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Evaluation of antimicrobial, antioxidant, and phytochemical properties of Joshanda, a traditional herbal formation



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Abstract

Humans have long used herbs to treat common health issues, such as the common cold, cough, and inflammation. Developing nations, such as India and Pakistan, continue to follow these practices. Joshanda is one of those herbs that is quite affordable and has few or no negative effects. This study aimed to screen and evaluate the biological activities of a commercial sample of Joshanda. Joshanda extract was obtained using ethanol solvent. The antimicrobial activity of Joshanda extract against pathogens, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Proteus mirabilis*, *Streptococcus mutans*, *Staphylococcus aureus*, *Bacillus subtilis*, and strain of fungi *Candida albicans*, was examined using the agar well-diffusion method. The antioxidant capability of Joshanda was assessed using a DPPH radical scavenging activity assay. The ethanolic extract of Joshanda exhibited significant antimicrobial activity against *S. typhi* (15–16 mm) and *P. aeruginosa* (12 mm); however, it was ineffective against *S. mutans* and *C. albicans*. The inhibition zones were approximately half the size of those of the controls (Ciprofloxacin and Azithromycin). Joshanda exhibited a DPPH radical scavenging activity of (51.57%), approximately two times lower than that of (97.9%) for the ascorbic acid standard. Numerous bioactive phytochemicals, including alkaloids, flavonoids, saponins, sugars, carbohydrates, glycosides, cardiac glycosides, proteins, and amino acids, have been identified in the Joshanda extract. Gas chromatography-mass spectrometry (GC-MS) analysis revealed a wide variety of bioactive components, with a high percentage of methyl esters. The results suggest that Joshanda possesses medicinal properties, probably due to the presence of bioactive compounds and phytochemicals. However, the antimicrobial and antioxidative capacities of Joshanda were far below the control standards. Future research should be conducted to determine the minimum inhibitory concentrations (MIC) and *in vivo* toxicity to ensure the safety of the utilization of Joshanda herbal formulation.

Keywords: Herbal medicine; Antimicrobial activity; Antioxidant activity; Phytochemical analysis

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INTRODUCTION

The global tendency to choose herbal treatments over synthetic medications has significantly changed in recent years, signifying a "Return to the Nature" (Chaughule & Barve, 2024; Iyiola & Adegoke, 2024; Shamsi et al., 2018). The global phytomedicine market was valued at approximately USD 40 billion in 2022 and is projected to reach USD 70 billion by 2030, with an annual growth rate of 7.2%, highlighting its global adaptation and acceptability (Soni et al., 2026). These herbal medicines use whole plants or plant parts to treat various illnesses and promote overall health (Latif and Nawaz, 2025; Mudondo et al., 2025).

Because of the increasing public interest in herbal medications and their acceptance for their positive characteristics with minimal or no side effects against diverse health-related issues, the traditional healthcare system is swiftly becoming more well-known worldwide (Chaachouay & Zidane, 2024; Balkrishna et al., 2024; Krsnik & Erjavec, 2024). Currently, 60% of the world's population uses herbal or conventional treatments as the primary means of treating malaria-related fevers. For a variety of diseases, herbal or alternative medicines are the first option for 80% of Africans, 30–50% of Chinese, 48% of Australians, 70% of Canadians, 80% of Germans, 42% of Americans, 39% of Belgians, and 76% of French people (Ansari et al., 2020).

Herbal remedies are used in South Africa, London and San Francisco. The use of herbal products is becoming increasingly popular among health professionals because of their affordability, and nations such as Malaysia are currently spending more on high-quality herbal medications than on allopathic medications (Sen & Chakraborty, 2017). The majority of people in developing nations are thought to utilize traditional commodities as their main source of Medicare, and medicinal uses, such as the use of herbs, are valued as essential components of the traditions of these nations (Orisatoki & Oguntibeju, 2010; Aggarwal et al., 2022; Saggat et al., 2022; Haider, 2023; Nguyen et al., 2025).

