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Antimicrobial Potential of Fagopyrum esculentum Monech Stem Extract: Phytochemical Characterization and Efficacy Against Drug-Resistant Pathogens

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ABSTRACT

The escalating crisis of antimicrobial resistance (AMR) demands novel sources of antimicrobial agents. This study investigates the antimicrobial potential of *Fagopyrum esculentum* (common buckwheat) stem, integrating phytochemical analyses with in vitro antimicrobial assays against a panel of pathogenic microbes. Comprehensive phytochemical screening of the buckwheat stem revealed abundant flavonoids and phenolics; quantitative assays showed high total phenolic (1.52 mg GAE/g) as well as flavonoid (0.14 mg QE/g) contents in extract that is methanolic. Pharmacognostic fluorescence analysis further corroborated the presence of these phytochemicals. Antimicrobial efficacy was evaluated via agar diffusion assays against Staphylococcus aureus, Enterococcus faecalis, Mycobacterium tuberculosis, and a representative Enterobacteriaceae strain (*E. coli/Klebsiella*). The buckwheat stem extract exhibited dose-dependent inhibition of all tested microbes. Notably, at the highest concentration (3 mg/disc), it produced substantial zones of inhibition against *S. aureus* (~15–16 mm) and *M. tuberculosis* (~15–18 mm), indicating significant activity comparable to standard antibiotics' susceptibility thresholds. Moderate activity was observed against *Enterococcus* (12–15 mm zone at 3 mg) and weaker effects against Gram-negative Enterobacteriaceae (8–12 mm at 3 mg). The extract's broad-spectrum antibacterial action, especially towards Gram-positive and acid-fast organisms, is attributed to its high polyphenolic content. Potential mechanisms, such as membrane disruption and enzyme inhibition by flavonoids, are discussed. These findings highlight *F. esculentum* stem as a promising natural source of antimicrobial compounds, meriting further investigation for development into plant-based antimicrobials in the fight against resistant infections.

Keywords: *Fagopyrum esculentum; Antimicrobial activity; Phytochemical screening; Phenolic compounds; Flavonoids; Agar diffusion assay; Drug-resistant pathogens*

1. INTRODUCTION

One of the biggest risks to world health today is antimicrobial resistance. Drug-resistant diseases now cause at least 700,000 deaths annually; if immediate action is not done, this number might rise to 10 million annually by 2050[1,2]. The situation is particularly alarming in countries like India, often dubbed the "AMR capital of the world" due to the high prevalence of multidrug-resistant pathogens[3,4]. In this context, there is an imperative need to explore new antimicrobial agents, including those derived from medicinal plants. Historically, plants have been a rich source of antibiotics and antiseptics, and many phytochemicals (e.g. phenolics, flavonoids, essential oils) possess antimicrobial properties[5,7].

Fagopyrum esculentum (common buckwheat) is widely known as a nutritious pseudocereal and traditional remedy. While buckwheat is renowned for its cardiovascular and antioxidant benefits, emerging evidence also points to its antimicrobial potential [8-11]. Buckwheat plants are rich in secondary metabolites: the seeds and hulls contain high levels of phenolic compounds like rutin and quercetin derivativesfoodandfeed.fins.uns.ac.rs, and the flowers yield essential oils with antibacterial activity[10,12,13]. These phytochemicals, especially flavonoids, are known to exert antimicrobial effects through diverse methods, that includesinterference with enzyme function, disruption of microbial membranes and inhibition of nucleic acid synthesis[6,14]. For instance, rutin and quercetin have demonstrated bacteriostatic effects against Gram-positive bacteria in prior studies. Additionally, buckwheat is in folk medicine to treat wounds and skin contaminations,

implying antimicrobial as well as wound-healing properties of the plant [15]. Despite this, the stem of *F. esculentum* has been relatively under-investigated for antimicrobial activity, as attention has largely focused on grain and flower extracts [11,16]. The current study aims for filling this gap by assessing the antimicrobial potential of *F. esculentum* stem. We first characterize the phytochemical profile of the stem extract, including total phenolic and flavonoid content and fluorescence analysis for compound classes. We then assess its antimicrobial efficacy against a spectrum of pathogens that are medically significant and often drug-resistant: *Staphylococcus aureus* (Gram-positive cocci, common cause of skin and systemic infections, often multi-resistant), *Enterococcus faecalis* (Gram-positive cocci, noted for hospital-acquired infections and vancomycin resistance), *Mycobacterium tuberculosis* (acid-fast bacillus, cause of TB, with rising drug resistance), and a representative Gram-negative Enterobacteriaceae (opportunistic pathogens like *E. coli/Klebsiella*, known for ESBL and carbapenem resistance) [17-19]. Zone of inhibition assays and corresponding CLSI interpretative standards are used to gauge the extract's efficacy at different concentrations. We hypothesize that the buckwheat stem extract will show significant antibacterial effects, particularly against Gram-positive organisms, due to its high polyphenolic content. Demonstrating such activity would support using*F. esculentum*like a natural agent that is antimicrobial in nature and possibly guide the isolation of specific anti-infective compounds from this plant. Ultimately, this research contributes to the search for plant-derived solutions to the AMR crisis, aligning with the "One Health" approach by exploring an agricultural resource (buckwheat) for addressing human infectious disease challenges [9,14,20].

