

Exploring the bioactive compounds and antimicrobial potentials in leaves and sprouts of common buckwheat (*Fagopyrum esculentum* Moench)

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ABSTRACT

Common buckwheat (*Fagopyrum esculentum* Moench) is a rich source of bioactive compounds such as rutin, quercetin, and polyphenolics that have been suggested to possess antimicrobial properties. This study aimed to evaluate *in vitro* antimicrobial activities of the methanolic extracts from leaves and sprouts of common buckwheat against clinically relevant bacterial and fungal strains, and to determine their chemical compositions using Fourier Transform Infrared Spectroscopy (FTIR). Methanolic extracts of buckwheat leaves and sprouts were studied against 10 bacterial strains (*Pseudomonas mosselii*, *Bacillus cereus*, *Escherichia coli*, *Serratia rubidaea*, *Klebsiella michiganensis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Enterococcus faecium*) and two fungal strains (*Candida albicans* and *Candida krusei*) using the agar well diffusion method. The highest zone of bacterial inhibition was observed with leaf extract (21.9 ± 0.4 mm, $p = 0.000000437$) against *S. rubidaea*, while the lowest suppression was shown against *B. cereus* with values of (16.3 ± 1.2 mm, $p = 0.0000126$), and there is no observable activity shown against *C. albicans* ($p = 0.00$). The sprout extract showed the highest activity against *S. pyogenes* (17.3 ± 0.7 mm, $p = 0.0000371$) and the lowest against *C. krusei* (15.7 ± 0.62 mm, $p = 0.0000437$). FTIR analysis shows the presence of alcohols, esters, ketones, nitro compounds, and carboxylic acids in leaf and sprout extracts of buckwheat. These functional groups are a part or function as bioactive compounds, including antimicrobials. These findings suggest that common buckwheat is a potential source of natural antimicrobial agents, which should be further investigated to identify the phytochemicals by GC-MS, LC-MS, synergistic effects, and toxicities before therapeutic applications.

INTRODUCTION

Common buckwheat (*Fagopyrum esculentum* Moench) is a pseudo cereal crop in the Polygonaceae family that has been widely cultivated for over 1,000 years [1]. Buckwheat is indigenous to southwestern China (Yunnan and Sichuan provinces) [2,3]. The plant is well-grown in Europe, North America, and Asia. Unlike other cereals and grasses (such as wheat), buckwheat is not a cereal or grass. Buckwheat is not related to wheat, as it is a member of the grass family. It has been used as a foodstuff for many years owing to its rich nutritional values, including essential amino acids, fibers, vitamins, and various bioactive phytochemicals such as rutin, quercetin, and polyphenolic compounds [4]. The phytochemicals are well documented in the literature for various activities like



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antioxidant, anti-inflammatory, and antimicrobial [5]. This common type of buckwheat is primarily cultivated in the northwestern part along the Thakurgaon, Panchagarh, and Dinajpur districts, including some places at the foothills of the Himalayas from Rangpur Division in Bangladesh [6]. Buckwheat is a type of edible seed containing such bioactive compounds as flavonoids or flavonoid-like compounds, which mostly belong to the higher plant category [7], and which include essential amino acids. Rutin is a major flavonoid that can strengthen blood vessels and may reduce the risk of neurodegenerative disease. The other important bioactive compounds include quercetin and D-chiro-inositol, which can aid in the treatment of diabetic issues [8]. Buckwheat contains a variety of phytochemicals and has shown effective antibacterial and antifungal properties, so it could be used as a potential natural drug candidate [9,10].

The growing global burden of antimicrobial resistance (AMR) has resulted in a growing search for new therapies. The interest in buckwheat is in part since it has been used as a traditional medicine, and the novel studies have found its antimicrobial activity [11]. Several assays are used for determining the potential of antimicrobials. The disk diffusion assay is commonly utilized to determine compound efficacy by evaluating the zone of inhibition on an agar plate against organisms. Furthermore, the minimum inhibitory concentration (MIC) specifies the lowest concentration required to inhibit bacterial growth [12]. Antifungal efficacy is the ability of a chemical to inhibit fungal cell growth or kill them and is critical for managing the practice of fungal infection that may be difficult to cure [13]. Antifungal activities can be quantitatively measured based on *in vitro* studies, such as the broth diffusion test, to determine the MIC of a phytochemical against fungi. Some antifungal medications disrupt the activity of fungal cell membranes, and some inhibit the synthesis of essential parts [14]. Buckwheat extracts have been shown to exhibit strong antibacterial activity against various pathogenic bacteria. Phenolic compounds like rutin and quercetin are also responsible for its antibacterial activity [4]. The methanolic extracts of buckwheat leaves and sprouts possess strong inhibitory activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*, as revealed through the results of scientific studies [15]. The action mode is mainly attributed to the damage to bacterial cell membranes and the inhibition of vital enzymes. Also, the high content of polyphenols in buckwheat increases its peptidase activity, helping it to inhibit bacterial growth and act as an antibacterial instead of chemical antibiotics [16]. Fagopyrin, the major active naphthodianthrone constituent of Fagopyrum species (buckwheat), has recently attracted substantial attention due to its diverse bioactive properties. Its activity against several pathogenic and spoilage fungi, as well as its photoinduced cytotoxicity, support the possibility of using it as a natural photosensitizer in photodynamic antifungal and anticancer therapies [17,18]. Buckwheat extracts are known for their anti-fungal activity against some fungal strains, such as *Candida albicans* and *Aspergillus niger* [19]. Buckwheat exerts its antifungal activity through, among others, disorganization of fungal cell walls and inhibition of ergosterol biosynthesis. On the other hand, the hydrophobic character of buckwheat phenolics could facilitate their membrane penetration into fungi and fungicidal role. As a green and harmless agent for controlling the fungal infection with the augmented antifungal resistance, buckwheat comes up as a feasible choice [7]. In the current study, the antimicrobial activity of methanolic extracts derived from common buckwheat leaves and sprouts was examined against ten bacterial and two fungal strains. We employed Fourier Transform Infrared Spectroscopy (FTIR) to examine the chemical composition of these extracts, which provides information about functional groups related to bioactivity and is a useful preliminary screening method for potential antimicrobial activity.