In addition to being widely used in many developed countries, herbal remedies are becoming increasingly significant in the UK, Australia, North America, and the European Union (Haider, 2023; Coulter & Willis, 2004; Chen et al., 2025; Izah et al., 2024; Ghosh et al., 2023). Given the global acceptance of herbal remedial medicines, it is essential to verify the strength and efficacy of these widely used herbal formulations through scientific research and reports to establish consumer trust and increase their market value. Some of these have been reported to have antimicrobial Ashraf et al. (2023), and Izah et al. (2023), antifertility Mili et al. (2025) and Mishra et al. (2025), antidiabetic Husen (2023), antiarthritic Wang et al. (2024), antiaging Raina et al., (2023) and Fan et al. (2025), antidepressant Haque et al. (2023), sedative, antispasmodic, antianxiety, anti-inflammatory, analgesic, vasodilatory Azmi et al. (2025), Aly et al. (2026), Khan et al. (2022), anti-HIV Shahrajabian & Sun, (2023), Ng'weshemi et al. (2025) & Hasan et al. (2023), hepatoprotective Gonfa et al.

(2025) properties, and to treat cirrhosis, acne, asthma, menopause, impotence, gallstones, migraine, Alzheimer's disease, chronic fatigue, and memory-enhancing activities. In the Indo-Pak region, a popular home remedy for colds and coughs accompanied by fever and pharyngeal inflammation is Joshanda, a polyherbal compound of *Unani (Greco-Arab)* provenance (Shamsi et al., 2018). It is easily produced and utilized in Pakistan to treat diverse forms of illnesses, including fever, flu, bronchitis, cough, and the common cold. It affects the smooth muscles of the bronchial tissue (Alam & Maimoona, 2022). It is evident that using herbal decoctions, such as Joshanda formulations, for self-medication is safer than using contemporary cold medicines (Azmi et al., 2010; Shah, 2024; Firdaus et al., 2024).

Joshanda is being used widely to treat URC (Upper Respiratory Catarrh), especially in chronic instances, following the global resurgence of interest and acceptance in the use of traditional medicines. A range of expectorant, anti-catarrhal, and respiratory demulcent herbs are used in this polyherbal blend to assist in relieving the crippling cough. The use of a combination of antitussive (tending or having the ability to act as a cough suppressant) agents with various modes of action, such as reflex and central expectorants, parasymimetic, stimulants of sensory cells, and secretomotorics, is the key to highly effective phytotherapy (Ahuja et al., 2009). However, there are limited reports in the literature validating the strength and efficacy of Joshanda formulations against the pathogens implicated in the health conditions addressed with Joshanda formulations. This study was conducted based on a threefold objective: (i) to qualitatively screen the presence of phytochemicals in commercial Joshanda formulation, (ii) to assess the in vitro antimicrobial potential of ethanolic extract of Joshanda against nine common human pathogens, and (iii) to evaluate the antioxidant capacity of Joshanda via DPPH radical scavenging assay. The main aim of this study was to evaluate the antibacterial and antioxidative properties of Joshanda to confirm its potential for treating common human health conditions. The bioactive compounds of Joshanda extract were also evaluated to ascertain the possible active ingredients responsible for the pharmacological properties of Joshanda.

Materials and Methods

Sample collection

Processed Joshanda sachets were purchased from local stores in Peshawar, with the composition as shown in Table 1.

Table 1. Ingredients and composition of Joshanda used for this study

S. No	Name of Ingredient	Common Name (Pakistan)	Amount Used (in mg per 5 g of sample)
1	Licorice Extract	<i>Mulethi</i>	212.76
2	Adhatoda vasica	<i>Arusa</i>	106.38
3	Starch	<i>Nashasta</i>	106.38
4	Poppy seeds	<i>KhashKhash</i>	106.38
5	Hyssop	<i>Zufah yabis</i>	53.19
6	Ephedra	<i>Ephedra</i>	53.19
7	Tea	<i>Chai</i>	53.19
8	Aspartame	-	17
8	Fennel oil	<i>Roghan Saunf</i>	30
9	Sodium Benzoate	-	1
10	Peppermint Extract	<i>Roghan Pudina</i>	Variable

Microbial species

The test organisms, *Escherichia coli* (ATCC# 8738), *Pseudomonas aeruginosa* (ATCC# 9721), *Proteus mirabilis* (ATCC# 12553), *Streptococcus mutans* (ATCC# 35668), *Staphylococcus aureus* (ATCC# 6538), *Bacillus subtilis*, *Klebsiella pneumoniae*, *Salmonella typhi*, and a strain of fungi, *Candida albicans*, were obtained (as clinical isolates) from the Pakistan Council for Scientific and Industrial Research laboratory, in Pakistan.

