcohol-based solution, hematite concentration is dramatically decreased which is attributed to the solubility of iron compounds which is typically more soluble in water [34]. The main function of phosphorus (P) in human body is in the formation of bones and teeth [35]. It plays an important role in how the body uses carbohydrates

and fats. It is also needed for the body to make protein for the growth, maintenance, and repair of cells and tissues. Beetroot as water-based solution shows a very high phosphorus with the concentration of 724.3 ppm compare to other solutions, indicating strong nutritional value. Water-based Beetroot solution, also shows higher potassium (K) content with the concentration of 12120 ppm compare to other solutions; this is essential for several biological function [36]. Moreover, significant calcium (Ca) content of 2400 ppm is observed in water-based Beetroot solution which is likely from processing or plant structure. Calcium and phosphorus, are two of the most abundant minerals in the body, play key roles in vital physiological functions. Maintaining balanced levels of both is essential for preventing musculoskeletal issues and other health problems, particularly in older adults [37]. The concentrations of extracted trace elements are very low (ppm range) and are therefore unlikely to independently account for the observed antibacterial activity. Their contribution is more reasonably synergetic, enhancing the effects of the primary bioactive compounds rather than acting as dominant antimicrobial agents. Trace metal oxides can facilitate reactive oxygen species (ROS) generation, protein oxidation, and cell membrane destabilization, which increase bacterial susceptibility to other active compounds [38,39].

In this study, the ions and traces quantities within a complex organic matrix suggesting that their effect is likely secondary or catalytic. The main antibacterial activity is attributed to plant-derived phytochemicals, such as cinnamaldehyde, punicalagin and betalains in. These compounds have well-documented mechanisms, including membrane disruption, enzyme inhibition, protein denaturation, and interference with bacterial DNA and RNA synthesis [40,41]. Therefore, we propose that the observed antibacterial effect primarily arises from these major phytochemicals, with trace metal oxides potentially contributing a minor synergistic enhancement through oxidative or membrane-mediated mechanisms.

To evaluate the optical properties of the extracted solutions from the Beetroot, Cinnamon and Pomegranate solutions, UV-Visible absorption spectroscopy is carried out and results are demonstrated in Fig. (2). For the water-based solution shown in Fig. (2A), the absorption peak of the Beetroot-water based solution at short UV wavelengths is likely due to the presence of betalains, especially betanin which is the most common betacyanin is betanin (Beetroot Red) [42]. This contains conjugated double bonds and chromophores that strongly absorb UV light. Betalains are water-soluble, nitrogen-containing pigments found in plants, primarily those in the order caryophyllales [42].

Such high absorbance makes beetroot suitable for UV-blocking or antioxidant-related applications. The beetroot water-based solution delivers a high amount of bio accessible antioxidants and may be a cost effective and convenient method of increasing antioxidant status. Cinnamon also shows significant UV absorbance, peaking a little later at around 200 nm, this peak suggests strong $\pi \rightarrow \pi^*$ transitions in UV range and attributed to its aromatic and phenolic compounds, especially cinnamaldehyde [43]. This peak is a signature of antioxidant and bioactive constituents in cinnamon extract. Another peak around 280 nm is also observed in the cinnamon which is attributed to cinnamaldehyde and other phenolic [43]. Cinnamaldehyde is an antibacterial through multiple mechanisms which vary according to the pathogen [44]. Moreover, Pomegranate displays a broader, flatter spectrum with lower absorbance around (~280 nm) which could be related to ellagitannins unique of Punica botanical gender and to a pattern of anthocyanin, typical of pomegranate [45].