By taking common buckwheat as the only object of study, and by comparing the biological activity of its leaves and sprouts, this study attempts to provide new

viewpoints into the antimicrobial activities of the plant. The results could contribute to the preparation of plant-derived antimicrobial agents and can serve as a reference to research that needs to be carried out using more sensitive analytical methods like GC-MS or LC-MS for metabolite profiling.

MATERIALS AND METHODS

Study site

The present research work was conducted from July to December 2024 in the Departments of Biochemistry and Molecular Biology and Pharmacy, Gono Bishwabidyalay, Dhaka-1344, and the Department of Microbiology at Khwaja Yunus Ali University (KYAU), Sirajganj-6751, Bangladesh.

Chemicals and reagents

All reagents used to perform the experiments were of analytical grade. Reagents, such as methanol (Munshi Scientific, Dhaka, Bangladesh, 99.99%), ethanol (Munshi Scientific, Dhaka, Bangladesh, 99.5%), Nutrient Agar (Labtex, Dhaka, Bangladesh), NaCl (Psyche International, Dhaka, Bangladesh), NaOH (Psyche International, Dhaka, Bangladesh), Na₂CO₃ (Psyche International, Dhaka, Bangladesh), and sodium hypochlorite (Psyche International, Dhaka, Bangladesh), were used.

Collection of plant samples, bacterial, and fungal species

Common buckwheat (*Fagopyrum esculentum*) seeds were obtained from the buckwheat germplasm center of Hajee Mohammad Danesh Science and Technology University, Bangladesh [20], and seeds were grown from November 2023 to March 2024 in the Department of Biochemistry and Molecular Biology of Gono Bishwabidyalay, Bangladesh (Figure 1). Methanolic extracts from leaves and sprouts harvested were used for antimicrobial analysis. Ten pathogenic bacterial strains were selected: five for leaves (*Pseudomonas mosselii*, *Bacillus cereus*, *Escherichia coli*, *Serratia rubidaea*, *Klebsiella michiganensis*) and five for sprouts (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Enterococcus faecium*). Additionally, two fungal species, *Candida albicans* (for leaves) and *Candida krusei* (for sprouts), were used for antimicrobial activities in buckwheat extracts. These bacterial isolates were identified through morphological, biochemical, and molecular characterization after being obtained from the Department of Microbiology at Khwaja Yunus Ali University, Sirajganj, Bangladesh.

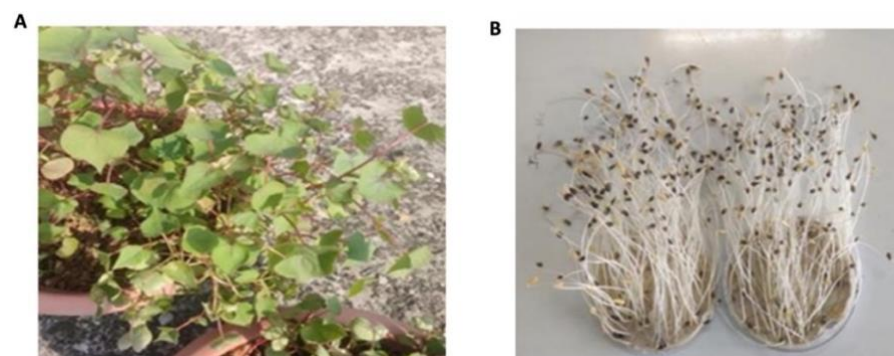


Figure 1. Plant samples of the common buckwheat. A) showing the leaves grown in the field conditions within plastic pots. B) represents the sprouts cultivated under laboratory conditions within plastic petri dishes.

Preparation of buckwheat leaves and sprout extract

Individual leaves and sprouts were removed from the branches, cut them into 1-2 mm size, properly cleaned with tap water, and dried the samples were dried at room temperature on newspaper in a shaded area, rotating them twice daily until they are fully dehydrated. The process involves preparing plant material for laboratory grinding by removing foreign compounds and sand particles from the foliage. Methanolic extraction of plant samples was performed according to the method as described earlier [21]. About 100 grams of coarsely powdered plants were macerated in 600 mL of 80% methanol for a week at room temperature. After 72 hours, the filtrate was collected with Whatman No. 1 filter paper, and the marc was macerated two more times. A rotary evaporator at 40°C removed the alcohol, and the extracts were dried in a water bath and stored at 4°C.

Media and disk preparation

To prepare Mueller-Hinton agar, 0.1 g of methylene blue dye was dissolved in 20 ml of distilled water by gently heating. Then 100 µl of this dye solution was added, and 20 g of glucose per liter of agar was suspended. Following the manufacturer's instructions, the autoclaving process was conducted. After that, once the temperature of the agar solution reached 45-50 °C, it was poured into Petri dishes to a depth of about 4 mm (67-70 ml for 150 mm plates, 28-30 ml for 100 mm plates). The pH should be maintained between 7.2 and 7.4, and the plates should be used within seven days unless sealed. Sterility check was performed by culturing a sample at 30-35 °C for 24 hours and conducting quality control testing [11].