2. MATERIALS AND METHODS

2.1 Plant Material and Pharmacognostic Evaluation

Collection and Authentication: Aerial parts (stems and leaves) of *F. esculentum* were collected from a suitable habitat and authenticated by a botanist. Herbarium specimen was deposited for reference [8].

Macroscopic and Microscopic Analysis: Pharmacognostic characterization of the stem was performed. Macroscopic features (color, texture, fracture, taste) were recorded. Microscopic examination of stem cross-sections and powdered stem was done using standard techniques [21].

Physicochemical Parameters: Standard physicochemical constants were determined for the dried stem powder: water, total ash, acid-insoluble ashand alcohol extractive values, moisture content, etc. [21,22].

Fluorescence Analysis: Stem (powdered) drug had been treated with many reagents as well as observed under UV (254 nm, 365 nm) and visible light [23].

2.2 Preparation of Extracts and Phytochemical Screening

Extraction Procedure: The dried stems were powdered and successively extracted with solvents of increasing polarity like chloroform, acetone, methanol and water using Soxhlet extraction and maceration for the aqueous extract [24].

Qualitative Phytochemical Tests: Standard chemical tests were performed on various extracts to detect major phytochemical classes. Tests indicated the presence of flavonoids (Shinoda test positive), phenolics/tannins (ferric chloride test yielding dark blue-green), alkaloids (Mayer's and Dragendorff's tests mild positive), carbohydrates (Benedict's test positive), and proteins (Biuret test positive), especially in the polar (methanol, water) extracts. Saponins were absent (no froth in foam test). These findings qualitatively confirmed a broad spectrum of phytoconstituents in *F. esculentum* stem, aligning with its reputed medicinal properties [25].

Total Phenolic Content (TPC): Using the Folin–Ciocalteu reagent, the TPC was measured and expressed as gallic acid equivalents [26].

Total Flavonoid Content (TFC): Quercetin was employed as a reference in the aluminium chloride colorimetric technique to measure the flavonoid concentration, which was then represented as quercetin equivalents [27].

HPTLC Fingerprint Analysis: High-performance thin-layer chromatography was performed on the methanolic stem extract to profile its constituents. Quercetin had been used like a reference marker compound. Extract and standard were applied on a silica gel plate and developed in an appropriate solvent system. The plate was scanned at 366 nm. The HPTLC chromatogram of the extract revealed multiple peaks; importantly, a prominent peak with Rf matching that of standard quercetin was observed (confirming quercetin presence in the extract) [28].

2.3. Antimicrobial activity

The procedure involves positioning paper disks imbued with antimicrobial agents onto a bacterial lawn cultivated on an agar medium. Following an overnight incubation of the plate, one measures absence or presence of a zone of inhibition neighbouring the disks. "Kirby–Bauer method" established standardized parameters for inoculum size, disk size, incubation duration, temperature. The findings were articulated in qualitative terms, categorized as resistant, intermediate, or susceptible. Bauer and his colleagues developed the disc diffusion technique, which has since been continuously improved and expanded upon by the National Committee for Clinical Laboratory Standards, which is now known as the Clinical and Laboratory Standards Institute (CLSI).

S. No.	Bacterial strain	Resistant
1.	*Staphylococcus aureus*	Methicillin
2.	*Enterococcus*	Vancomycin
3.	*Mycobacterium tuberculosis*	Multi-drug-resistant
4.	*Enterobacteriaceae*	Carbapenem

3. RESULTS AND DISCUSSION

3.1 Pharmacognostical study

3.1.1 Biological source

Plant collected form Mainpat region in surguja district of Chhattisgarh.It consists of aerial parts of *Fagopyrumesculentum*belonging to family Polygonaceae [8].

3.1.2 Macroscopy

With paired opposing leaves and bulged nodes, the stem displays thin twigs of aerial branches that range in thickness from 2 to 2.5 mm(Figure 3.1). Its bark is delicate, exhibiting a dark brown hue interspersed with lighter brown lenticels that are uniformly distributed. The surface of the stem feels slightly textured, while its fracture is irregular and fibrous, imparting an astringent taste devoid of any notable scent. Leaves exhibit a dorsiventral structure, characterized by distinct upper and lower epidermal layers, with lower epidermis cells often presenting a papillose configuration. The hypodermis is located beneath the upper epidermis of midrib region. In the lamina region, palisade tissue is notably prominent, organized into two distinct layers. The epidermal hairs are unicellular and densely packed in the midrib area, situated beneath the palisade tissue. The spongy parenchyma is present, encompassing intercellular spaces. Both the palisade and spongy parenchyma are rich in chloroplasts, thereby making the mesophyll tissue capable of photosynthesis [8,21].

Figure 3.1: Fagopyrumesculentum

3.1.3 Microscopy

The cross-sectional view in figure 3.2 of the stem presents a circular configuration. The outer cork is composed of several coatings of dark brown, parenchymatous cells (irregular), while inner cork consists of some layers of radially organized lignified parenchymatous cells, organized in regular rows. The cortex is made of multiple layers of tangentially elongated and rounded cells, interspersed with a prominent belt of sclereids arranged in clusters of two to four. Numerous cells within the cortex, particularly in the outermost layers, are rich in tannins, exhibiting a spectrum of colors from yellow and orange to dark brown. Additionally, groups of pericyclic fibers are situated outside the phloem, which is observed in many thin patches encircling the xylem that is well-developed. Xylem constitutes approximately one-third of the transverse section and is consistently interspersed with one to four series of radially elongated lignified medullary ray cells. It comprises well-formed vessels, xylem fibers, tracheids, as well as xylem parenchyma. Small clusters of sclereids are scattered throughout the medullary ray cells, while thin-walled, rounded or polygonal lignified parenchymatous cells make up the pith, which is located near the centre of the stem. Prismmatic crystals linked to both sclereids and medullary ray cells are also seen in this region, along with tiny clusters of sclereids. [8,21].