Antioxidant activity

Agilent Cary 60 UV-Vis Spectrophotometer, Sigma-Aldrich® HPLC grade methanol, distilled water, Daejung® Ascorbic acid (99.9%), Aldrich® DPPH (98%)

GC-MS analysis

GCMS-QP2010 Plus (Shimadzu, Japan)

Phytochemical screening

Hager's reagents, Mayer's reagent, Seliwanoff's reagent, Barfoed's reagent, glacial acetic acid, 5% FeCl₃, conc. H₂SO₄, ferric chloride, Baljet's reagent, copper sulfate, 95% ethanol, KOH pellets, Millon's reagent, distilled water, olive oil, chloroform, 10% ferric chloride, and dilute HCl Ammonia solution were used.

Extraction of active metabolites from Joshanda

A separating funnel (1 L) containing ethanol as the extracting solvent was filled with 72 g of the Joshanda sample. The mixture was left for a full day to allow ethanol to operate as an extracting solvent and remove all active metabolites from the Joshanda sample. After removing excess ethanol from the solution, only the beneficial phytoconstituents from Joshanda remained, and the extract was produced using a rotary evaporator.

Extraction was performed at room temperature and pressure. The extract ratio was 4.16%, and 3 g of extract was obtained from 72 g of the Joshanda sample.

Antimicrobial activity of Joshanda extract

The agar well-diffusion method was used to assess the antimicrobial properties of Joshanda extract (Shamsi et al., 2018, Fazal et al., 2012, Güler et al., 2023, Erhonyota et al., 2023, Hossain, 2024). One litre of distilled water was used as a solvent to dissolve 28 g of agar to create one litre of agar gel. The mixture was autoclaved at 120 °C and a pressure of 1.5 bar for 15 min. After sanitizing the agar plates by wiping with ethyl alcohol, the medium was poured into agar plates inside a laminar hood flow (LHF). To verify sterility, the prepared plates were incubated for 24 hours at 37°C.

Samples of bacterial and fungal strains were collected in test tubes containing normal saline solution. Strains of bacteria, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Proteus mirabilis*, *Streptococcus mutans*, *Staphylococcus aureus*, *Bacillus subtilis*, and a strain of fungi, *Candida albicans* were assessed in this study.

To achieve consistent bacterial growth on the test plates, the aforementioned microbial species were transferred from standard saline-containing test tubes and applied to agar plates using a sterile glass spreader. The wells were punched over the agar media in plates using sterilized cork borer. Joshanda extract was dissolved in Dimethyl Sulfoxide (DMSO) solvent to obtain a solution with concentration of 100 mg/ml. Using a sterile syringe, 100µl of the prepared solution was applied to each of the two wells in each test plate in duplicate. The plates were then allowed to sit at room temperature for two hours to allow for diffusion. Ciprofloxacin and azithromycin, 25µg for each, were used as reference antibiotics. The activity was carried out in an ethyl alcohol-sterilized laminar hood flow. The inoculated agar plates were then incubated at 37 °C for 24 h. The diameter of the inhibitory zone (mm) was determined using a meter rule. The inhibition zones produced by Joshanda were compared with the inhibition values of the common antibiotics (ciprofloxacin and azithromycin). To show experimental consistency, data were collected in duplicate, and the results were taken as individual readings (R1 and R2).

Phytochemical screening of Joshanda extract

Following the procedures by Shaikh, J. R., & Patil, M. (Shaikh & Patil, 2020), a number of standardized qualitative chemical tests were conducted in triplicate to qualitatively determine the various phytoconstituents present in the ethanolic extract of Joshanda.

Alkaloids

Hager's Test: Few mL filtrate + 1-2 mL Hager's reagent. A creamy white precipitate was formed.

Mayer's/ Bertrand's/ Valser's Test: Few mL filtrate + 1-2 drops of Mayer's reagent (along the sides of test tube). A creamy white/yellow precipitate was obtained.

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Carbohydrates

Seliwanoff's Test: 1mL extract solution + 3mL Seliwanoff's reagent + heated in a water bath for 1 min. Rose-red color (ketoses).

Barfoed's Test: 1mL filtrate + 1mL Barfoed's reagent + Heated for 2 min. red precipitate (monosaccharides).

Glycosides

Concentrated H₂SO₄ Test: 5 ml plant extract + 2mL glacial acetic acid + a drop of 5% FeCl₃ + conc. H₂SO₄. Brown ring formed in the tube.