Estimation of the antimicrobial activity of the *F. esculentum* leaves and sprouts methanolic extracts by agar cap method

Methanolic extracts of the buckwheat leaves and sprouts were evaluated at three concentrations (50 µl, 100 µl, and 150 µl) using the agar well diffusion method. Ciprofloxacin (10 µg/disc) and Nystatin (100,000 Units/ml) were used as positive controls for bacteria and fungi, respectively, based on their broad-spectrum efficacy and established standard use in antimicrobial assays [22]. Control concentrations were matched with experimental conditions. Bacterial strains were standardized according to the diffusion guidelines established by the Clinical and Laboratory Standards Institute (CLSI) [23]. New colonies were suspended in sterile saline and calibrated to a 0.5 McFarland standard (about 2×10^8 CFU/mL). A sterile swab was used to streak the suspension in three directions, creating a confluent lawn on Mueller–Hinton agar; the plates were permitted to dry for approximately 5 to 10 minutes. Sterile agar caps (6 mm in diameter) were subsequently positioned on the infected surface and infused with the test plant extracts at designated doses (50, 100, and 150 µl per cap), in conjunction with the suitable positive control. Plates were incubated at 37°C for 24 hours, and inhibition zones were measured in millimetres. The entire process of the antimicrobial susceptibility test and FTIR analysis of the methanolic extract is depicted in Figure 2.



Figure 2. Schematic representation of all procedures for isolating bioactive compounds and determining antibacterial potential of buckwheat (*Fagopyrum esculentum* Moench).

Determination of antimicrobial compounds from extracted samples by FTIR

Fourier Transform Infrared (FTIR) spectra were acquired using a vintage JASCO FT/IR-400Plus (JASCO corporation, Tokyo, Japan) spectrometer across the range of 400-4000 cm^{-1} , applying the sodium chloride pellet method [24]. In total, 100 mg of chloroform and 1 mg of the sample were mixed for analysis, with the intensity plotted against the wave number.

Statistical analysis

The data were used to explore mean, standard deviation, one-way ANOVA, plotting, and Tukey HSD analyses were performed using R Studio v. 4.2.3 based on the zone (mm) of inhibition of the methanolic extracts of buckwheat leaves and sprouts.

RESULTS

Antimicrobial activity of buckwheat leaf extracts

Crude methanolic leaf extracts of buckwheat showed significant inhibitory activity against five pathogenic bacteria (four being Gram-negative bacteria, namely *P. mosselii*, *E. coli*, *S. rubidaea*, and *K. michiganensis*) and one Gram-positive bacterium (*B. cereus*). *S. rubidaea* was most sensitively inhibited in a concentration-dependent manner. The maximum inhibition zone found was $(21.9 \pm 0.4 \text{ mm})$ at 150 μL concentration (Table 1). As for the inhibitory activity against *P. mosselii*, this was observed at 150 μL (15 ± 0.1), whereas the methanolic extract showed a reduced zone of inhibition at 50 μL . The inhibitory zones observed for *K. michiganensis* were $8.93 \pm 0.9 \text{ mm}$, $14 \pm 1 \text{ mm}$, and $16.2 \pm 1.31 \text{ mm}$ at concentrations of 50, 100, and 150 μL , respectively. The lowest activity against *E. coli* was seen for the common buckwheat methanolic extract, with a mean $\pm$ SD of 11.9 ± 1.01 at a concentration of 150 μL , and we did not find any activity against *C. albicans*.

One-way ANOVA indicated statistically significant differences in zone of inhibition among all the bacterial species tested ($p < 0.001$). The maximum F value of 299.90 was

observed in *S. rubidaea*, followed by *B. cereus* and *K. michiganensis*. In contrast, no inhibition was detected for *C. albicans*, suggesting that our therapeutic did not have a relevant antifungal effect against this pathogen (Table 2). The antibacterial activity of three concentrations (50, 100, and 150 μ L) was evaluated against five bacterial strains. The results showed a clear trend of $150 > 100 > 50 \mu\text{L}$ in terms of inhibition zone diameters (Table 3 and Figure 3).

Table 1. The MIC values of the crude methanolic extract for common buckwheat leaves against bacterial and fungal species.

Organism	Minimum inhibitory zone (mm)			Ciprofloxacin (5 μ g)/ Nystatin (1 lakh unit/mL)
	50 μ L (Mean $\pm$ SD)	100 μ L (Mean $\pm$ SD)	150 μ L (Mean $\pm$ SD)	
<i>Pseudomonas mosselii</i>	8.9 $\pm$ 0.36	14 $\pm$ 0.2	15 $\pm$ 0.1	26.28 $\pm$ 0.275
<i>Bacillus cereus</i>	6.3 $\pm$ 0.66	10.9 $\pm$ 0.85	16.3 $\pm$ 1.2	20.25 $\pm$ 0.25
<i>Escherichia coli</i>	4.7 $\pm$ 0.7	7.5 $\pm$ 0.5	11.9 $\pm$ 1.01	20.18 $\pm$ 0.18
<i>Serratia rubidaea</i>	7.8 $\pm$ 0.72	15.2 $\pm$ 0.68	21.9 $\pm$ 0.4	27.125 $\pm$ 0.125
<i>Klebsiella michiganensis</i>	8.93 $\pm$ 0.9	14 $\pm$ 1	16.2 $\pm$ 1.31	29.23 $\pm$ 0.225
<i>Candida albicans</i>	0 $\pm$ 0.00	0 $\pm$ 0.00	0 $\pm$ 0.00	0 $\pm$ 0.00

Table 2. Summary of the one-way ANOVA performed for the zone of inhibition of the methanolic extract of common buckwheat leaves against bacterial and fungal species.

Organism	F Value	Df (Between, Within)	p Value
<i>Pseudomonas mosselii</i>	40.23	2, 6	0.000367
<i>Bacillus cereus</i>	146.25	2, 6	0.0000126
<i>Escherichia coli</i>	79.82	2, 6	0.0000745
<i>Serratia rubidaea</i>	299.90	2, 6	0.000000437
<i>Klebsiella michiganensis</i>	83.32	2, 6	0.000478
<i>Candida albicans</i>	0.00	0.00	0.00

Table 3. Results of Tukey's HSD test for multiple comparisons of zone of inhibition values among leaf extract concentrations.