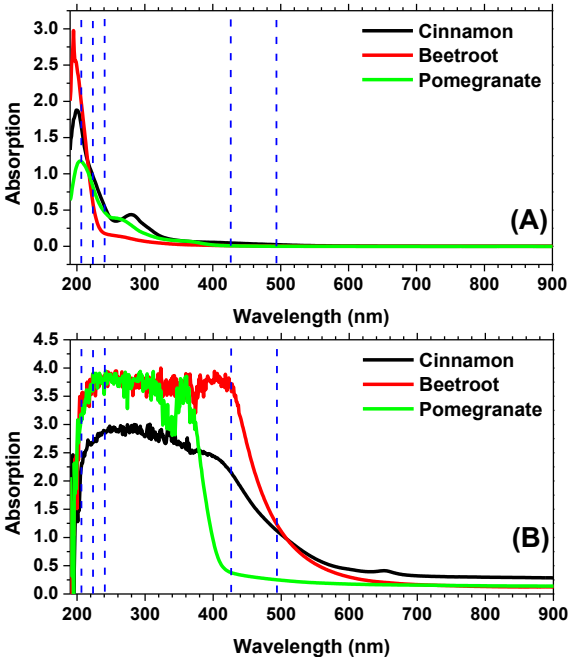

Fig. (2) UV-Visible absorption spectra of the Beetroot, Pomegranate and Cinnamon plants in (A) Water and (B) Alcohol, based solutions

On the other hand, for the Alcohol based solutions shown in Fig. (2B), the absorption peaks for all samples demonstrate different features with broader bands extending up to 500 nm with red-shifted and less sharp; these characteristics often associated with surface plasmonics resonance (SPR). The change in the dielectric environment surrounding the nanoparticles shifts the plasmonics resonance toward longer wavelengths [46]. Also, these differences may arise due to solvent effects on the electronic transitions of plant extract compounds. Solvent polarity and hydrogen bonding ability significantly influence UV-visible spectra [47]. Water is a highly polar protic solvent

(strong hydrogen bonding capacity), whereas alcohol is less polar but still protic, with moderate hydrogen bonding [48]. In water-based solution (Fig. 2A), strong hydrogen bonding stabilizes ground states more than excited states lead to blue-shift (hypsochromic shift). On the other hand, in Alcohol bases solution (Fig. 2B), weaker hydrogen bonding than water resulting in less stabilization of ground states compared to water and causes red-shift (bathochromic shift). The observed spectral shifts between water and alcohol extracts demonstrate the influence of solvent polarity on compound selectivity. Polar solvents like water favor extraction of hydrophilic phenolic compounds, whereas less polar solvents such as alcohol preferentially extract hydrophobic bioactive compounds [49].

Calculating the bandgap for non-crystalline plant extracts provides insight into their electronic structure, light absorption properties, and potential photochemical or biological activity. Although bandgap is traditionally used for crystalline semiconductors, in these extracts it reflects the average energy required to excite electrons from occupied to unoccupied molecular orbitals of the main bioactive compounds. The Tauc plot is appropriate here, as it effectively estimates the onset of optical transitions in disordered or molecular systems, allowing quantitative comparison of extracts and prediction of their functional behavior. Lower bandgap values indicate molecules with extended conjugation or electron-donating groups, which absorb longer-wavelength light and may exhibit higher photochemical or biological activity [50].

The optical bandgap (E_g) likely corresponds to the energy difference between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) of the dominant chromophores' compounds in the extract in the water and alcohol-based solutions. It was measured by plotting $(\alpha h\nu)^2$ vs. $(h\nu)$ photon energy using Tauc's equation: $(\alpha h\nu)^n = \alpha h\nu - E_g$ [51]. Figure (2) shows the Tauc's relation to evaluate bandgaps for the extracted solutions. Cinnamon shows an E_g of 5.2 eV in water (Fig. 3A) and this has reduced to 2.6 eV in alcohol (Fig. 3D), while Beetroot shows an E_g of 5.6 eV in water (Fig. 3B) and reduced to 2.62 eV in alcohol (Fig. 3E). Furthermore, Pomegranate shows E_g of 5.25 eV in water (Fig. 3C) and reduced to 3.1 eV in alcohol (Fig. 3F). This reduction in the alcohol-based solutions is attributed to the enhanced solubility and dispersion in alcohol compared to water which changes the electronic environment due to different solvent polarities resulting in aggregation or molecular interaction differences in alcohol [52].