Cardiac Glycosides

Keller-Killani Test: 1mL filtrate + 1.5mL glacial acetic acid + 1 drop of 5% ferric chloride + conc. H₂SO₄ (along the side of the test tube). A blue solution (in the acetic acid layer).

Baljet Test: 2mL of extract + a drop of Baljet's reagent. A yellow-orange color indicated a positive result.

Proteins and amino acids

Biuret Test: 2mL of filtrate + one drop of 2% copper sulfate solution. + 1mL of 95% ethanol + KOH pellets. A pink-colored sol (in the ethanolic layer).

Millon's Test: 2mL filtrate + few drops of Millon's reagent. A white precipitate was formed.

Saponins

Olive oil test: Aqueous extract + 5mL distilled water; shaken vigorously + few drops of olive oil + shaken vigorously. A foam appeared on the surface.

Steroids

Hesse's Response 5mL aqueous extract + 2mL chloroform + 2mL conc. H₂SO₄. Pink ring/red color (in the lower chloroform layer).

Flavonoids

Ferric chloride test: Aqueous extract + few drops of 10% ferric chloride solution. A green precipitate was formed.

Ammonia Test: Filtrate + 5mL dil. Ammonia solution + conc. HCl H₂SO₄. A yellow color indicated a positive result.

Antioxidant activity of Joshanda extract

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay was used to assess the antioxidant potential of the Joshanda extract sample (Hatano et al., 1988; Nisar Ahmad et al., 2011; Gulcin & Alwasel, 2023; Rubab et al., 2022), using ascorbic acid as the standard. A 25% solution of Joshanda extract was prepared by adding 3.0 mg of extract to 12.0 ml methanol. Similarly, ascorbic acid (standard) was prepared (3.3 mg in 13.2 ml methanol). A 0.1 M solution of DPPH was prepared and kept in a dark and cool place until use. An aliquot of 2.0 ml of DPPH was mixed with 3.0 ml of Joshanda and ascorbic acid solutions, and the mixtures were incubated for 30 min at room temperature. The mixtures were kept in the dark. The absorbance of the DPPH mixture was measured using a was 517 nm. Methanol was used as the blank. The absorbance values were determined for the DPPH-added mixtures of Joshanda extract and ascorbic

acid. The experiment was conducted twice to demonstrate the consistency of the results.

The percentage DPPH radical scavenging activity for each sample was calculated using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = \frac{(\text{Control Absorbance} - \text{Sample Absorbance})}{(\text{Control Absorbance})} \times 100 \dots \dots \dots (1)$$

GC-MS analysis of bioactive compounds of Joshanda

GC-MS analysis of the ethanolic extract of Joshanda was performed using the GCMS-QP2010 Plus instrument. The column used in the equipment was DB5 MS with a column length of 30m, diameter of 0.25 mm, and film thickness of 0.24µm. Helium gas was used as the carrier gas. Splitless injection mode was employed. The column oven temperature was 80 °C and allowed to increase with a ramp rate of 5 °C per minute until it reached 220°C, and was retained at this temperature for a hold time of 4 min. The injection was started at 250°C. The injection volume was 1 µL. The ion-source temperature was 280 °C, and interface temperatures were both set to 280 °C. The GC column flow was 1.22 mL/min, and the total flow was 40mL/min. The total run time for the analysis was 30 min. The NIST05 library was used to identify the compounds.

Results and Discussions

Antimicrobial activity

The Joshanda extract showed marked antimicrobial activity against all the microbial strains tested, except for *C. albicans* and *S. mutans* (Figure 1.). The antimicrobial activities elicited by the standard drugs against the test organisms, expressed as zones of inhibition, were much higher than those obtained using the Joshanda extract. Some of the test organisms, such as *S. aureus*, *B. subtilis*, and *B. cereus*, are known human pathogens and are implicated in upper respiratory tract infections (Bottone, 2010; Pettigrew et al., 2008; Yablonsky, 1983; Paul, 2024; Koca, 2024; Dastgir et al., 2025). This suggests that Joshanda could potentially be used to treat upper respiratory tract infections in humans and animals. Shamsi et al. also reported the antibacterial activity of Joshanda extract against microorganisms such as *E. coli*, *B. subtilis*, *S. aureus*, and *P. aeruginosa* (Shamsi et al., 2018; Koca, 2024; Dastgir et al., 2025). Although *C. albicans* and *S. mutans* showed resistance to Joshanda extract in the current study, further tests were not performed to ascertain whether the resistance was concentration-dependent. The antimicrobial activity could be attributed to the presence of phytochemicals and bioactive compounds, such as flavonoids and methyl esters, detected in Joshanda. These compounds have been documented for their biological actions, including antimicrobial activity (Olivia et al., 2021, Jane et al., 2025, Joshi et al., 2026). According to some authors, these phytochemicals initiate antibiotic actions either by preventing lipid peroxidation, iron deprivation, hydrogen bonding, or interactions with specific important enzymes