Organism	Comparison	Mean Difference (mm)	p Value	Significant Status	Significant Order
<i>Pseudomonas mosselii</i>	50 vs 100	5.7	0.0011	Significant ($p < 0.01$)	150 > 100 > 50 μ L
	50 vs 150	6.83	0.0004	Highly significant ($p < 0.01$)	
	100 vs 150	1.14	0.41	No significance ($p > 0.01$)	
<i>Bacillus cereus</i>	50 vs 100	4.6	0.00076	Significant ($p < 0.01$)	150 > 100 > 50 μ L
	50 vs 150	9.8	0.0000094	Highly significant ($p < 0.01$)	
	50 vs 100	5.2	0.00036	Significant ($p < 0.01$)	
<i>Escherichia coli</i>	50 vs 150	2.8	0.0099	Significant ($p < 0.01$)	150 > 100 > 50 μ L
	100 vs 150	7.2	0.000061	Highly significant ($p < 0.01$)	
	100 vs 150	4.4	0.00095	Significant ($p < 0.01$)	
<i>Serratia rubidaea</i>	50 vs 100	7.4	0.000014	Significant ($p < 0.01$)	150 > 100 > 50 μ L
	50 vs 150	14.1	0.0000005	Highly significant ($p < 0.01$)	
	100 vs 150	6.7	0.000027	Significant ($p < 0.01$)	
<i>Klebsiella michiganensis</i>	50 vs 100	5.06	0.0029	Significant ($p < 0.01$)	150 > 100 > 50 μ L
	50 vs 150	7.26	0.00043	Highly significant ($p < 0.01$)	
	100 vs 150	2.2	0.104	No significance ($p > 0.01$)	

There were significant differences ($p < 0.01$) for *P. mosselii* between the two 100-50 and 150-50 μ L groups, and no significant differences between the two 150 and 100 μ L groups. For *B. cereus*, *E. coli*, and *S. rubidaea*, increases were statistically significant in all comparisons. When comparing the volumes of 150 and 50 μ L, *S. rubidaea* provided the largest mean increase of 14.1 mm, while *K. michiganensis* showed a similar trend, with the comparison between 150 and 100 μ L being non-significant ($p > 0.01$). A strong dose-dependent response associated with antibiotic action was observed with the highest impact in *S. rubidaea* and the lowest in *P. mosselii*.

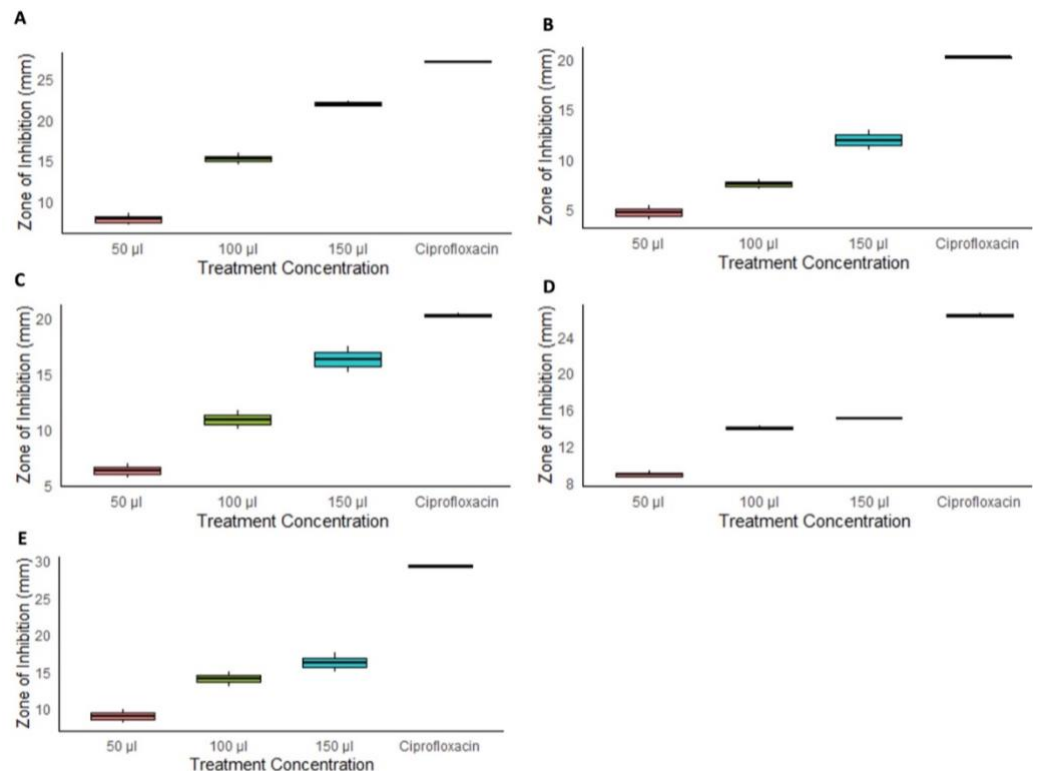


Figure 3. Illustrations showing the inhibitory efficacy (mm) of the three concentrations (50, 100, and 150 µl) of methanolic extract from common buckwheat leaves against several bacterial species. A) *Pseudomonas mosselii*, B) *Bacillus cereus*, C) *Escherichia coli*, D) *Serratia rubidaea*, and E) *Klebsiella michiganensis*. Ciprofloxacin was used as a positive control, demonstrating the most significant antimicrobial efficacy in all cases. The 150 µl extract concentration exhibits the most significant antibacterial efficacy against all selected pathogens.

