Antibacterial Activity and Phytochemical Screening of Different Solvent Extracts of a Polyherbal Kahwa Formulation Against Escherichia coli

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Abstract

The increasing prevalence of antimicrobial resistance has prompted the exploration of plant-based products as alternative antimicrobial agents (WHO, 2024; Cowan, 1999). Polyherbal formulations contain a wide range of phytochemicals known for their biological activities (Sofowora, 2008; Saxena et al., 2013). The present study investigated the antibacterial activity of different solvent extracts and evaluated whether the detected phytochemicals may contribute to the observed antibacterial activity against *Escherichia coli*. Five solvent extracts were prepared using distilled water, ethanol, methanol, propanol, and chloroform. Antibacterial activity was evaluated using the agar well diffusion method following CLSI guidelines (CLSI, 2023), while phytochemical screening was performed using standard qualitative procedures described by Harborne (1998) and Trease and Evans (2009). Among all extracts tested, only the aqueous extract demonstrated antibacterial activity, producing inhibition zones of 30 mm, 32 mm, and 33 mm at concentrations of 50 µL, 75 µL, and 100 µL, respectively. The antibiotic control produced a zone of inhibition of 14 mm. Phytochemical analysis revealed the presence of alkaloids, saponins, carbohydrates, phenolic compounds, coumarins, and oils and resins in the aqueous extract. The findings suggest that water-soluble phytochemicals present in the Kahwa formulation may contribute significantly to antibacterial activity against *E. coli* (Cushnie & Lamb, 2011; Daglia, 2012). Further studies involving replicated experiments and characterization of active compounds are required to validate these findings.

Keywords: Kahwa, Antibacterial Activity, *Escherichia coli*, Phytochemical Screening, Herbal Formulation, Aqueous Extract.

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Introduction

Antimicrobial resistance has become one of the most serious public health concerns worldwide (WHO, 2024). The excessive and indiscriminate use of antibiotics has led to the emergence of resistant bacterial strains, thereby reducing the effectiveness of conventional antimicrobial therapies (WHO, 2024). Consequently, there is growing interest in exploring
medicinal plants and herbal products as alternative sources of antimicrobial compounds (Cowan, 1999; Chassagne et al., 2021).

Plant-derived products are rich in secondary metabolites such as alkaloids, tannins, flavonoids, phenolic compounds, saponins, coumarins, and essential oils. These phytochemicals are known to possess antimicrobial, antioxidant, anti-inflammatory, and therapeutic properties (Harborne, 1998; Sofowora, 2008; Kumar & Pandey, 2013). Numerous studies have reported that these compounds can inhibit microbial growth through mechanisms including membrane disruption, enzyme inhibition, interference with nucleic acid synthesis, and oxidative damage (Daglia, 2012; Bouarab-Chibane et al., 2019; Cushnie et al., 2014).

Polyherbal beverages such as Kahwa are prepared using a combination of herbs, spices, and tea leaves. Common ingredients include green tea, ginger, cinnamon, clove, black pepper, cardamom, and tulsi, all of which have been reported to possess antimicrobial properties (Vaou et al., 2021; Chassagne et al., 2021). Green tea catechins, particularly epigallocatechin gallate, have shown significant inhibitory effects against various pathogenic bacteria including *Escherichia coli* (Taylor et al., 2005).

Escherichia coli is a Gram-negative bacterium commonly associated with gastrointestinal infections, urinary tract infections, and foodborne diseases (CLSI, 2023). Due to its clinical importance and widespread occurrence, *E. coli* is a bacterial strain frequently used as a model organism in antimicrobial studies.

Therefore, the present study aimed to evaluate the antibacterial activity of different solvent extracts of a polyherbal Kahwa formulation against *Escherichia coli* and to identify the phytochemical constituents associated with the observed antimicrobial effects.

1. Materials and Methods

1.1 Sample Collection

A commercially available polyherbal Kahwa formulation was procured from the local market and used for the study.

Formulation of Kahwa

The formulation of this Kahwa contains a blend of green tea, spices, and medicinal herbs traditionally used in Ayurveda and supported by modern scientific studies for their therapeutic properties.

Green Tea (*Camellia sinensis*)

Green tea is rich in polyphenolic compounds, particularly catechins such as epigallocatechin gallate (EGCG), which possess strong antioxidant and anti-inflammatory activities (Taylor et al., 2005; Daglia, 2012). Research suggests that green tea may help reduce oxidative stress, improve cardiovascular health, enhance metabolic function, and support weight management. Additionally, its bioactive compounds have been associated with neuroprotective and chemopreventive effects.

Black Pepper (*Piper nigrum*)
Black pepper contains piperine, a bioactive alkaloid known for enhancing the bioavailability of various nutrients and phytochemicals. Piperine exhibits antioxidant, anti-inflammatory, antimicrobial, and digestive-stimulatory properties (Taylor et al., 2005; Daglia, 2012). It has also been reported to improve gastrointestinal function and nutrient absorption.