The FTIR spectra of the extracted solutions displayed several characteristic absorption bands corresponding to functional groups commonly present in plant-derived compounds are presented in Fig. (4), and the corresponding vibration peaks are presented in table (3).

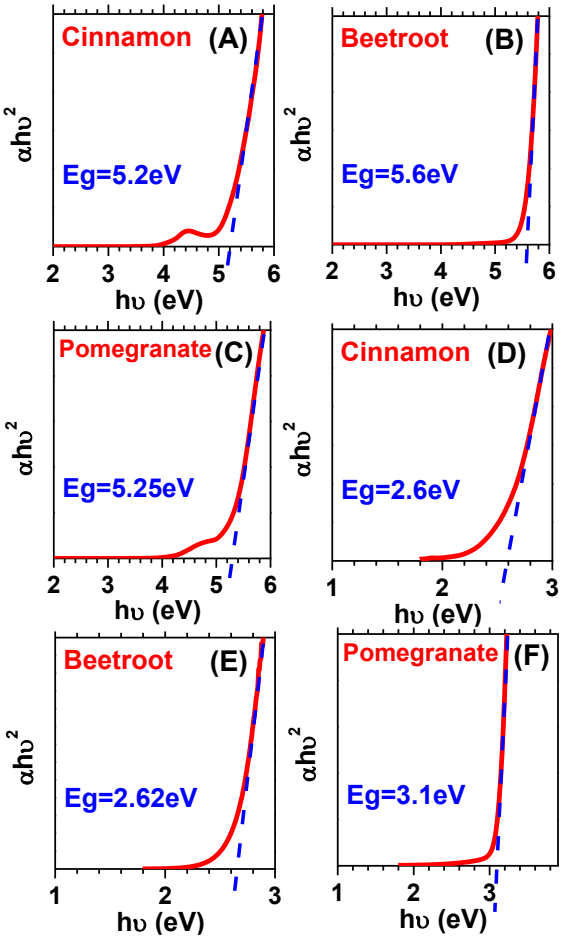

Fig. (3) Tauc relation for the water-based solution, (A) Cinnamon (water), (B) Beetroot (water), (C) Pomegranate (water), (D) Cinnamon (alcohol), (E) Beetroot (alcohol) and (F) Pomegranate (alcohol)

Table (3) FTIR functional groups for the Beetroot, Cinnamon and Pomegranate as water and alcohol-based solutions

Wavenumber (cm^{-1})	Vibration Type	Bond
3300–3400	Stretching (broad)	O-H / N-H
2900–2950	Stretching	C-H
1600–1700	Stretching	C=O / C=C
1400–1450	Bending (scissoring)	C-H
1200–1300	Stretching	C-O
1000–1100	Stretching	C-O / C-N
600–650	Out-of-plane bending	Aromatic C-H

A broad absorption between 3300-3400 cm^{-1} was observed, which was attributed to O-H and N-H stretching vibrations. This band was broader in water-based extracts due to stronger hydrogen bonding interactions in aqueous solutions [53]. Bands in the 2900-2950 cm^{-1} region corresponded to aliphatic C-H stretching vibrations of methyl and methylene groups. These peaks were often more intense in alcohol extracts, consistent with the higher solubility of hydrophobic moieties in organic solvents [54].

Absorptions in the 1600-1700 cm^{-1} range were assigned to C=O stretching of carbonyl groups and C=C stretching of aromatic rings. Slight shifts in peak positions were noted depending on the solvent environment, reflecting the effect of solvent polarity on vibrational frequencies [55]. The 1400-1450 cm^{-1} region showed C-H bending associated with both aliphatic and aromatic groups. These peaks were relatively consistent across solvents, indicating minimal sensitivity to extraction medium.