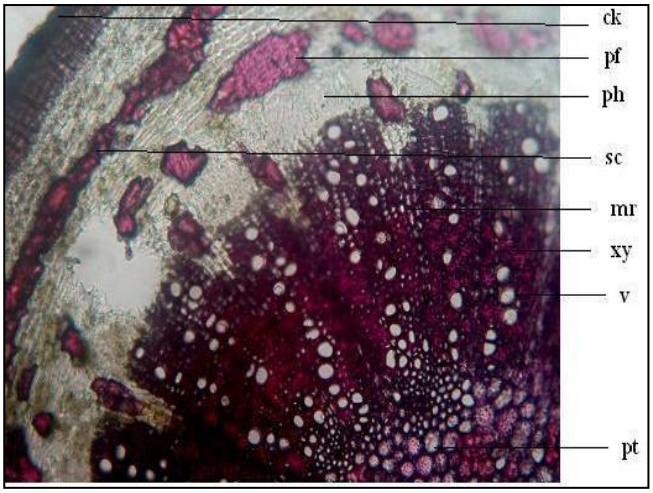

Figure 3.2: Transverse section of stem

ck- cork, **pf**- pericyclic fibres, **ph**- phloem, **sc**- stone cells,**mr**- medullary rays, **xy**- xylem, **v**- vessels, **pt**- pith

3.1.4 Powder analysis

The analysis of the powder derived from the stem unveiled the existence of vessels exhibiting simple pitted thickenings, sclereid clusters harboring crystals (prismatic), and remnants of parenchyma cells enriched with tannins. The analysis revealed the existence of tracheids, fibers, and cork cells [29].(Figure 3.3)

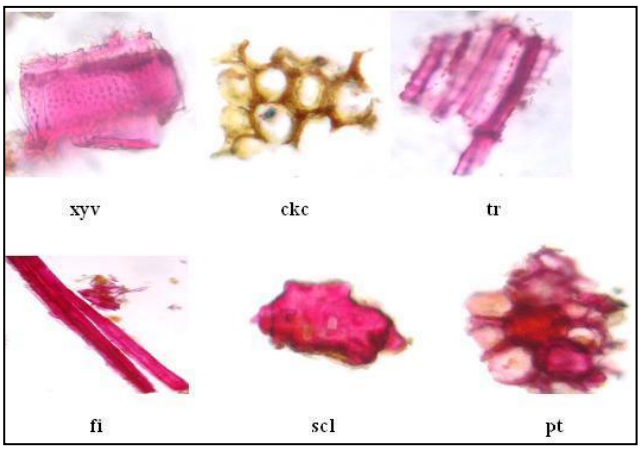

Figure 3.3: Powder characteristics of stem

xyv- xylem vessels, **ckc**- cork cells, **tr**- tracheids, **fi**- fibres, **scl**- sclereids, **pt**- pith

The powder microscopy of leaves(Figure 3.4) doesn't show much detail expect the occurrence of the mesophyll cells, in general. On special staining with iodine, the presence of starch is noted as starch content develops below colorations. Stomata on the surface view of the lower epidermis