Ginger (*Zingiber officinale*)
Ginger is widely recognized for its anti-inflammatory, antioxidant, and antiemetic properties (Sofowora, 2008; Vaou et al., 2021). Active constituents such as gingerols and shogaols contribute to its effectiveness in alleviating nausea, promoting digestion, reducing gastrointestinal discomfort, and modulating inflammatory responses.

Tulsi (*Ocimum tenuiflorum / Ocimum sanctum*)
Tulsi, commonly known as Holy Basil, is considered an adaptogenic herb. Studies indicate that it possesses antioxidant, immunomodulatory, antimicrobial, and anti-stress activities (Sofowora, 2008; Chassagne et al., 2021). Tulsi may help enhance immune function, reduce stress-induced physiological changes, and improve respiratory health.

Asafoetida (*Ferula asafoetida*)
Asafoetida contains sulfur-containing compounds and volatile oils that contribute to its medicinal properties (Vaou et al., 2021). It is traditionally used as a carminative and digestive aid. Scientific investigations suggest potential antimicrobial, antispasmodic, and anti-inflammatory effects, particularly in gastrointestinal disorders.

Clove (*Syzygium aromaticum*)
Clove is rich in eugenol, a phenolic compound with potent antioxidant, antimicrobial, analgesic, and anti-inflammatory activities (Daglia, 2012; Vaou et al., 2021). Clove has been extensively used in oral healthcare and may contribute to reducing microbial growth and inflammatory responses.

Cardamom (*Elettaria cardamomum*)
Cardamom contains essential oils such as cineole and terpinyl acetate, which exhibit antioxidant and antimicrobial properties (Vaou et al., 2021). Research indicates that cardamom may aid digestion, support cardiovascular health through blood pressure regulation, and contribute to respiratory wellness.

Cinnamon (*Cinnamomum verum / Cinnamomum cassia*)
Cinnamon is known for its antioxidant and anti-inflammatory properties (Khameneh et al., 2021). Bioactive compounds, including cinnamaldehyde, have been associated with improved glucose metabolism, antimicrobial activity, and potential cardiovascular benefits. Several studies suggest its role in supporting glycemic control.

Nutmeg (*Myristica fragrans*)
Nutmeg contains compounds such as myristicin and elemicin, which possess antioxidant and antimicrobial activities (Vaou et al., 2021). Traditionally, nutmeg has been used to support digestion and improve sleep quality. Experimental studies indicate potential neuroprotective and anti-inflammatory effects.

Acidity Regulator (INS 330 – Citric Acid)
Citric acid functions primarily as an acidity regulator and preservative. Collectively, the ingredients present in the Girnar Detox Desi Kahwa formulation contain bioactive phytochemicals that have been reported to possess antioxidant, anti-inflammatory, digestive, immunomodulatory, and antimicrobial properties (Vaou et al., 2021; Chassagne et al., 2021).

Antibiotic Control: Cefixime (Zifi-200) was used as the positive control. A Zifi-200 tablet containing cefixime was finely crushed, and the required quantity was dissolved in sterile distilled water to prepare a 1 mg/mL solution. One hundred microlitres (100 µL) of the prepared antibiotic solution was dispensed into the agar well and used as the positive control in the agar well diffusion assay (CLSI, 2023).

Statistical Analysis: All experiments were performed in triplicate (n = 3), and the results are expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) to compare the antibacterial activity among the different treatment groups. A p-value of < 0.05 was considered statistically significant.

1.2 Preparation of Solvent Extracts

Five grams (5 g) of commercially available Kahwa powder was separately extracted with 50 mL each of distilled water, ethanol, methanol, propanol, and chloroform, maintaining a solvent-to-sample ratio of 1:10 (w/v). The mixtures were extracted using a rotary shaker at 120 rpm for 72 hours under ambient laboratory conditions (approximately 35–37°C, as the laboratory was not temperature-controlled). Following extraction, the aqueous and chloroform extracts were filtered through sterile muslin cloth because of their higher viscosity, whereas the ethanol, methanol, and propanol extracts were filtered using Whatman No. 1 filter paper (nominal pore size: 11 µm). The filtrates were transferred to sterile Petri dishes and concentrated by evaporation in a hot air oven maintained at 55–60°C until constant weight was achieved (approximately 7–8 days). The dried extracts were collected and stored for subsequent phytochemical and antibacterial analyses and then transferred to sterile, airtight containers and stored at −20°C until further phytochemical screening and antimicrobial evaluation. All extracts were maintained under the same storage conditions throughout the study. The extraction yield (%) was not determined in the present study.

Fig. 1 Extraction of polyherbal Kahwa using different solvents in a rotary flask shaker at 120 rpm for 72 h.



Fig. 2 Solvent extracts of polyherbal Kahwa prepared using ethanol (95%), methanol, propanol, chloroform, and distilled water after concentration.