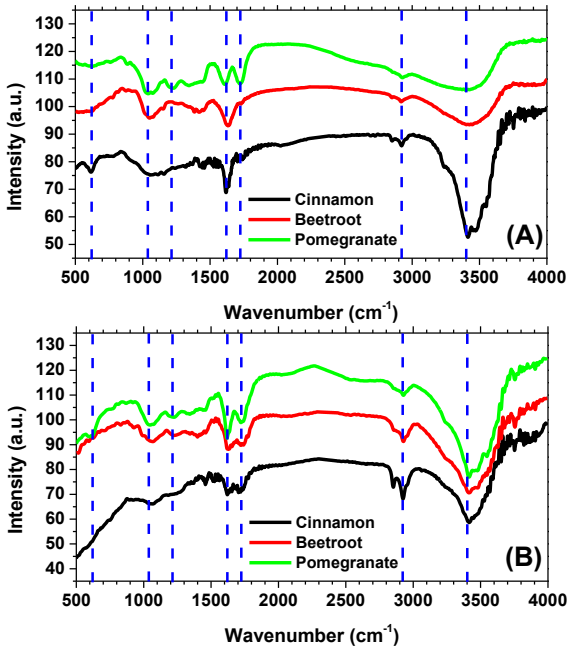

Fig. (4) FTIR spectra of the Beetroot, Pomegranate and Cinnamon plants in (A) Water and (B) Alcohol, based solution

The absorptions between 1200-1300 cm^{-1} were assigned to C-O stretching vibrations of ethers, esters, and phenolic groups. Variations in intensity and band sharpness were observed between aqueous and alcoholic extracts, suggesting solvent-dependent differences in the extraction of oxygenated compounds [56]. In the 1000-1100 cm^{-1} range, bands were attributed to C-O and C-N stretching, often associated with alcohols, carbohydrates, and amines. Moderate solvent effects were visible, particularly in the overall band definition. Finally, characteristic absorptions appeared in the 600-650 cm^{-1} region, corresponding to out-of-plane C-H bending and ring skeletal vibrations typical of aromatic structures. These peaks were generally varied between the solution-based extracts, reflecting the differences in solubility of aromatic compounds between solvents [57].

The bacteria *Escherichia coli* (*E. coli*) (see Fig. 5) and *P. aeruginosa* (see Fig. 6) were isolated from samples of two patients admitted in local hospital in Baghdad, Iraq. To evaluate the antibacterial activity of some plant extracts, extracts of pomegranate, beetroot, and cinnamon were used in their aqueous and alcoholic forms with different concentration (1%, 2%, 3% and 4%, and stock) and compare them with the inclusion of standard antibiotics Imipenem (IPM) and Aztreonam (ATM) validated the antibacterial assays. The susceptibility test was performed using the agar diffusion method, and the efficacy of the concentrates of aqueous and alcoholic extracts was tested in comparison with the antibiotics Aztreonam (ATM) and Imipenem (IPM).

Results revealed clear variations in activity depending on both the plant source and the extraction solvent. In case of *E. coli*, results are illustrated in Fig. (5) and table (4), the control plates containing only water or alcohol showed no inhibition zones, confirming that the solvents themselves did not affect bacterial growth. Cinnamon extracts demonstrated the lowest effective antibacterial activity, particularly when extracted with water. While, ethanol-based cinnamon solutions produced larger inhibition zones across all tested concentrations, suggesting that alcohol is highly effective in extracting the bioactive constituents responsible for antibacterial action, such as cinnamaldehyde and phenolic compounds [58], which were detected by UV-Visible and FTIR analyses, and are known to disrupt bacterial membranes and metabolic pathways [43]. Trace amounts of CuO identified by XRF may also work in combination with cinnamaldehyde by generating reactive oxygen species (ROS), enhancing antibacterial potency [16].