Antimicrobial activity of the buckwheat sprout extracts

Three doses (50, 100, and 150 µL) of the extracts were examined against six pathogens for antimicrobial activity. For all species, the zone of inhibition increased in a dose-dependent manner. *K. pneumoniae* was moderately sensitive to the selected concentrations of sprouts extracts with inhibition zones of 9.83 ± 0.57 mm at 50 µL and 14.9 ± 0.9 mm at 150 µL, and much greater than that of ciprofloxacin, which had zones of inhibition of 28.22 ± 0.225 mm (Table 4). The bacteria *S. aureus* and *P. aeruginosa* showed moderate sensitivity at 50 µL (around 5.4 and 5.3 mm, respectively) but became markedly more sensitive at 150 µL; the strongest inhibition was found in the case of *S. pyogenes*, where inhibition reached 17.3 ± 0.7 mm at that dose and *E. faecium*, which had a notable rise from 3.67 ± 0.59 mm to 15.4 ± 0.72 mm (both effective, $P \leq 0.01$), indicating potentially dose-responsive behaviour. The only no-biofilm-forming found to be susceptible (*C. krusei*) had larger inhibition zones at high doses, but nystatin (24.32 ± 0.325 mm) was more effective.

The results of the one-way ANOVA comparing the zone of inhibition revealed a statistically significant ($p < 0.01$) difference for all microbes analyzed at three concentrations of the methanolic extract (Table 5). *E. faecium* showed the highest F-value (217.02), suggesting a strong dose-dependent response, followed by *P. aeruginosa* (142.74) and *S. aureus* (107.44). The mean score of inhibition shows a pronounced variation by methanolic extract against *C. krusei* ($F = 59.91$), indicating the antifungal activity of the extract as well. The results showed that common buckwheat sprouts extract inhibited the growth of bacteria, and the methanolic extract of common buckwheat sprouts

exhibited effective antimicrobial activity, and this activity increased with increasing concentration. Although Tukey's HSD test revealed that the methanolic extract exhibited a more pronounced antimicrobial effect that correlated with increasing volume (50-150 μ L) across all tested organisms, all doses were relatively effective against all organisms. Between 50 and 100 μ L and between 50 and 150 μ L, the differences were also significant ($p < 0.01$) (Table 6 and Figure 4). However, for *K. pneumoniae*, *E. fecium*, and *C. krusei*, no significant difference was detected for 150 and 100 μ L. Overall, the best activity was shown with 150 μ L, particularly for *E. faecium* and *P. aeruginosa*.

Table 4. The MIC values for the crude methanol extract of common buckwheat sprouts against bacterial and fungal species.

Organism	Minimum inhibitory zone (mm)			Ciprofloxacin (5 μ g)/ Nystatin (1 lakh unit/mL)
	50 μ L (Mean $\pm$ SD)	100 μ L (Mean $\pm$ SD)	150 μ L (Mean $\pm$ SD)	
<i>Klebsiella pneumoniae</i>	9.83 $\pm$ 0.57	12.7 $\pm$ 1.08	14.9 $\pm$ 0.9	28.22 $\pm$ 0.225
<i>Pseudomonas aeruginosa</i>	5.43 $\pm$ 0.51	11.4 $\pm$ 0.6	15.1 $\pm$ 0.85	25.38 $\pm$ 0.375
<i>Staphylococcus aureus</i>	5.33 $\pm$ 0.8	10.2 $\pm$ 0.57	13.8 $\pm$ 1.27	22.24 $\pm$ 0.24
<i>Streptococcus pyogenes</i>	9.4 $\pm$ 0.87	14.5 $\pm$ 0.64	17.3 $\pm$ 0.7	23.28 $\pm$ 0.275
<i>Enterococcus faecium</i>	3.67 $\pm$ 0.59	11.9 $\pm$ 0.31	15.4 $\pm$ 0.72	22.43 $\pm$ 0.425
<i>Candida krusei</i>	9.63 $\pm$ 0.65	14.2 $\pm$ 0.53	15.7 $\pm$ 0.62	24.32 $\pm$ 0.325

Table 5. Summary of the one-way ANOVA performed for the zone of inhibition of the methanolic extract of common buckwheat sprouts against bacterial and fungal species.

Organism	F Value	Df (Between, Within)	p Value
<i>Klebsiella pneumoniae</i>	39.22	2, 6	0.00117
<i>Pseudomonas aeruginosa</i>	142.74	2, 6	0.00000645
<i>Staphylococcus aureus</i>	107.44	2, 6	0.0000952
<i>Streptococcus pyogenes</i>	96.42	2, 6	0.0000371
<i>Enterococcus faecium</i>	217.02	2, 6	0.000000674
<i>Candida krusei</i>	59.91	2, 6	0.0000437

Table 6. Tukey's HSD post-hoc test showing pairwise differences in the zone of inhibition between sprout extract concentrations.

Organism	Comparison	Mean difference (mm)	p Value	Significant status	Significant order
<i>Klebsiella pneumoniae</i>	100–50	2.86	0.016	Significant ($p < 0.01$)	150 > 100 > 50 μ L
	150–50	5.1	0.00094	Highly significant ($p < 0.01$)	
	150–100	2.23	0.046	No significance ($p > 0.01$)	
<i>Pseudomonas aeruginosa</i>	100–50	5.96	0.000088	Highly significant ($p < 0.01$)	150 > 100 > 50 μ L
	150–50	9.66	0.0000049	Highly significant ($p < 0.01$)	
	150–100	3.7	0.0012	Significant ($p < 0.01$)	
<i>Staphylococcus aureus</i>	100–50	4.83	0.0016	Significant ($p < 0.01$)	150 > 100 > 50 μ L
	150–50	8.43	0.000076	Highly significant ($p < 0.01$)	
	150–100	3.6	0.0074	Significant ($p < 0.01$)	
<i>Streptococcus pyogenes</i>	100–50	5.13	0.00036	Significant ($p < 0.01$)	150 > 100 > 50 μ L
	150–50	7.9	0.000031	Highly significant ($p < 0.01$)	
	150–100	2.76	0.0092	Significant ($p < 0.01$)	
<i>Enterococcus faecium</i>	100–50	8.26	0.0000044	Significant ($p < 0.01$)	150 > 10 > 50 μ L
	150–50	11.7	0.0000008	Highly significant ($p < 0.01$)	
	150–100	3.43	0.00074	No significance ($p > 0.01$)	
<i>Candida krusei</i>	100–50	4.56	0.00021	Significant ($p < 0.01$)	150 > 100 > 50 μ L
	150–50	6.06	0.00004	Highly significant ($p < 0.01$)	
	150–100	1.5	0.051	No significance ($p > 0.01$)	