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in microbial cells (Shamsi et al., 2018). The results (Table 2.) suggest that Joshanda should be viewed as a complementary treatment in normal cases rather than a replacement for primary antibiotics because of its significantly lower activity compared with Ciprofloxacin and Azithromycin.

Phytochemical screening

Qualitative screening of the ethanolic extract of Joshanda revealed the presence of a diverse range of biologically important phytochemicals, including alkaloids, carbohydrates, proteins, amino acids, glycosides, cardiac glycosides, sugars, and saponins (Table 3.). Steroids were not detected in the phytoconstituents. Other researchers have also reported the presence of similar phytochemicals in plant-based extracts (Shamsi et al., 2018; Olivia et al., 2021; Dubale et al., 2023). The presence of these phytochemicals has been linked to a wide range of biological activities. For instance, alkaloids are used as analgesics, antiseptics, sedatives, stomachics, uterine muscle depressants, and for eye and bleeding disorders in Indian and Islamic traditional medicine (Da-Cunha et al., 2005; Pinjari et al., 2024; Mohammed et al., 2022). They also exhibit anti-HIV, antifungal, cardioprotective, immunoregulatory, antimalarial, anti-inflammatory, and antioxidant properties (Ma et al., 2017; Zuo et al., 2006). Flavonoids have a wide range of pharmacological effects, including anti-Parkinson, anti-ulcer, spasmolytic, antidepressant, antibacterial, antihypertensive, anti-diabetic, anti-inflammatory, and anti-cancer (Rana & Gulliya, 2019; Ullah et al., 2020; Abou Auda, 2025; Fathi et al., 2022). Various glycosides possess anti-inflammatory, anti-arthritis, anti-allergic, anti-thrombotic, vasoprotective, hemorrhagic, anti-fungicidal, and anticancer properties (Kowsalya et al., 2025). In contrast, these glycosides improve the stability and quality of food, act as natural sweeteners, and enhance flavor (Saraiva et al., 2020). These biological activities demonstrated by flavonoid-rich vegetables or plants, coupled with their good bioavailability, confer the potential use of flavonoids as functional foods. The ability of cardiac glycosides to bind to and block the sodium pump makes them a diverse family of naturally occurring chemicals (Joubert, 2023; Umarova, 2026; Ponce et al., 2025). Arab physicians potentially used plant extracts containing cardiac glycosides to cure cancer in the eighth century CE. The application of extracts with various cardiac glycosides is an ancient Chinese medical treatment for cancer that is still in use today. Numerous studies have demonstrated the effectiveness of cardiac glycosides as non-proliferative treatments for various cancer types, including human colon, colorectal, and breast cancers (Nabil et al., 2024; Ainembabazi et al., 2023). Saponins and their derivatives are versatile glycosidic compounds distinguished by their soap-forming nature. They play a pivotal role in the food, agricultural, and pharmaceutical industries, ranging from being a natural additive in food and having their role in traditional medicines to various applications in the pharmaceutical sector. Saponins have been reported to possess hypoglycemic, anti-asthmatic,

antioxidant, antihypertensive, and antimicrobial activities (Sharma et al., 2023; Elish et al., 2023; Sakna et al., 2023). The considerable structural diversification of saponins is linked to their anticancer properties. The functions of saponins in cancer and their mechanisms of action, such as cell cycle arrest, antioxidant activity, inhibition of cellular invasion, activation of apoptosis, and autophagy, have been documented in many studies (Elekofehinti et al., 2021).