1.3 Preparation of Test Solutions

For antimicrobial testing, the ethanol, methanol, propanol, and chloroform extracts were prepared at a concentration of 1 mg/mL by dissolving 1 mg of each dried extract in 1 mL of the respective solvent. As the aqueous extract was maintained as a liquid extract, 1 µL was diluted in 1 mL of sterile distilled water before testing. The antibiotic control consisted of cefixime (Zifi-200) prepared at 1 mg/mL in sterile distilled water, and 100 µL of this solution was used as the positive control in the agar well diffusion assay.

1.4 Preparation of LB Agar Medium

LB agar medium was prepared using: Tryptone – 5 g, Yeast Extract – 2.5 g, Sodium Chloride – 5 g, Agar – 8 g, Distilled Water – 500 mL. The medium was sterilized at 121°C and 15 psi before pouring into sterile Petri plates under aseptic conditions.

1.5 Bacterial Strain

Escherichia coli MTCC 723 stock cultures preserved at −20°C were revived in Luria–Bertani (LB) medium prior to the antibacterial assay. A freshly revived culture was used for each experiment. The bacterial inoculum was prepared directly from the revived culture and spread uniformly on the agar plates before performing the agar well diffusion assay.

1.6 Agar Well Diffusion Assay

Antibacterial activity was determined using the agar well diffusion method with minor modifications as described by Parekh and Chanda (2007) and Balouiri et al. (2016). Agar wells of 10 mm diameter were prepared using a sterile cork borer to accommodate the required extract volumes. Aliquots of 50, 75 and 100 µL of each extract were aseptically
dispensed into the respective wells. Cefixime (Zifi-200; 1 mg/mL) was used as the positive control, and 100 µL of the prepared antibiotic solution was added to the control well. The inoculated plates were incubated at 37°C for 48 h, after which the diameters of the zones of inhibition were measured in millimetres. All experiments were performed under identical experimental conditions to ensure valid comparative evaluation of the antibacterial activity. An overview of all plates is shown in Fig. 3, with individual plates shown in Fig. 4-6.

Fig. 3 Agar well diffusion assay showing antibacterial activity of different solvent extracts of polyherbal Kahwa against *Escherichia coli*. Only the aqueous extract exhibited measurable zones of inhibition.

1.7 Phytochemical Screening

Qualitative phytochemical analysis was performed according to the methods described by Harborne (1998), Trease and Evans (2009), and Sofowora (2008). The extracts were qualitatively screened for alkaloids, tannins, saponins, flavonoids, coumarins, phenolic compounds, carbohydrates, and oils and resins. Representative phytochemical test results are shown in Figs. 7 to14.

Table 1. Zone of inhibition (mm) against *Escherichia coli* (triplicate observations).

Sample/Concentration	Replicate1 (R1)	Replicate2 (R2)	Replicate3 (R3)	Mean ± SD(mm)
DW Extract (50 µL)	30.0	29.5	30.5	30.00 ± 0.50
DW Extract (75 µL)	32.0	31.0	33.0	32.00 ± 1.00
DW Extract (100 µL)	33.0	33.5	32.5	33.00 ± 0.50
Antibiotic Control (Zifi – 200)/ (100 µL)	14.0	14.5	13.5	14.00 ± 0.50

NOTE – Organic solvent extracts (ethanol, methanol, propanol, and chloroform) consistently showed 0 mm zones of inhibition in all three replicates and were therefore excluded from the ANOVA to avoid distortion of variance estimates.

Table 2. One-way ANOVA summary for antibacterial activity.

Source of Variation	Sum of Squares (SS)	Degree of Freedom (df)	Mean Square (MS)	F-Value	P-Value
Between groups	681.00	3	227.00	–	–
Within groups (error)	4.00	8	0.50	–	–
Total	685.00	11	–	454.00	<0.0001

*** Statistically highly Significant (P<0.01)**

2. Result

To evaluate the concentration-dependent antibacterial

activity of the aqueous Kahwa extract, a one-way analysis of variance (ANOVA) was performed to compare the inhibition zones produced by the three extract concentrations (50, 75, and 100 µL) and the antibiotic control (Table 2). The analysis revealed a highly significant difference among the groups ($F(3,8) = 454.00$, $p < 0.0001$), with the calculated F-value greatly exceeding the critical F-value (Fcrit = 4.07). These findings indicate that the antibacterial activity of the aqueous Kahwa extract increased with increasing extract concentration under the experimental conditions employed. Furthermore, the aqueous Kahwa extract at higher concentrations produced significantly larger zones of inhibition than the antibiotic control.

2.1 Antibacterial Activity

Only the aqueous extract exhibited antibacterial activity against *E. coli* (Fig. 3, Fig. 4). The aqueous extract demonstrated concentration-dependent antibacterial activity, with zones of inhibition increasing from 30 mm at 50 µL to 33 mm at 100 µL (Table 3). The antibiotic control (Zifi-200) produced a zone of 14 mm. No inhibition zones were observed for ethanol, methanol, propanol, or chloroform extracts (Fig. 5a-d), or for the crude Kahwa solution (Fig. 6).