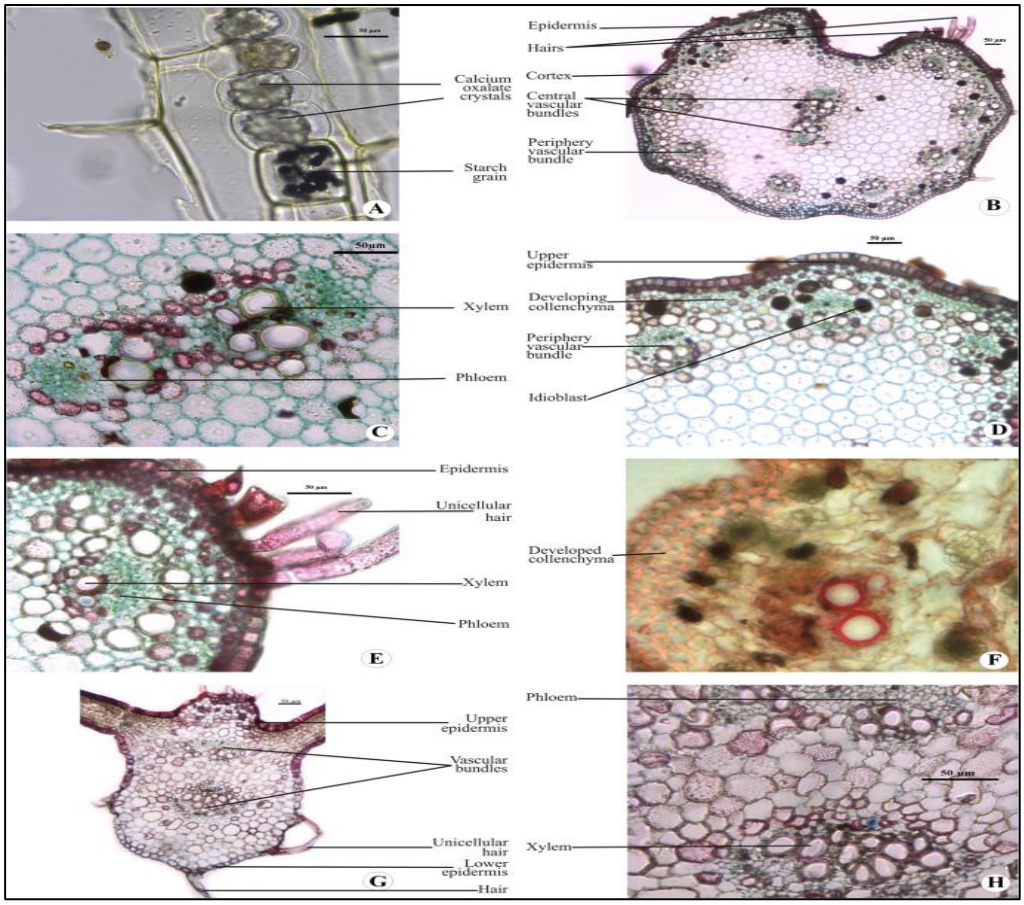

Figure 3.4: Powder microscopy of leaves

3.2 Physicochemical constants of stem of *Fagopyrumesculentum*

3.2.1 Ash values

The stem powder's total ash contenthad been found to be 3.7% w/w, which represents total amount of inorganic material (mineral content) present(Table 3.1). This is within the acceptable range for crude plant drugs, suggesting a moderate presence of mineral matter and no excessive contamination.The acid-insoluble ash value was 0.65% w/w, indicating minimal contamination with siliceous materials such as sand or soil. This low value confirms the purity of the raw material and its proper handling during collection and processing.The water-soluble ash was 2.37% w/w, suggesting that a substantial portion of the total ash consists of water-soluble minerals, possibly potassium, calcium, or magnesium salts. This is significant as it gives an idea of the physiological availability of minerals. The sulphated ash, measured at 2.80% w/w, reflects the total content of inorganic residues after complete oxidation of organic matter, further confirming the consistency of total ash findings [21].

Table 3.1: Ash values

Parameters	Value
Total ash	3.7 %w/w
Acid insoluble ash	0.65 %w/w
Water soluble ash	2.37 %w/w
Sulphated ash	2.80 %w/w

3.2.2 Extractive values

The water-soluble extractive value had been 16.7% w/w, which shows a rich content of polar constituents like sugars, tannins, glycosides, and flavonoids. This high value suggests that water is an effective solvent for extracting active phytochemicals from the stem. The ethanol-soluble extractive value had been 5.6% w/w, showing moderately polar compounds' presencelike phenolics, alkaloids, as well as resins. The ether-soluble extractive value had been 3.2% w/w, which shows presence of non-polar constituents like lipids, waxes, as well as essential oils, although in lower amounts compared to the polar constituents (Table 3.2).

Table 3.2: Extractive values

Parameters	Value
Water soluble extractive	16.7 %w/w
Ethanol soluble extractive	5.6 %w/w
Ether soluble extractive	3.2 %w/w

3.2.3 Moisture content

Moisture content that is loss on drying at 105 °C was 13.45% w/w, which is slightly above the ideal threshold (~10–12%) and could influence the storage stability of the raw drug. Higher moisture content may lead to microbial growth or degradation during storage.

3.2.4 Foaming index

The foaming index was recorded as NIL, indicating the absence of saponins or other foaming agents, which are often used as markers for certain pharmacologically active groups [22].

3.2.5 Tannin content

Tannin content had been found to be 9.0% w/w, suggesting significant amount of astringent polyphenolic compounds. Tannins contribute to antioxidant, antimicrobial, and wound-healing properties, making the stem pharmaceutically valuable.

3.2.6 Swelling index

The swelling index was 3, which provides information about the mucilage or hydrophilic fibre content. A moderate swelling index indicates some potential for gastrointestinal health or water retention [30].

3.3 Phytochemical study of stem of *Fagopyrumesculentum*

3.3.1 Preliminary phytochemical extraction:

The preliminary phytochemical extraction through successive solvent extraction revealed varying yields and colors of the plant extracts, indicating the differential solubility of phytoconstituents in solvents of increasing polarity(Table 3.3). Petroleum ether (60–80 °C), being a non-polar solvent, extracted a small amount of dull yellowish-green residue (1.17 g), suggesting non-polar compounds' limited presence such as oils, fats, or waxes. Benzene, which is other non-polar solvent, yielded a slightly higher amount (1.24 g) with a dark greenish-brown coloration, possibly indicating the presence of certain lipophilic pigments or low-polarity compounds.

Chloroform and acetone, which are moderately polar solvents, resulted in increased yields of 1.73 g and 1.64 g, respectively, with dark green and dirty brown colors. This suggests occurence of medium-polarity constituents like alkaloids, terpenoids, as well as certain phenolics. Ethanol (95%), a highly polar solvent, produced a substantial blackish-brown extract weighing 4.23 g, reflecting the extraction of a broad range of polar compounds, including flavonoids, tannins, saponins, and polyphenolic compounds.