Antioxidant activity

The antioxidant potential of Joshanda extract (approximately 51%) compared favorably to the standard, ascorbic acid (approximately 98 %) (Figure 2.). Antioxidants can prevent free radicals from having an oxidative effect (Chandimali et al., 2025, Kiran et al., 2023). Joshanda's antioxidant properties may be attributed to the phytochemicals present in Joshanda, including alkaloids, phenolic acids, flavonoids (Rana & Gulliya, 2019; Ullah et al., 2020; Cai et al., 2006), and saponins (Sharma et al., 2023, Elekofehinti et al., 2021). The synergistic effects of these phytochemicals may also contribute to their effectiveness. The antioxidant properties of the ingredients in Joshanda are widely recognized (Tungmunnithum et al., 2018). The antioxidant activity of Joshanda was assessed using ascorbic acid as a reference. According to the current study findings, the antioxidant activities of Joshanda and ascorbic acid are (51.57%) and (97.9%), respectively. These results (Table 4.) suggest that Joshanda exhibited moderate DPPH radical scavenging activity; however, it was significantly lower than that of standard ascorbic acid. The antioxidant activity of Joshanda in the current study was lower than the maximum value reported by Shamsi et al. (Shamsi et al., 2018). The variations observed in this report may be concentration-dependent. The variations in the composition and state of the Joshanda samples used may have also contributed to the variations in the obtained results. According to the authors, commercially available Joshanda samples could be in a raw or processed state (Saddiqe & Ahmad, 2022).

Table 3: Test results for phytochemical screening

S. No	Phytochemicals tested	Present/Absent
1	Alkaloids	+
2	Steroids	-
3	Carbohydrates	+
4	Cardiac Glycosides	+
5	Glycosides	+
6	Protein and Amino Acids	+
7	Flavonoids	+
8	Sugar	+
9	Saponins	+

'+' = present, '-' = absent

Table 4. Comparative antioxidant activity of Joshanda and ascorbic acid

S. No	Sample	% Activity
1	Joshanda	51.57 ± 1.4
2	Ascorbic acid	97.9 ± 1.2

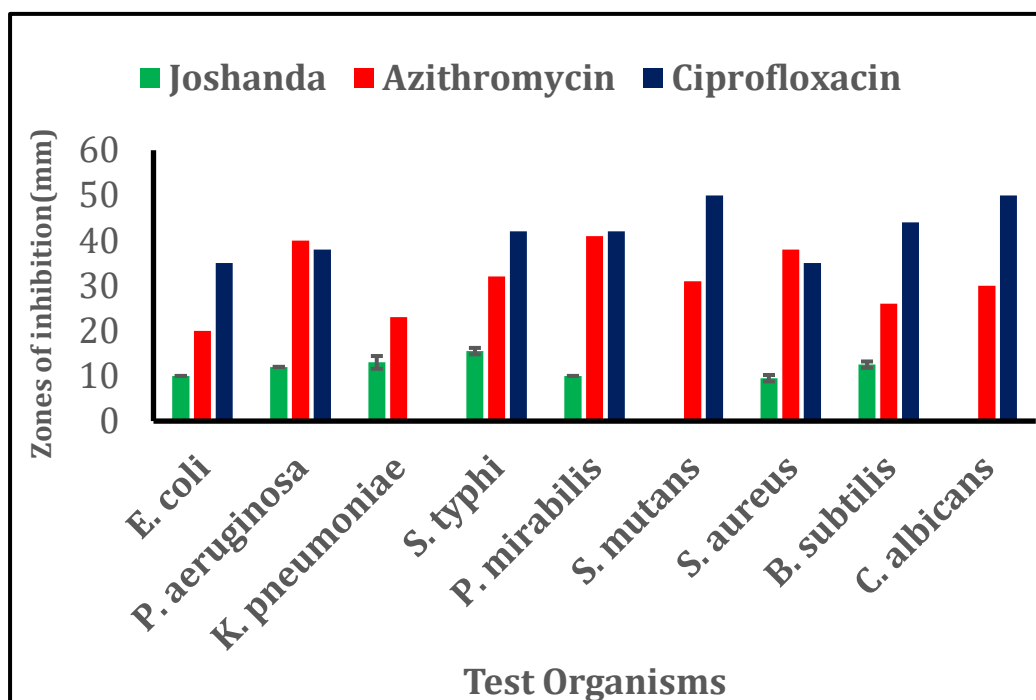


Figure 1: Antimicrobial activity of Joshanda against different microbial pathogens