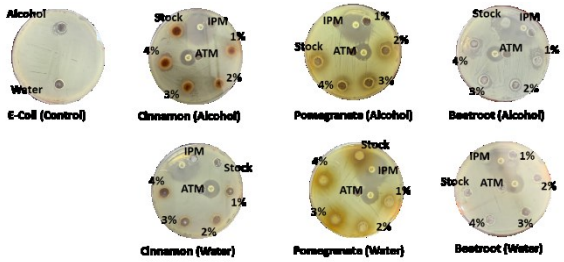

Fig. (5) *E. coli* bactrial inhibition behaviour for the plants solution extractions (Water and Alcohol)

In contrast, water-based cinnamon extracts have not inhibited *E. coli* as produced zero zones, indicating lower bioavailability of these hydrophobic compounds in aqueous media. Pomegranate extracts show higher activity with almost the same inhibition zones in both cases, water and alcohol solutions. The aqueous pomegranate extracts produced slightly higher inhibition zones even at lower concentrations, suggesting that phenolic compounds such as punicalagin and ellagic acid, which are more soluble in water and are associated with membrane-disrupting activity [45], play a dominant role in antibacterial action [58]. The presence of trace Ag_2O bioactive compounds further supports antibacterial activity since silver ions are known to strongly inhibit *E. coli* growth [23]. Beetroot extracts demonstrated no inhibition against *E. coli* when extracted by water. This can be

explained by the dominance of betalains, which act primarily as antioxidants rather than strong antimicrobials [42]. This observation aligns with previous reports that beetroot bioactivity is mainly attributed to betalains and other hydrophilic antioxidants, which are more efficiently extracted in aqueous solutions [59]. Although beetroot contained notable amounts of iron oxides and CoO as bioactive compounds, their limited diffusion in agar likely restricted their contribution to antibacterial activity. Overall, beetroot appears highly effective against *E. coli* compared to cinnamon and pomegranate, especially when extracted by alcohol. Taken together, these findings highlight the critical influence of extraction solvent on the antibacterial performance of plant-derived compounds. Such differences can be explained by the varying solubility of active phytochemicals: hydrophobic molecules (e.g., cinnamaldehyde) are better extracted with organic solvents, while hydrophilic polyphenols (e.g., punicalagin, betalains) are preferentially recovered in water. The *P. aeruginosa*, on the other hand, is known for its natural resistance mechanisms, including efflux pumps and biofilm formation, which often make it harder to inhibit than *E. coli* [8]. Despite this, the plant extracts demonstrated measurable inhibitory effects. The inhibition assay against *P. aeruginosa* revealed solvent and plant dependent differences in antibacterial efficacy, results are illustrated in Fig. (6) and table (5). The control plates containing only water or alcohol showed no inhibitory effects, confirming that any antibacterial activity observed was due to the plant extracts themselves. Cinnamon extracts again displayed the lowest antibacterial activity, with alcohol-based extracts showing small inhibition zones across all concentrations. This suggests that cinnamaldehyde and phenolic compounds are less effective even against a resilient bacterium like *P. aeruginosa* [8].

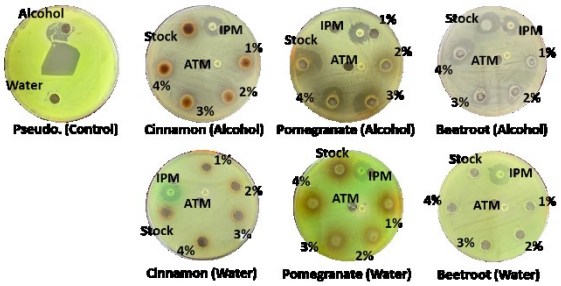

Fig. (6) *P. aeruginosa* bactrial inhibition behaviour for the plants solution extractions (Water and Alcohol)