The highest yield was obtained using a chloroform:water mixture (1:99), which produced 5.60 g of a brown-colored extract. This result indicates a dominant presence of highly polar, water-soluble phytoconstituents in the plant material [31,32].

Table 3.3: Successive Solvent extraction

S. No.	Solvent	Color	Weight of the extract(g)
1.	Petroleum ether (60-80 °C)	Dull yellowish green	1.17
2.	Benzene	Dark greenish brown	1.24
3.	Chloroform	Dark green	1.73

4.	Acetone	Dirty brown	1.64
5.	Ethanol (95%)	Blackish brown	4.23
6.	Chloroform:Water (1:99)	Brown	5.60

3.3.2 Phytochemical analysis of different extracts (Qualitative chemical tests)

The qualitative phytochemical analysis of successive solvent extracts in table 3.4 reveals significant variation in the solubility and presence of bioactive compounds depending on the polarity of the solvents used. Petroleum ether, being non-polar, extracted limited phytochemicals like phytosterols, carbohydrates, as well as fixed oils and fats, indicating the presence of lipophilic and slightly polar compounds. Benzene also showed a limited profile, yielding only fixed oils and fats and proteins, which suggests its relatively poor extraction capability for most phytochemicals. Chloroform, a solvent of intermediate polarity, was positive only for alkaloids, reflecting its moderate ability to dissolve specific bioactive compounds, particularly alkaloids. Acetone showed the presence of flavonoids and proteins, indicating its ability to extract certain moderately polar constituents. Methanol, a highly polar solvent, was far more effective, giving positive results for phenolic compounds, alkaloids, carbohydrates, phytosterols and tannins, as well as flavonoids, demonstrating its broad-spectrum solubility for various polar phytochemicals [10,12]. Water, the most polar solvent in the study, was found to extract the widest range of compounds. It tested positive for fixed oils, alkaloids, carbohydrates, phytosterolsas well as fats, phenolic compounds and tannins, proteins, and flavonoids, indicating that a large proportion of the plant's bioactive constituents are highly polar and water-soluble. Notably, saponins and gums as well asmucilageshad been absent in all extracts, suggesting either their absence in the plant material or the ineffectiveness of the employed solvents in extracting them. Overall, methanol and water emerged as the most efficient solvents for a broad range of phytochemicals, which is valuable for guiding future extraction and bioactivity studies [32].

Table 3.4: Qualitative chemical tests of successive extracts

Test	Petroleum ether	Benzene	Chloroform	Acetone	Methanol	Water
Alkaloids	-	-	+	-	+	+
Carbohydrates	+	-	-	-	+	+
Phytosterols	+	-	-		+	+
Fixed oils and fats	+	+	-	-	-	+
Saponins	-	-	-	-	-	-
Phenolic compounds and tannins	-	-	-	-	+	+
Proteins	-	+	-	+	-	+
Gums and mucilages	-	-	-	-	-	-
Flavonoids	-	-	-	+	+	+

(+ Present, - Absent)

3.3.3 Total Phenolic content

It was assessed with the use of gallic acid as the standard, as well as results are shown in mg of gallic acid equivalent (GAE) per gram of extract shown figure 3.5. The methanolic extract exhibited a phenolic content of 1.522 mg/g, while the aqueous extract showed a slightly lower value of 1.1 mg/g. This suggests that methanol is a solvent that extracts phenolic chemicals more effectively than water, likely due to its intermediate polarity which facilitates better solubility of various polyphenolic structures.

The antibacterial, anti-inflammatory, and antioxidant properties of phenolic compounds are well recognised, and moderate levels observed here suggest the *F. esculentum* stem may have beneficial pharmacological potential, especially when extracted with methanol [26].

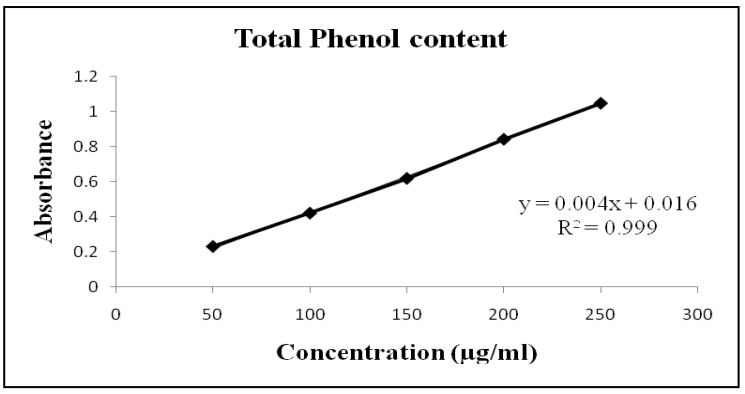

Figure 3.5: Standard plot of Gallic acid

3.3.4 Total Flavonoid content

It is expressed as quercetin equivalents, was determined to be 0.140 mg/g for methanolic extract, as well as 0.029 mg/g for aqueous extract. These results shown in figure 3.6, highlight a marked difference in flavonoid solubility, with methanol again proving to be a more effective solvent than water.Flavonoids are a subclass of polyphenols with potent antioxidant and anti-carcinogenic properties, often contributing to vascular health, immune modulation, and UV protection. Although the absolute flavonoid content is relatively low, the higher yield in the methanolic extract supports its preferential use for isolating such bioactives from the plant material [27].