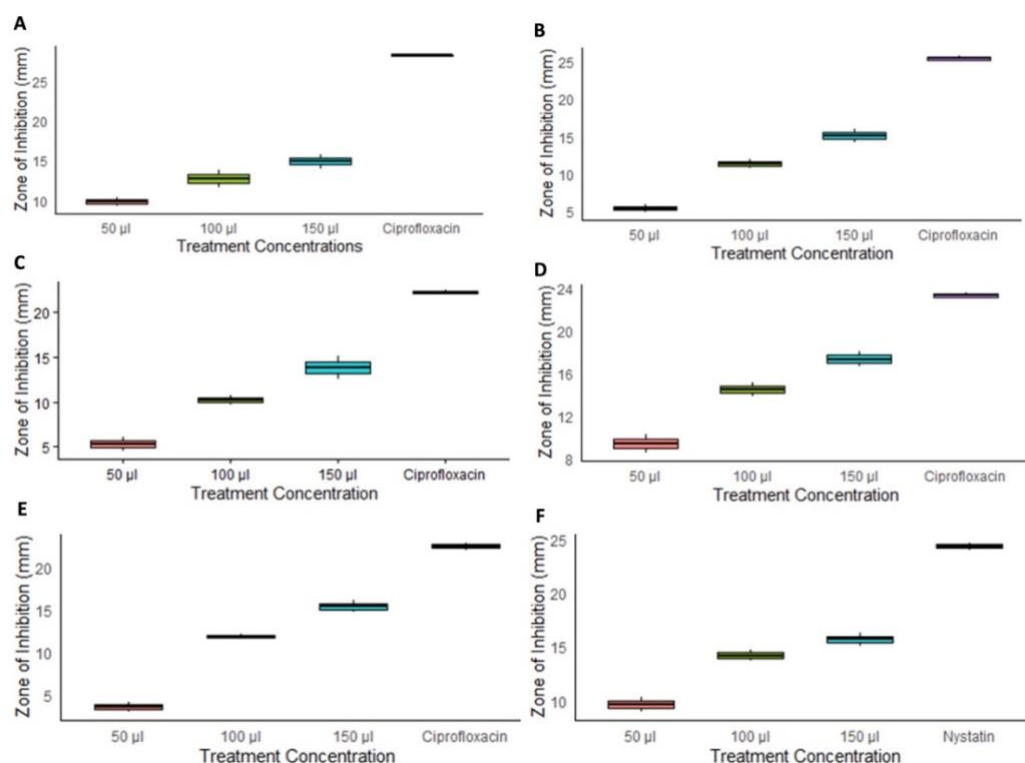


Figure 4. Illustrations showing the inhibitory efficacy (mm) of the three concentrations (50, 100, and 150 µl) of methanolic extract from common buckwheat sprouts against several bacterial and fungal species. Panels: (A) *Klebsiella pneumoniae*, (B) *Pseudomonas aeruginosa*, (C) *Staphylococcus aureus*, (D) *Streptococcus pyogenes*, (E) *Enterococcus faecium*, and (F) *Candida krusei*. Ciprofloxacin (bacterial) and Nystatin (fungal) served as positive controls, demonstrating the most significant antimicrobial efficacy in all cases. The 150 µl extract concentration exhibits the most significant antibacterial efficacy against all selected pathogens.

Characterization of bioactive compounds in buckwheat leaf and sprout extracts using FTIR spectroscopy

FTIR Spectroscopy analysis was an essential part of this study, as it systematically facilitated the identification and characterization of the functional groups in the methanolic extracts of buckwheat leaves and sprouts. Strong peaks at 1712 cm^{-1} and 3379 cm^{-1} were also seen due to the presence of C=O functional groups and O-H stretching vibrations, respectively, which indicated the presence of bioactive compounds with recorded antibacterial activities. In addition to providing support for the observed patterns of microbiological inhibition, this molecular-level characterization lays the groundwork for investigations into the structure–activity relationship, which enables the targeted isolation and pharmacological optimization of putative antimicrobial components.

Table 7 shows the peaks at 3394.72, 3008.95, 2924.09, 2854.65, 2360.87, 1735.93, 1651.07, 1512.19, 1350.17, 1249.87, 1103.28, 833.25, 756.10, 671.23, and 509.21 cm^{-1} . These correspond to functional groups including phenols, alcohols, and minor amine contribution (O-H stretching); aromatic or alkene C-H stretching (=C-H); aliphatic $-\text{CH}_2/-\text{CH}_3$ stretching; atmospheric CO_2 asymmetric stretch; ester or carboxylic acid C=O stretching; conjugated/aromatic C=C with possible amide I or C=N contribution; aromatic C=C or nitro group N-O stretching; phenolic O-H bending, nitro group N-O bending, or C-N stretching; alkyl aryl ether C-O stretching; aliphatic ether C-O stretching; and aromatic C-H out-of-plane bending for monosubstituted rings. However, this assignment is tentative and could also represent other low-frequency skeletal modes

(Figure 5A). The FTIR spectral study of buckwheat leaves, therefore, indicates the presence of phenolic/alcoholic groups, aromatic rings, aliphatic chains, carbonyl-containing functionalities (esters/acids), and nitro groups.