Table 3. Zone of inhibition produced by different solvent extracts of polyherbal Kahwa against *Escherichia coli*.

Sample	Zone of Inhibition (mm)
DW Extract (50 µL)	30
DW Extract (75 µL)	32
DW Extract (100 µL)	33
Antibiotic Control	14
Ethanol Extract	No inhibition (0 mm)
Methanol Extract	No inhibition (0 mm)
Propanol Extract	No inhibition (0 mm)
Chloroform Extract	No inhibition (0 mm)

Fig. 4 Agar well diffusion assay for distilled water extract showing clear concentration-dependent zones of inhibition at 50 µL (30 mm), 75 µL (32 mm), and 100 µL (33 mm) against *E. coli*. Ab = antibiotic control showing 14 mm zone.

Fig. 5 Agar well diffusion assay for organic solvent extracts. (a) Ethanol, (b) Methanol,

(c) Propanol, and (d) Chloroform extracts showed no detectable zones of inhibition against

E. coli at 50 µL, 75 µL, or 100 µL concentrations. Ab = antibiotic control.

(a) E

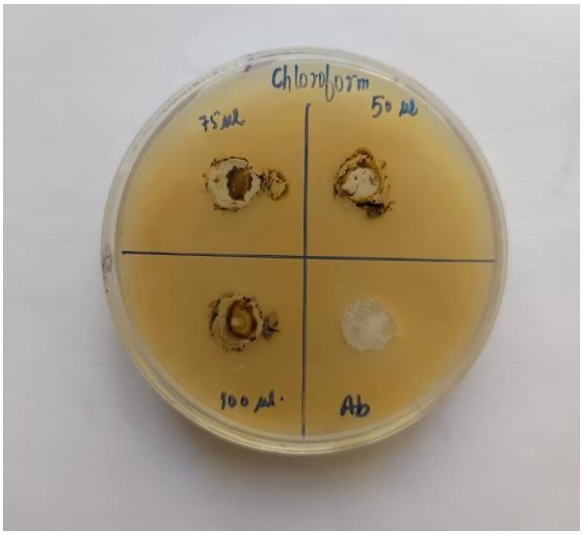
thanol

(b) M

ethanol

(c) P

ropanol



(d) Ch

loroform

Fig. 6 Agar well diffusion assay for unextracted Kahwa solution showing no zone of inhibition against *E. coli*. D.W = distilled water control, Ab = antibiotic control.

2.2 Preliminary Phytochemical Screening

Preliminary phytochemical screening is a qualitative analytical approach used to detect the presence of major classes of secondary metabolites in plant extracts. It serves as an important initial step in phytochemical investigations, providing rapid insight into the chemical composition of medicinal plants and their derived preparations. These screening methods are based on specific chemical reactions that produce characteristic color changes or precipitate formation, thereby indicating the presence of compounds such as alkaloids, flavonoids, phenolics, tannins, saponins, coumarins, oils, and resins. The selection of extraction solvent plays a crucial role in the detection of phytochemicals, as different solvents vary in polarity and extraction efficiency, thereby influencing the availability of bioactive constituents in the final extract. Such studies are widely employed in pharmacognosy to correlate traditional medicinal uses of plants with their potential bioactive compounds (Harborne, 1998; Trease & Evans, 2009; Kokate, 2010; Saxena et al., 2013).

These qualitative methods provide valuable preliminary information; however, they do not offer structural identification or quantification of individual compounds. Therefore, further advanced analytical techniques such as high-performance liquid chromatography (HPLC) and mass spectrometry-based methods are required for detailed phytochemical characterization (Trease & Evans, 2009; Sofowora, 2008).

To further characterize the phytochemical profile of the Kahwa extracts, individual qualitative assays were carried out to detect the presence of specific classes of bioactive compounds using standard phytochemical procedures.

Phytochemical screening revealed differential distribution of secondary metabolites across solvent extracts (Table 4). The observed variation in phytochemical distribution among solvent extracts is

consistent with differences in solvent polarity and extraction efficiency reported in previous phytochemical studies (Harborne, 1998; Kumar & Pandey, 2013). The aqueous extract tested positive for alkaloids, saponins, carbohydrates, phenolic compounds, coumarins, and oils and resins. Saponins were detected in both distilled water and chloroform extracts as evidenced by persistent froth formation. Tannins were present only in ethanol and methanol extracts, while flavonoids were detected in methanol and chloroform extracts. Coumarins were detected in both distilled water and chloroform extracts