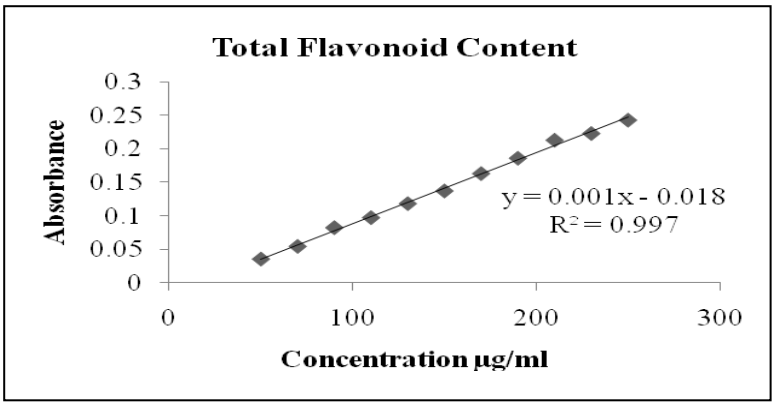

Figure 3.6: Standard plot of quercetin

3.3.5Fluorescence Analysis

The powdered stems' (*Fagopyrum esculentum)*fluorescence analysisprovided valuable insights into the presence of various phytochemicals through color changes observed under short UV light, long UV light, and daylight after treatment with different chemical reagents(Table 3.5). The untreated powder exhibited a yellowish-brown color under short UV, light brown under long UV, and dark brown in daylight, establishing the base characteristics of the sample. Upon treatment with 1N hydrochloric acid (HCl), the powder changed to brown, pale black, and brown respectively, suggesting occurence of acid-reactive compounds like alkaloids or tannins. Treatment with 1N sulfuric acid (H_2SO_4) produced light lemon yellow under short UV, light violet under long UV, and no color in daylight, possibly indicating the presence of acid-sensitive compounds like flavonoids or phenolics. When treated with 1N nitric acid (HNO_3), the powder showed light blue under short UV, violet under long UV, and dark blue in daylight, hinting at oxidative transformations of phenolic structures.

Alkaline treatments also revealed distinct reactions. The sample treated with 1N aqueous sodium hydroxide (NaOH) exhibited blackish green under short UV, black under long UV, and orange brown in daylight, while alcoholic NaOH treatment resulted in light greenish yellow, light brown, and light yellow respectively. These results are consistent with occurence of flavonoids, coumarins, as well as other phenolic compounds that are known to fluoresce under alkaline conditions. Additional treatments with picric acid and ferric chloride ($FeCl_3$) resulted in greenish yellow to blackish green under UV light and yellow to bright yellow in daylight, further supporting the presence of phenolic groups. Iodine treatment produced brownish green, reddish brown, and brown, possibly indicating the presence of terpenoids or starch-like compounds. Overall, the fluorescence behavior of the powdered drug in various chemical environments suggests a complex phytochemical profile rich in flavonoids, phenolics, and possibly alkaloids [28].

Table 3.5: fluorescence analysis of the powdered stem of *Fagopyrum esculentum*

S. No.	Treatment	Short UV light	Long UV light	Day light
1.	Powder as such	Yellowish Brown	Light brown	Dark brown
2.	Powder + 1N HCl	Brown	Pale black	Brown
3.	Powder + 1N H_2SO_4	Light lemon yellow	Light violet	No colour
4.	Powder + 1N HNO_3	Light blue	Violet	Dark blue
5.	Powder + 1N Aq. NaOH	Blackish green	Black	Orange brown
6.	Powder + 1N AlcNaOH	Light greenish yellow	Light brown	Light yellow
7.	Powder + Picric acid	Greenish yellow	Blackish green	Bright yellow
8.	Powder + 5% $FeCl_3$	Greenish yellow	Blackish green	Yellow
9.	Powder + Iodine	Brownish green	Reddish brown	Brown

3.3.6 HPTLC fingerprint profile of *Fagopyrumesculentum*

The HPTLC fingerprinting of the methanolic extract of *Fagopyrum esculentum* stem further confirmed the phytochemical composition, particularly the presence of flavonoids. The extract was analyzed using quercetin, a known flavonoid, as the reference standard. The 3D chromatogram (Figure 3.7) of the extract scanned at 366 nm revealed multiple peaks, indicating the presence of several phytoconstituents. When compared with the HPTLC chromatogram of standard quercetin shown in figure 3.8, the chromatogram of the extract showed matching peaks at similar Rf values, confirming the presence of quercetin or structurally related flavonoids within the sample. This correlation validates the extract's flavonoid content and aligns with the results observed in fluorescence analysis. The presence of quercetin in the methanolic extract in figure 3.9, supports the traditional use of *Fagopyrum esculentum* for its antioxidant and pharmacological properties and provides a reliable chromatographic fingerprint for quality control and standardization [33].