Table 8 shows the peaks at 3379.29, 2924.09, 2854.65, 1712.79, 1651.07, 1512.19, 1373.32, 1172.72, 1056.99, and 671.23 cm^{-1} . These correspond to functional groups including broad O-H stretching of alcohols or phenols; aliphatic $-\text{CH}_2/-\text{CH}_3$ stretching modes of alkanes; strong C=O stretching from aldehydes, ketones, esters, or carboxylic acids; conjugated C=C stretching in alkenes or aromatic rings; N-O stretching of nitro groups; C-H bending of methyl groups; C-O stretching in ester or ethers; strong S=O stretching from sulfoxides; and aromatic C-H out-of-plane bending. The substantial broad absorption around 3350-3400 cm^{-1} is most consistent with hydrogen-bonded O-H groups, though some N-H contribution from amines or amides cannot be excluded. Overall, the FTIR profile of buckwheat sprout extract suggests the presence of alcohols/phenols, aliphatic chains, carbonyl-containing compounds, nitro groups, sulfoxides, and aromatic structures (Figure 5B). While these group-level identifications are consistent with plant-derived metabolites such as phenolic acids, flavonoids, and alkaloids, FTIR alone cannot confirm specific compounds without complementary analytical methods.

Table 7. Identified compounds using FTIR of buckwheat leaves.

Peak Position	Group	Class	Peak Details
3394.72	O-H Stretching	Phenols, alcohols, amines (minor)	Medium
3008.95	=C-H stretch	Aromatics/alkenes	Strong, Broad
2924.09	CH_2/CH_3 asymmetric stretch	Alkanes, fatty chains	Strong, Broad
2854.65	CH_2/CH_3 symmetric stretch	Alkanes, fatty chains	Weak, Broad
2360.87	CO_2 (asymmetric stretch)	Atmospheric contaminant	Strong, Broad
1735.93	C=O Stretching	Esters, carboxylic acids	Strong
1651.07	C=C (conjugated/aromatic) $\pm$ amide I; C=N tentative	Aromatics, proteins, imines	Strong
1512.19	Aromatic C=C / N-O Stretching	Aromatics, nitro compounds	Strong
1350.17	Phenolic O-H bending/N-O (nitro)/ C-N	Phenols, nitro compounds, amines	Medium
1249.87	C-O Stretching	Alkyl Aryl Ether	Strong
1103.28	C-O Stretching	Aliphatic Ether	Strong
833.25	C-H out-of-plane bend	Aromatics (mono-substituted)	Medium
756.1	C-H out-of-plane bend	Aromatics (mono-substituted)	Strong
671.23	C-H out-of-plane bend	Alkene	Medium
509.21	C-I Stretching	Halo Compound	Strong

Table 8. Identified compounds using FTIR of buckwheat sprouts.

Peak Position	Group	Class	Peak Details
3379.29	O-H stretching	Alcohol	Strong, Broad
2924.09	C-H stretching	Alkane	Strong, Broad
2854.65	C-H stretching	Alkane	Strong, Broad
1712.79	C=O stretching	Aldehyde / Ketone / Ester / Carboxylic Acid	Strong
1651.07	C=C Stretching	Alkene/Aromatic	Strong
1512.19	N-O Stretching	Nitro Compound	Strong
1373.32	C-H Bending	Alkane ($-\text{CH}_3$)	Medium
1172.72	C-O Stretching	Ester/Ether	Strong
1056.99	S=O Stretching	sulfoxide	Strong
671.23	C-H bending (out of plane)	Aromatic	Strong

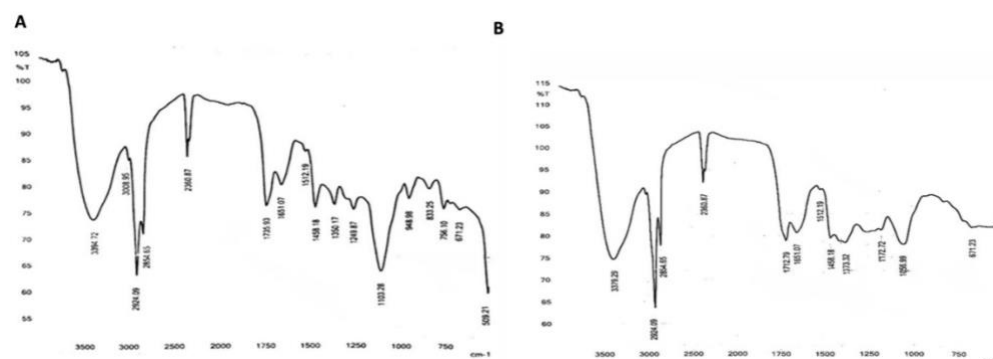


Figure 5. Comparative FTIR spectra analysis of the methanolic extract of Buckwheat leaves. This leaf extract contains many phytochemical components. A), leaf extracts FTIR spectra, which mostly consist of hydroxyl, carbonyl, aromatic, and alkene functional groups. B) sprout extract's FTIR spectra, which demonstrate that it contains several phytochemical components due to the abundance of alkene, aromatic, nitro, and sulfoxide functional groups.

DISCUSSION

Antimicrobial resistance is often regarded as one of the most significant public health threats to global humanity. Therefore, it is crucial to understand the emergence, development, and consequences of the disease and its controlling measures. The current study explored the antioxidant capacity, antimicrobial activity, and chemical composition of methanolic extracts of buckwheat leaves and sprouts. It was shown by evaluating the results of the MIC assay that the leaf and sprout extracts were effective against several pathogenic bacteria and fungi species. Furthermore, FTIR spectroscopy indicated the presence of different chemical groups characteristic of a variety of bioactive compounds. MIC values of methanolic extracts from buckwheat leaves and sprouts against various Gram-positive and Gram-negative bacterial strains, as well as filamentous fungi, indicate high antimicrobial activity.