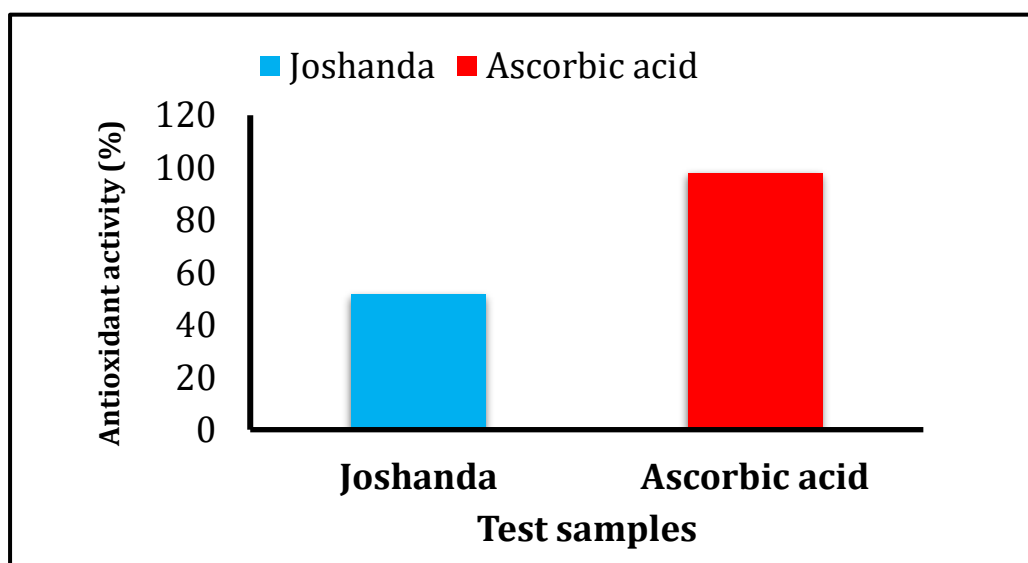


Figure 2: Radical scavenging (antioxidant) activity of Joshanda

GC-MS analysis of Joshanda

Gas chromatography-mass spectrometry (GC-MS) analysis of the Joshanda extract revealed the presence of several biochemical components, up to 18 in number. Formic acid, 4-o-methylmannose, 5-hydroxymethylfural, caffeine, and D1-menthol were the major compounds, with area percentages of 21.26, 16.13, 12.02, 6.19, and 5.5, respectively (Table 5). The GC-MS chromatogram is shown in (Figure 3). In another study, approximately 21 bioactive compounds were identified in the Joshanda extract. used, as against the 18 used in this study (Shareef & Masood, 2022). Although the reasons for the variations in reports are

unknown, the biological activities of Joshanda are attributed to the presence of these bioactive components. For instance, formic acid has been reported to be highly effective against some enteropathogenic bacteria, *E. coli* and *Salmonella* sp., among several organic acids tested (Gómez-García et al., 2019). In poultry production, formic acid is supplemented in poultry diets as an antimicrobial agent to limit foodborne pathogens, especially *Salmonella* spp. (Ricke et al., 2020). 4-o-methylmannose, in another report, exhibited antioxidant properties and antimicrobial action against *S. aureus* and *P. aeruginosa* (Nurhayat et al., 2023). There have also been reports of biologically active compounds from other plant-derived extracts

using GC-MS, whereby the number of bioactive compounds was greater [Olivia et al. \(2021\)](#) or less

[Gomathi et al. \(2015\)](#) than that reported in the current study, although the bioactive compounds were similar.