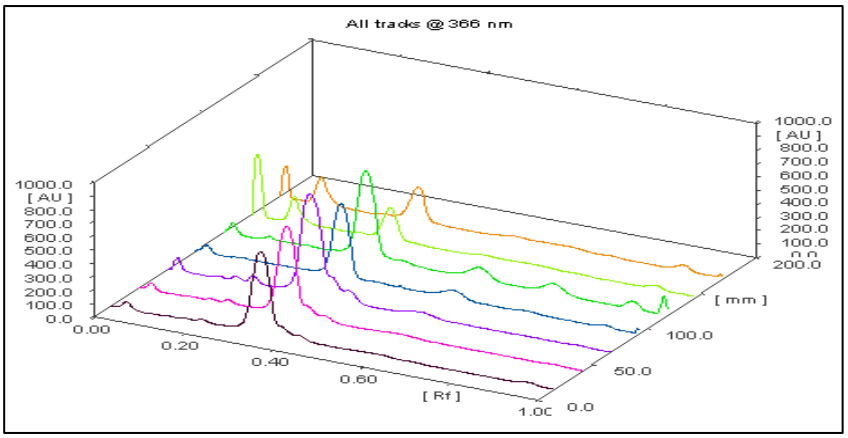

Figure3.7:3D chromatogram of quercetin and methanolicextract of *Fagopyrumesculentum* scanned at 366 nm

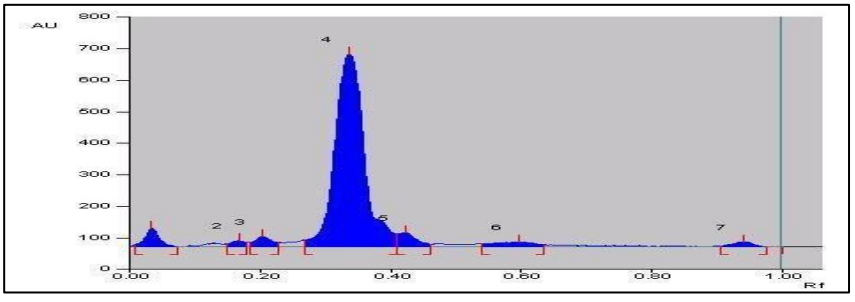

Figure3.8 HPTLC chromatogram of quercetin

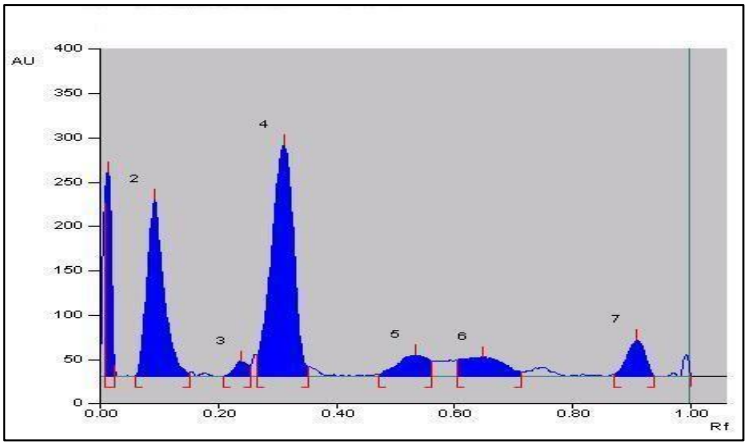

Figure3.9: HPTLC chromatogram of quercetin in methanolic extract of *F. esculentum*

3.4 *In-vitro* estimation of antimicrobial activity

The *Fagopyrum esculentum* stem extract's antimicrobial potential was evaluated against various pathogens, that includes*Staphylococcus aureus*, *Enterococcus*, *Mycobacterium tuberculosis*, and *Enterobacteriaceae*, using zone of inhibition standards compared to known antibiotics.

3.4.1 Zone diameter interpretative standards for *Staphylococcus aureus*

The stem extract of *Fagopyrum esculentum* demonstrated in table 3.6 with antibacterial activity against *Staphylococcus aureus*, with a clear dose-dependent response. At the lowest tested concentration of 500 µg, the extract produced a zone of inhibition measuring 5–7 mm, indicating intermediate sensitivity. As the concentration increased to 1000 µg, the inhibition zone expanded to 10–12 mm, suggesting a moderate antimicrobial effect. At 2000 µg, the zone reached 13–14 mm, moving closer to the defined threshold for susceptibility. Most significantly, at 3000 µg, the extract produced a zone of 15–16 mm, which approaches the susceptibility criteria set for standard antibiotics. These results indicate that the extract possesses promising antibacterial activity against *S. aureus*, especially at higher concentrations, and may be comparable to standard antibiotics in efficacy when sufficiently concentrated [34,35].

Table 3.6: Demonstrated notable antibacterial activity against *Staphylococcus aureus*for *Fagopyrum esculentum*extracts

	Resistant	**Intermediate**	**Susceptible**
Clidamycin (2 µg)	≤15	16-18	≥19
Erythromycin (15µg)	≤14	15-20	≥22
Penicillin G (10µg))	≤24	24-28	≥29
Vancomycin (30 µg)	--	--	≥15
Extract (500 µg)	--	5-7	≥8
Extract (1000 µg)	--	10-12	≥13
Extract (2000 µg)	--	13-14	≥14
Extract (3000 µg)	--	15-16	≥17