The significant inhibition of leaf extract against *P. mosselii*, *S. rubidaea*, *E. coli*, and *K. michiganensis* suggests that the buckwheat leaf extract possesses potential and is active even against pathogenic bacteria, but not against *C. albicans*, revealing that its potential can vary from organism to organism. For *E. coli*, *B. cereus*, and *S. rubidea*, no MIC was shown, which indicates that the volume of the test solution could affect the efficiency of the antimicrobial [3, 8, 25]. At lower concentrations, MICs were not detected, indicating that the sensitivity limit dropped below the minimum inhibitory concentration of the doses administered, or that these doses only induced partial resistance. These results suggest that further testing using narrower concentration ranges is needed. Likewise, the extract of the sprout exhibited antimicrobial potential against a wider range of bacteria, including *K. pneumoniae*, *P. aeruginosa*, *S. pyogenes*, *S. aureus*, *E. faecium*, and *C. krusei*. These results suggest that buckwheat leaves and sprouts are a promising source of bioactive substances with antimicrobial activity. The data show that the concentration of the medium during the experiment might influence the antimicrobial action, as higher volumes may provide better diffusion and homogenization rates of the antimicrobial.

A study by Zhong *et al.* [25] reported that the methanolic extract of the sprouts exhibited potent antibacterial activity against *P. lachrymansa*, *B. subtilis*, *S. albus*, and *S. aureus*, consistent with our results. A study by Zhou *et al.* [26] also reported research and extended the spectrum of activity of the antimicrobial as MIC values for additional microorganisms, including *Serratia rubidaea* and *Klebsiella michiganensis*, were provided. Moreover, Atambayeva *et al.* [27] also reported that crude methanol extract of new tertiary buckwheat leaves exhibited strong inhibitory potential against various bacteria,

including Gram-negative (*P. lachrymal*, *S. Typhimurium*) as well as Gram-positive (*B. subtilis*, *B. albus*, and *S. aureus*), with a MIC. The recorded antimicrobial activity is in accordance with previous work and other studies published on several extracts of buckwheat rich in flavonoids and phenolic acids, possessing an intensive antimicrobial nature [28]. Further, the antimicrobial properties of these compounds could be due to their antioxidant function, reducing oxidative stress, which destroys the cell wall of microorganisms [29]. The contents of rutin, quercetin, and kaempferol, as well as their antibacterial activity, were determined in sprouts formed from common seeds of buckwheat. The differences in microorganism inhibition may be due to the extraction process, solvent used, or existing environmental conditions [30]. Further, extracts of the above sprouts exhibited moderate antifungal activity against *C. krusei*, indicating that phytochemicals (phenolic acids, flavonoids, and fagopyrin) led to fungal cell integrity disruption through an antioxidant-mediated oxidation stress mechanism [17, 29].

FTIR analysis of the methanolic extracts of the leaves and sprouts of common buckwheat revealed the existence of various bioactive functional groups having significant therapeutic potential. The characteristic O-H stretching peaks confirmed the existence of Phenols, alcohols, and amines (trace). We observed ester groups, which are indicative of flavonoid glycosides that have anti-inflammatory and antimicrobial activities. Further, the peaks in the nitro group may also be due to alkaloids. Alkaloids are known for their diverse biological activities, such as bactericidal and tumoricidal properties.

A report by Kokalj Ladan *et al.* [31] also observed the same functional characteristics in buckwheat extracts (alcohols, carboxylic acids, esters), and these compounds were associated with phenolic and flavonoid presence. However, Dumitru *et al.* [32] also performed a complete study of the functional groups in buckwheat leaves and sprouts and pointed out that, depending on the stage of growth, the chemical composition might differ between both systems. This is a particular output since it indicates that different parts of the buckwheat plant may not contain the same concentration and distribution of bioactive compounds, which can affect their medicinal value.

The potent antimicrobial activity shown by the crude extracts in this study suggests that these medicinal plants can be used as an alternative natural remedy for common infections. Promotion of such plant-based alternatives can lead to healthcare practices that are more environmentally friendly and culturally sensitive, reduce dependence on imported pharmaceuticals, and stimulate the growth of a local herbal medicine industry. Thus, while FTIR analysis confirms the bioactive ingredients, it cannot determine an extract's chemical makeup. Further novel quantification of antimicrobial substances is required using modern analytical techniques such as GC-MS, HPLC, or LC-MS. These tools are crucial for quantifying new or novel metabolites, which can be used as therapeutic agents in the pharmaceutical industry.

CONCLUSIONS

This study explores the potential of leaves and sprouts of common buckwheat (*F. esculentum*) as novel sources of natural antimicrobial agents. Methanolic extracts of leaves and sprouts possessed significant inhibitory activity against a wide range of bacterial and fungal pathogens, with the maximum activity exhibited by the leaf extract for *S. rubidaea* and sprout extract for *S. pyogenes*. FTIR analysis showed the existence of certain bioactive functional groups, including alcohols, ketones, esters, and nitro groups that correlated with the antimicrobial properties, respectively. Although FTIR spectra can be readily interpreted in terms of functional groups, future work should involve analysis using compound-specific techniques (GC-MS, LC-MS) for greater clarity and

precision in compound characterization and quantification. Furthermore, combination assays with reference antibiotics and cytotoxicity studies are needed to clarify their therapeutic efficacy. The results also reveal the prospect of using common buckwheat as a source for plant-based novel antimicrobial agents used in pharmaceuticals, food preservatives, and healthcare benefits.

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AUTHOR CONTRIBUTIONS

MRS: conceptualization, investigation, methodology, formal analysis, writing original draft, writing, review and editing; MA: writing original draft, writing, review and editing; MZA: validation, resources; MEU: supervision; AH: writing original draft; MARR: formal analysis; MMRS: resources; MAS: supervision, validation, resources. All authors read and approved the final version of the manuscript.

DECLARATION ON USING AI-ASSISTED TOOLS

The author(s) declare that no AI-assisted tools have been used during preparation of this manuscript.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

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