The additional role of trace ZnO and CuO bioactive compounds (with antimicrobial nanoparticle-like effects) may explain cinnamon's consistent potency as alcoholic solution compare to water-based solution [16]. Water extracts of cinnamon also showed no antibacterial activity, which aligns with the lower solubility of these hydrophobic active compounds in aqueous solvents. Pomegranate extracts showed consistent antibacterial activity against *P. aeruginosa*, with ethanol extracts producing slightly larger inhibition zones than aqueous extracts. This pattern mirrors what was observed against *E. coli*, further highlighting the role of hydrophilic polyphenols, including punicalagin and ellagic acid, as the principal antibacterial constituents in pomegranate [58]. The presence of Ag_2O and CoO bioactive compounds further enhances antibacterial activity by damaging bacterial membranes through ion release and ROS generation [22]. Beetroot extracts produced weak inhibition against *P. aeruginosa.*, while betalains contribute antioxidant properties, they do not strongly suppress bacterial growth. Trace amounts of CoO and MnO_2 bioactive compounds might play a minor role, but overall, beetroot was the least effective plant extract tested. These findings are in line with reports suggesting that betalains and other beetroot antioxidants possess mild antimicrobial effects, but their potency against Gram-negative pathogens such as *P. aeruginosa* is limited [59]. Interestingly, compared to *E. coli*, the overall inhibition zones produced by all plant extracts were smaller, reflecting the higher intrinsic resistance of *P. aeruginosa*. This bacterium possesses a robust outer membrane and efflux pump systems, which contribute to its reduced susceptibility to many natural antimicrobials [60]. Nevertheless, all extracted alcohol-based solutions demonstrated measurable inhibitory effects, suggesting their potential as natural adjuncts in managing *P. aeruginosa* infections. The results indicate that water-based extracts, particularly from pomegranate, produced comparable inhibition zones compared to their alcohol-based counterparts. This highlights the importance of choosing an appropriate extraction solvent based on the chemical nature of bioactive compounds. Water is especially effective in extracting hydrophilic phytochemicals such as polyphenols, tannins and betalains, which are well-documented for their antibacterial activity [58, 59]. For instance, punicalagin and ellagic acid in pomegranate are highly water-soluble, explaining the superior inhibition zones observed in the aqueous extracts compared to ethanol-based extracts. Similarly, betalains in beetroot are hydrophilic antioxidants that are preferentially extracted in water, leading to better antibacterial effects. Beyond chemical solubility, the use of water as a solvent carries practical advantages. Water is non-toxic, inexpensive, environmentally friendly, and more suitable for medical, pharmaceutical, and food-related applications. Alcohol-based extracts may concentrate certain hydrophobic compounds (such as cinnamaldehyde in cinnamon), but their application is limited in cases where solvent toxicity or volatility is a concern [61]. Therefore, the strong inhibition zones obtained with water-based extracts underline not only

the chemical compatibility between solvent and active phytochemicals but also the feasibility of translating these extracts into safe, natural antibacterial agents for therapeutic and industrial purposes. The inclusion of standard antibiotics Imipenem (IPM) and Aztreonam (ATM) validated the antibacterial assays. Against E. coli, both produced large inhibition zones, confirming the sensitivity of the strain. For *P. aeruginosa*, IPM produced a much larger zone than ATM, reflecting clinical evidence that *P. aeruginosa* is often more resistant to aztreonam but remains susceptible to carbapenems like Imipenem [8]. Compared with these antibiotics, the plant extracts showed measurable but smaller inhibition zones, indicating that while these extract solutions are promising, they cannot match the potency of clinical antibiotics. Instead, their role may be more complementary, as adjunctive or preventive antimicrobial agents, particularly in food preservation or nutraceutical contexts [62]. Alcohol extracts were more potent than water extracts, though water remains more suitable for practical applications. Standard antibiotic controls (IPM and ATM) confirmed bacterial sensitivity and highlighted the relative strength of pharmaceuticals over plant-derived compounds. These findings support the potential use of these solutions as complementary natural antimicrobials, especially where mild inhibition or synergistic effects with existing treatments are desirable.