3.4.2 Zone diameter interpretative standards for *Enterococcus*

The antibacterial activity of the extract against *Enterococcus* was comparatively less potent but still exhibited a concentration-dependent trend. No inhibition was observed at the lowest concentration of 500 µg, indicating no activity at this dose. However, beginning at 1000 µg, the extract started to exhibit mild antibacterial effects, with a zone of inhibition measuring 9–11 mm. The effectiveness improved slightly at 2000 µg, reaching 10–12 mm, and further at 3000 µg, where the zone increased to 12–15 mm. Although the extract did not reach the defined threshold for full susceptibility (≥16 mm), the gradual improvement across concentrations suggests that it has moderate activity against *Enterococcus*, especially at

higher doses. (Table 3.7)

Table 3.7: Demonstrated notable antibacterial activity against *Enterococcus* for *Fagopyrum esculentum*extracts

	Resistant	Intermediate	Susceptible
Ampicillin (10 µg)	≤12	14-17	≥18
Chloramphenicol (30µg)	≤14	14-18	≥19
Ciprofloxacin (05 µg))	--	---	≥21
Doxycycline (30µg)	≤12	13-16	≥17
Extract (500 µg)	--	--	--
Extract (1000 µg)	--	9-11	≥11
Extract (2000 µg)	--	10-12	≥14
Extract (3000 µg)	--	12-15	≥16

3.4.3 Zone diameter interpretative standards for *Mycobacterium tuberculosis*

When tested against *Mycobacterium tuberculosis*, the stem extract showed in table 3.8, a progressive increase in antimicrobial activity with rising concentrations. At 500 µg, there was no observable inhibition, indicating no effective action at this level. However, at 1000 µg, the zone of inhibition measured 7–9 mm, signifying the onset of antimycobacterial activity. This effect became more pronounced at 2000 µg, with an increased inhibition zone of 11–14 mm. At the highest concentration tested, 3000 µg, the extract produced a zone of 15–18 mm. Although this does not meet the defined susceptibility threshold of ≥20 mm, the consistent upward trend points toward significant antimycobacterial potential at higher doses. These findings suggest that the extract could serve as a natural source for antimycobacterial agents after further optimization and active component isolation.

Table 3.8: Demonstrated notable antibacterial activity against *Mycobacterium tuberculosis* for *Fagopyrum esculentum*extracts

	Resistant	Intermediate	Susceptible
Amikacin (30)	≤21	22-25	≥27
Ciprofloxacin (5)	≤19	19-22	≥25
Clarithromycin (15)	≤23	24-27	≥28
Rifampin (5)	--	10-15	≥18
Extract (500 µg)	--	--	--
Extract (1000 µg)	--	7-9	≥10
Extract (2000 µg)	--	11-14	≥16
Extract (3000 µg)	--	15-18	≥20

3.4.4 Zone diameter interpretative standards for Enterobacteriaceae

The extract exhibited the least antimicrobial activity against *Enterobacteriaceae*, though a concentration-dependent response was still evident. At 500 µg, no zone of inhibition was observed, indicating that the extract lacked activity at lower concentrations. Upon increasing the dose to 1000 µg, a zone of 5–8 mm appeared, signifying mild activity. The inhibition zone further expanded to 7–9 mm at 2000 µg and 8–12 mm at 3000 µg. Despite not reaching the susceptibility threshold of ≥12 mm in most cases, the data suggest that the extract does have weak to moderate antibacterial effects against this group of Gram-negative bacteria [10].

Table 3.9: Demonstrated notable antibacterial activity against *Enterobacteriaceae* for *Fagopyrum esculentum*extracts

	Resistant	Intermediate	Susceptible
Cefuroxime (30 µg)	≤19	20-22	≥23
Amoxicillin-clavulanic acid (20/10µg)	≤13	14-17	≥18
Ertapenem (10µg))	≤18	19-21	≥22
Levofloxacin (5µg)	≤13	14-17	≥18
Extract (500 µg)	--	--	--
Extract (1000 µg)	--	5-8	≥10
Extract (2000 µg)	--	7-9	≥11
Extract (3000 µg)	--	8-12	≥12

This lower efficacy may be attributed to the complex and resistant outer membrane structure of Gram-negative organisms. Nonetheless, the increasing trend with concentration indicates a potential that warrants further exploration [14,36]. (Table 3.9)

4. CONCLUSION

The present study highlights the antimicrobial potential of *Fagopyrum esculentum* (common buckwheat) stem extract against various drug-resistant pathogens. Pharmacognostic and phytochemical investigations confirmed the presence of bioactive constituents, particularly flavonoids and phenolics, with methanolic extracts showing high levels of both [6,9,14]. HPTLC fingerprinting validated the presence of quercetin, supporting the plant's medicinal relevance. In vitro antimicrobial assays demonstrated dose-dependent inhibitory effects of the stem extract, particularly against *Staphylococcus aureus* and *Mycobacterium tuberculosis*, with zones of inhibition comparable to standard antibiotics at higher concentrations. Moderate activity was also observed against *Enterococcus faecalis*, while the least activity was recorded against Gram-negative Enterobacteriaceae strains, likely due to their more resistant cell wall structures [19,36]. Overall, the results support the use of *F. esculentum* stem as a potential source of plant-based antimicrobials, particularly effective against Gram-positive and acid-fast bacteria. Its rich polyphenolic content may be responsible for the observed antimicrobial effects, suggesting a mechanism involving membrane disruption and enzyme inhibition. These findings encourage further investigation into the isolation and characterization of active constituents for developing novel therapeutics to combat antimicrobial resistance

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