Table 4. Phytochemical Constituents of Different Extracts

Phytochemical	Distilled water	Ethanol	Methanol	Propanol	Chloroform
Alkaloids (*Mayer's test)*	+	–	–	–	–
Proteins (*Ninhydrin test*)	–	–	–	–	–
Quinones (conc. HCl test)	–	–	–	–	–
Tannins (*Bramayers test*)	–	+	+	–	–
Saponins	+	–	–	–	+
Carbohydrates (Iodine test)	+	–	–	–	–
Phenolic Compounds (Potassium dichromate test)	+	+	–	–	–
Flavonoids (conc. H SO test)	–	–	+	–	+
Coumarins (NaOH test)	+	–	–	–	+
Oils and Resins (Filter paper test)	+	–	–	–	–

Detection of Alkaloids

The presence of alkaloids in the Kahwa extracts was investigated using Mayer's test. Mayer's reagent was prepared by mixing Solution A, consisting of 1.358 g mercuric chloride dissolved in 60 mL distilled water, with Solution B, consisting of 5 g potassium iodide dissolved in 10 mL distilled water, and subsequently diluting the mixture with distilled water to a final volume of 100 mL. For the assay, a few milliliters of the filtrate were treated with 1–2 drops of Mayer's reagent added carefully along the sides of the test tube. The formation of a creamy white to yellow precipitate was considered indicative of the presence of alkaloids.

Among the solvent extracts tested, only the distilled water extract produced the characteristic creamy white/yellow precipitate, indicating a positive result for alkaloids. In contrast, the ethanol, methanol, chloroform, and propanol extracts did not exhibit precipitate formation and were therefore considered negative under the conditions of the assay.

The positive response observed in the aqueous extract suggests that alkaloid constituents present in the Kahwa sample were effectively extracted into the distilled water fraction as shown in (Fig. 7). The absence of a detectable reaction in the remaining solvent extracts may be attributed to differences in the extraction efficiency, solubility characteristics, or concentration of alkaloid compounds in the respective solvent systems. The variation in alkaloid detection among the extracts highlights the influence of solvent selection on the recovery of phytochemical constituents.

Alkaloids are an important class of naturally occurring nitrogen-containing compounds known for their diverse pharmacological properties, including antimicrobial, antioxidant, anti-inflammatory, and therapeutic activities. Alkaloids have been reported to possess antimicrobial, antioxidant, and anti-inflammatory activities and may contribute to the observed antibacterial activity of the aqueous Kahwa extract (Cushnie et al., 2014).

Fig. 7 Alkaloid test in distilled water extract showing precipitate formation

Detection of Proteins

The presence of proteins in Kahwa solution was investigated using the Ninhydrin test, a qualitative phytochemical assay commonly employed for the detection of amino acids and proteinaceous compounds. For this analysis, 2 mL of filtrate obtained from each solvent extract of Kahwa (distilled water, ethanol, methanol, propanol, and chloroform) was taken separately in clean test tubes. To each filtrate, 2 drops of Ninhydrin solution were added, and the reaction mixtures were observed for the development of a characteristic color.

A positive Ninhydrin reaction is indicated by the formation of a purple or violet coloration, which results from the reaction between Ninhydrin and free amino groups present in amino acids or proteins. However, in the present study, none of the tested extracts exhibited the characteristic purple coloration after the addition of Ninhydrin reagent. The reaction mixtures remained unchanged, indicating a negative result for proteins and free amino acids in all solvent extracts.

The absence of a positive reaction in the distilled water, ethanol, methanol, propanol, and chloroform extracts suggests that proteinaceous compounds were either absent or present in concentrations below the detectable

limit of the test. These findings indicate that the Kahwa solution does not contain significant amounts of proteins or free amino acids extractable by the solvents employed in this study.

Overall, the Ninhydrin test demonstrated negative results for all solvent extracts, confirming the absence of detectable proteins in the Kahwa solution under the experimental conditions used.

Detection of Quinones

The presence of quinones in the Kahwa extracts was evaluated using the concentrated hydrochloric acid test. In this method, concentrated hydrochloric acid was added to the plant extract, and the development of a green coloration was considered indicative of the presence of quinones. None of the solvent extracts, including distilled water, ethanol, methanol, chloroform, and propanol, exhibited the characteristic green color. Therefore, all extracts were considered negative for quinones.

The absence of a positive reaction in all solvent extracts suggests that quinone compounds were either absent from the Kahwa sample or present in concentrations below the detection limit of the qualitative assay employed. The negative results obtained across all extraction solvents indicate that detectable levels of quinones were not recovered under the extraction conditions used in this study.

Quinones are a class of bioactive secondary metabolites known for their diverse biological activities, including antimicrobial, antioxidant, and cytotoxic properties. However, the present findings suggest that quinones do not constitute a significant phytochemical component of the investigated Kahwa extracts. Since qualitative phytochemical tests provide only preliminary information, the absence of a positive reaction should not be interpreted as definitive proof of the complete absence of quinone-related compounds (Harborne, 1998).