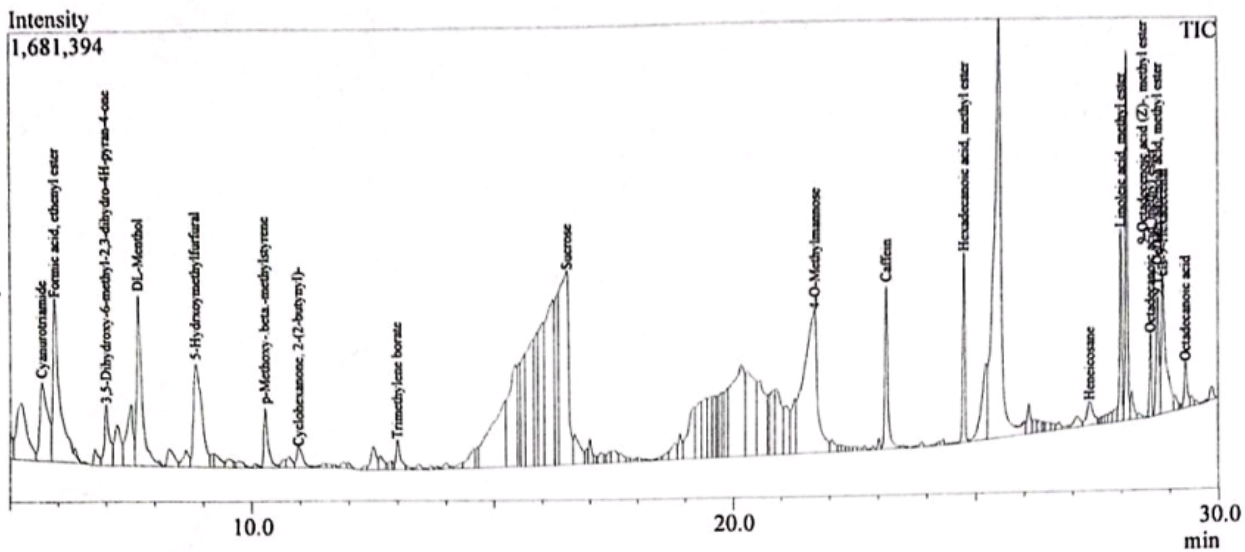


Figure 3: GC-MS chromatogram of ethanolic extract of Joshanda

Table 5: Bioactive compounds found in ethanolic extract of Joshanda

ID	Name	R. Time	Area	Conc. (%)
1	Cyanurotriamide	5.667	939817	11.11
2	Formic acid, Ethenyl ester	5.935	1797325	21.26
3	3,5-Dihydroxy-6-methyl-2,3-dihydro- 4H-pyran-4-one	6.999	319993	3.78
4	DL-Menthol	7.680	465467	5.50
5	5-Hydroxymethylfurfural	8.857	1016577	12.02
6	p-Methoxy-beta-methylstyrene	10.283	172959	2.05
7	Cyclohexanone, 2-(2-Butynyl)-	10.964	43722	0.52
8	Trimethylene borate	13.001	79689	0.94
9	Sucrose	16.542	259087	3.06
10	4-O-Methylmannose	21.691	1364182	16.13
11	Caffeine	23.160	523379	6.19
12	Hexadecenoic acid, Methyl ester	24.753	373570	4.42
13	Heneicosane	27.332	67050	0.79
14	Linoleic acid, Methyl ester	27.982	164168	1.94
15	9-Octadecenoic acid(Z)-, Methyl ester	28.101	273014	3.23
16	Octadecanoic acid, Methyl ester	28.594	166724	1.97
17	9,12-Octadecadienoic acid, Methyl ester	28.742	170247	2.01
18	Cis-9-Hexadecenal	28.856	214863	2.54

Limitation

All biological tests were performed at one extract concentration and only in duplicates. This prevents the calculation of MIC, IC₅₀, error bars, or any valid statistical comparison. Without triplicate measurements and a full dose–response series, the results remain descriptive rather than conclusive.

Conclusion

Herbal treatments, such as Joshanda, provide a hopeful alternative to manmade drugs, which are increasingly being criticized for their high cost, adverse effects, and resistance. Joshanda expressed

antibacterial activity against the selected bacterial pathogens such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Proteus mirabilis*, *Staphylococcus aureus*, and *Bacillus subtilis*, and comparable antioxidant activity with ascorbic acid using DHPP assay. Phytochemical screening and GC-MS analysis revealed the presence of a variety of phytoconstituents, including flavonoids, phenols, saponins, tannins, and terpenoids, with prominent bioactive compounds such as formic acid, 4-o-methylmannose, 5-hydroxymethylfufural, caffeine, and D1-menthol. These compounds are

believed to be responsible for the antimicrobial and antioxidant activities of Joshanda. The findings of this study show that Joshanda might have medicinal properties, as claimed by its traditional users, and provide scientific evidence for the use of Joshanda in the treatment of some human diseases. However, these findings are preliminary because of the study's descriptive nature. In future studies, the minimum inhibitory concentrations (MIC) of antibiotic activity should be determined and in vivo toxicity assessments should be conducted to ensure safe utilization as an antimicrobial product.

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