4. Conclusion

This study demonstrated that extracted solutions from pomegranate, cinnamon, and beetroot possess notable antibacterial activity against *E. coli* and *P. aeruginosa*, influenced significantly by the extraction solvent. The presence of phenolic and flavonoid compounds was confirmed, while functional groups such as hydroxyl, carbonyl, and aromatic rings associated with antimicrobial activity were identified. An enhanced electron transfer potential in alcoholic extracts was indicated, which may contribute to their higher antibacterial efficiency. Aqueous pomegranate and alcoholic cinnamon extracts showed the strongest inhibition, whereas beetroot extracts exhibited moderate effects linked to antioxidant constituents. Trace metal oxides detected at low concentrations may play only a minor synergistic role. Solvent polarity strongly affected phytochemical composition and biological performance, highlighting plant extracts as promising, safe, and eco-friendly alternatives for antimicrobial applications.

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Table (1) The Heavy Metals (Toxic trace) extracted from Beetroot, Cinnamon and Pomegranate as water and Alcohol based solutions (data extracted using XRF analysis); all the ratios in ppm

		Beetroot		Pomegranate		Cinnamon	
Traces	Effect	Water	Alcohol	Water	Alcohol	Water	Alcohol
ZnO	Toxic in excess [15]	30.9	14.2	26.4	17.8	18.2	18.4
CuO	Toxic in excess [16]	11.9	10.6	7.5	14.0	9.9	14.1
CdO	Carcinogen, toxic [17]	10.9	0	32.0	0	40.5	43.8
CrO_3	Carcinogen, toxic [18]	9.0	14.8	12.7	13.3	12.6	17.8
NiO	Allergen, toxic [19]	5.1	4.4	1.8	6.0	11.8	5.8
As_2O_3	Carcinogen [20]	1.0	0.9	0.3	0.9	2.1	0.5
MnO_2	Carcinogen, toxic [21]	40.7	3.7	15.7	5.2	58.0	6.4
CoO	Carcinogen, toxic [22]	402	14.1	8.3	14.4	16.0	33.3

Table (2) The higher concertation elements extracted from Beetroot, Cinnamon and Pomegranate as water-based juices (data extracted using XRF analysis)

Sample	Solvent	Hematite(ppm)	P(ppm)	K(ppm)	Ca (ppm)	Ag(ppm)
Beetroot	Water	502.7	724.3	12120	2400	25400
	Alcohol	19	84.6	0.326	211.7	28500
Cinnamon	Water	23.5	50.3	817.6	920	68500
	Alcohol	29.2	25.5	161.8	130	61500
Pomegranate	Water	33.9	140.2	0.32	0.29	68500
	Alcohol	21	103.2	0.23	294.1	61500

Table (4) The antibacterial effects and the inhibition zones of the extracted solution on E-cloi

E. coli										
Isolate	Solution	Diameter of inhibition zone (mm)								
		Solution concentrations					Control positive (mm)		Control negative (mm)	
		1%	2%	3%	4%	Stock	IPM	ATM	DW	Alcohol
Pomegranate	Water	10	10	12	13	14	15	8	NO	NO
	Alcohol	8	12	12	12	14	15	8	NO	NO
Cinnamon	Water	NO	NO	NO	NO	NO	15	8	NO	NO
	Alcohol	5	5	6	7	7	15	8	NO	NO
Beetroot	Water	NO	NO	NO	NO	NO	15	8	NO	NO
	Alcohol	15	15	18	20	22	15	8	NO	NO

Table (5) The antibacterial effects and the inhibition zones of the extracted solution on E-cloi

P. aureginosa										
Isolate	Solution	Diameter of inhibition zone (mm)								
		Solution concentrations					Control positive (mm)		Control negative (mm)	
		1%	2%	3%	4%	Stock	IPM	ATM	DW	Alcohol
Pomegranate	Water	12	12	13	15	15	15	NO	NO	NO
	Alcohol	2	20	20	25	28	15	NO	NO	NO
Cinnamon	Water	NO	NO	NO	NO	NO	15	NO	NO	NO
	Alcohol	5	5	6	7	7	15	NO	NO	NO
Beetroot	Water	NO	NO	NO	NO	NO	15	NO	NO	NO
	Alcohol	8	8	11	20	20	15	NO	NO	NO