Detection of Tannins

The presence of tannins in Kahwa solution was investigated using Braymer's test, a qualitative phytochemical assay commonly employed for the detection of tannin compounds. For the analysis, 1 mL of each Kahwa extract prepared in different solvents (distilled water, methanol, ethanol, propanol, and chloroform) was taken in separate test tubes. To each test tube, 3 mL of distilled water was added, followed by the addition of 2–3 drops of 10% ferric chloride solution. The mixtures were gently shaken and observed for the development of characteristic coloration.

A distinct blue-green coloration was observed in the methanolic and ethanolic extracts of Kahwa solution, indicating a positive test for tannins. The formation of this color is attributed to the reaction between ferric ions and the phenolic hydroxyl groups present in tannin molecules, resulting in the formation of colored complexes (Harborne, 1998). The positive response suggests that tannins were effectively extracted by methanol and ethanol, highlighting the suitability of these polar organic solvents for the extraction of phenolic phytochemicals from Kahwa.

In contrast, no significant color change was observed in the distilled water, propanol, and chloroform extracts. This negative result indicates either the absence of detectable tannins or their insufficient extraction in these solvent systems. The findings demonstrate that the distribution and extraction efficiency of tannins from Kahwa are highly dependent on solvent polarity. Overall, the Braymer's test confirmed the presence of tannins predominantly in the methanolic and ethanolic extracts of Kahwa solution, suggesting that methanol and ethanol may be more efficient solvents for extracting tannin-containing phytoconstituents under the conditions employed in this study as shown in (Fig. 8).



Fig. 8 Positive Braymer's test showing blue-green coloration in methanolic and ethanolic extracts, indicating the presence of tannins.

Detection of Saponins

The presence of saponins in Kahwa solution was determined using the foam test, a commonly employed qualitative method for the detection of saponins. For this analysis, 1 mL of each extract prepared in different solvents (distilled water, methanol, ethanol, propanol, and chloroform) was taken in separate test tubes. The extracts were vigorously shaken by hand for a short duration and then allowed to stand undisturbed. The formation and persistence of foam were carefully observed as an indicator of saponin content as shown in (Fig. 9).

A stable layer of bubbles and froth was formed at the top of the distilled water and chloroform extracts after vigorous shaking. The foam persisted for approximately one minute, indicating a positive result for saponins. The ability of saponins to reduce surface tension and produce stable foam in aqueous media is a characteristic property that serves as the basis for this test. The persistence of the froth confirmed the presence of saponin compounds in these extracts. Saponins have been reported to possess antioxidant, antimicrobial, and anti-inflammatory properties and may contribute to the biological activity of plant extracts (Arabski et al., 2012; Vaou et al., 2021).

In contrast, the methanol, ethanol, and propanol extracts did not exhibit persistent foam formation, indicating a negative result for saponins. The absence of stable froth suggests that these solvents either failed to extract detectable amounts of saponins or that the compounds

were present in very low concentrations. Overall, the foam test confirmed the presence of saponins predominantly in the distilled water and chloroform extracts of Kahwa solution, while the methanol, ethanol, and propanol extracts showed negative results.

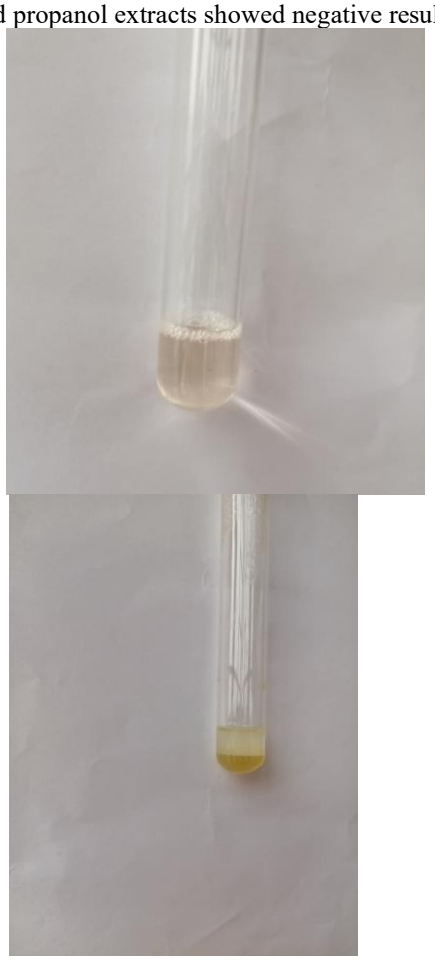


Fig. 9 Foam test showing persistent froth formation in distilled water and chloroform extracts, indicating the presence of saponins.

Detection of Carbohydrates

The presence of carbohydrates in Kahwa solution was evaluated through the iodine test using the aqueous extract. For this analysis, 1 mL of the aqueous extract was taken in a clean test tube and treated with iodine solution. The reaction mixture was gently mixed and observed for any characteristic color change indicating the presence of carbohydrate constituents, particularly starch.

Upon the addition of the reagent, a distinct blue-black coloration was observed in the aqueous extract. The development of this characteristic color indicated a positive result for the presence of starch, thereby confirming the occurrence of carbohydrate compounds in the Kahwa solution. The color change is attributed to the interaction of the reagent with starch molecules present in the extract, resulting in the formation of a colored complex. The intensity of the blue-black color suggested that starch was present in detectable amounts within the aqueous fraction of the sample.

The positive result obtained from the starch test demonstrates that the aqueous extract of Kahwa contains carbohydrate constituents. Carbohydrates are important phytochemical components that contribute to the nutritional value of plant-based beverages and may serve as a source of energy. The findings of the present study therefore confirm the presence of starch-based carbohydrates in the aqueous extract of Kahwa solution. This result highlights the effectiveness of water as an extraction medium for carbohydrate compounds and supports the phytochemical profile of Kahwa as a beverage containing nutritionally significant constituents as in (Fig. 10).



Fig. 10 Iodine test showing a positive blue-black coloration in the distilled water extract, indicating the presence of starch

Detection of Phenolic Compounds

The presence of phenolic compounds in the Kahwa extracts was assessed using the potassium dichromate test. In this method, a few drops of potassium dichromate solution were added to the plant extract, and the development of a dark coloration was considered indicative of the presence of phenolic compounds. Among the solvent extracts evaluated, the distilled water and ethanol extracts exhibited a distinct dark color, confirming a positive result for phenolic compounds. In contrast, the methanol, chloroform, and propanol extracts did not show any noticeable color change and were therefore considered negative.

The positive reaction observed in the aqueous and ethanolic extracts suggests that phenolic constituents present in the Kahwa sample were effectively extracted under the experimental conditions employed. The variation in phenolic compound detection among the different solvent extracts may be attributed to differences in the solubility, distribution, and extraction efficiency of these compounds in solvents of varying polarity. The occurrence of phenolic compounds in both distilled water and ethanol extracts indicate the presence of constituents that are compatible with relatively polar extraction media as shown in (Fig. 11).

Phenolic compounds are widely recognized for their antioxidant and antimicrobial properties and have been reported to disrupt bacterial cell membranes, which may contribute to antibacterial activity (Bouarab-Chibane et al., 2019; Daglia, 2012).



Fig. 11 Potassium dichromate test showing positive reactions for phenolic compounds in distilled water and ethanol extracts.

Detection of Flavonoids

The presence of flavonoids in the Kahwa extracts was evaluated using the concentrated sulfuric acid test. In this method, concentrated sulfuric acid was added to the plant extract, and the development of an orange coloration was considered indicative of the presence of flavonoids. Among the solvent extracts examined, only the methanolic and chloroform extracts exhibited a distinct orange color, confirming a positive result for flavonoids. In contrast, the distilled water, ethanol, and propanol extracts did not show any observable color change and were therefore considered negative.

The positive response observed in the methanolic and chloroform extracts indicates that flavonoid constituents present in the Kahwa sample were effectively extracted under the experimental conditions employed as shown in (Fig. 12). The variation in flavonoid detection among the different solvent extracts may be attributed to differences in the solubility and extractability of these compounds in solvents of varying polarity. The presence of flavonoids in both methanol and chloroform extracts suggests that the Kahwa formulation may contain flavonoid compounds with diverse physicochemical characteristics.

Flavonoids have been widely reported to possess antioxidant and antibacterial activities and may contribute to the observed biological activity of the aqueous Kahwa extract (Cushnie & Lamb, 2011; Kumar & Pandey, 2013).



Fig. 12 Concentrated sulfuric acid test showing positive flavonoid reactions in methanol and chloroform extracts.

Detection of Coumarins

The presence of coumarins in the Kahwa extracts was investigated using the sodium hydroxide test. In this assay, the plant extract was treated with 10% sodium hydroxide solution followed by the addition of chloroform. The development of a yellow coloration was considered indicative of the presence of coumarins. Among the extracts tested, the distilled water and chloroform extracts exhibited a distinct yellow color, confirming the presence of coumarins, whereas the ethanol, methanol, and propanol extracts did not show any noticeable color change and were therefore considered negative.

The positive reaction observed in the aqueous and chloroform extracts suggests that coumarin compounds present in the Kahwa formulation were preferentially extracted into these solvent systems. Coumarins are a diverse group of benzopyrone derivatives whose solubility depends on their structural characteristics and degree of substitution as shown in (Fig. 13). The detection of coumarins in the distilled water extract may be attributed to the presence of relatively polar coumarin derivatives, while their occurrence in the chloroform extract indicates the presence of less polar constituents that are readily soluble in organic solvents.

Coumarins have demonstrated broad-spectrum antimicrobial, antioxidant, and anti-inflammatory

activities and may contribute to the biological properties of medicinal plants (Souza et al., 2020). The variation in coumarin detection among different solvent extracts highlights the influence of solvent polarity on the extraction efficiency of phytochemical constituents.

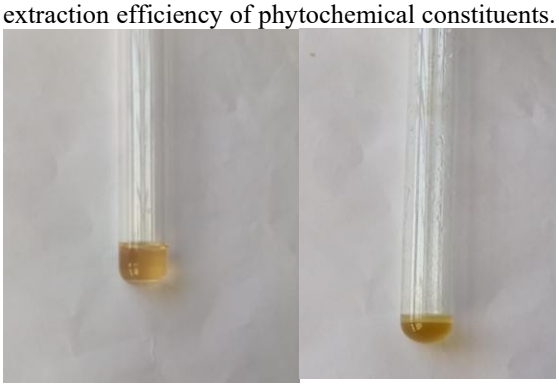


Fig. 13 Sodium hydroxide test showing positive coumarin reactions in distilled water and chloroform extracts.

Detection of Oil and Resins

The presence of oils and resins in the Kahwa extracts was assessed using the filter paper test. A few drops of each extract were placed separately on filter paper and allowed to dry. The formation of a persistent translucent or transparent spot was considered indicative of the presence of oils and resins. Among the extracts tested, only the distilled water extract produced a distinct translucent spot, indicating a positive result, whereas the ethanol, methanol, chloroform, and propanol extracts did not exhibit such an appearance and were therefore considered negative.

The positive reaction observed in the aqueous extract suggests the presence of compounds capable of producing a characteristic translucent stain on filter paper. This may be attributed to water-extractable resinous substances or emulsified lipid-containing constituents derived from the ingredients of Kahwa. The absence of a positive response in the organic solvent extracts may indicate that the concentration of extractable oils and resins in these fractions was below the detection limit of the assay or that the extracted constituents did not produce a sufficiently persistent translucent spot under the experimental conditions employed shown in (Fig. 14).

Kahwa is prepared from a blend of spices and medicinal plants that contain volatile oils, terpenoids, and resinous compounds known to contribute to their characteristic aroma and biological properties (Sofowora, 2008; Vaou et al., 2021). The detection of oils and resins in the distilled water extract may therefore contribute to the characteristic aroma and phytochemical profile of the Kahwa formulation.

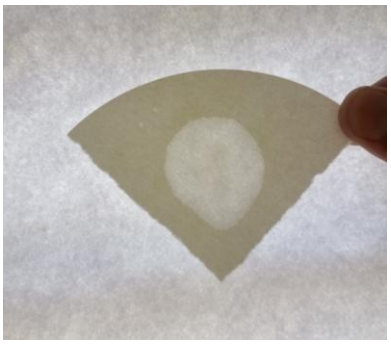


Fig. 14 Filter paper test showing a persistent translucent spot in the distilled water extract, indicating the presence of oils and resins.

3. Discussion

The present investigation demonstrated that only the aqueous extract of the polyherbal Kahwa formulation exhibited antibacterial activity against *Escherichia coli*. (Fig. 3, Fig. 4). Similar observations have been reported in studies where water-soluble phytochemicals were responsible for significant antimicrobial effects (Cowan, 1999; Vaou et al., 2021). The absence of activity in all organic solvent extracts (Fig. 5a-d) and crude solution (Fig. 6) strongly suggests that the principal antimicrobial constituents of the Kahwa formulation are predominantly water soluble.

Phytochemical screening revealed the presence of alkaloids, saponins, phenolic compounds, coumarins, carbohydrates, and oils and resins in the aqueous extract. Alkaloids have been reported to interfere with bacterial DNA replication and protein synthesis, which may contribute to antimicrobial activity (Cushnie et al., 2014). Phenolic compounds have been reported to disrupt bacterial membranes and inactivate essential enzymes and enzyme inactivation in bacterial cells (Daglia, 2012, Bouarab-Chibane et al., 2019). Saponins, confirmed in both aqueous and chloroform extracts by froth tests, may increase membrane permeability, resulting in leakage of intracellular contents and eventual cell death (Sofowora, 2008; Arabski et al., 2012). The presence of coumarins may contribute to the observed antibacterial activity because these compounds have demonstrated broad-spectrum antimicrobial effects in previous investigations (Souza et al., 2020).

The observed antibacterial activity may therefore result from the synergistic action of multiple phytochemicals (Khameneh et al., 2021) extracted into the aqueous phase. Although the inhibition zones observed in this study were larger than those produced by the antibiotic control, additional studies involving replicated experiments, statistical analysis, MIC determination, MBC determination, and chromatographic characterization of active compounds are required to confirm these findings.

These findings align closely with the limited existing literature specifically on Kahwa. A study developing a concentrate from Kahwa leaves (green tea base with saffron and cardamom) reported inhibition zones of 0.75–1.1 cm against *E. coli* using the aqueous extract, together with measurable antioxidant activity,

corroborating the present observation that the aqueous phase of Kahwa-type formulations carries the principal antibacterial burden (Bhat et al.,). This convergence across two independently prepared Kahwa formulations strengthens the inference that the polarity of the active constituents, rather than the specific proportions of individual ingredients, governs extractability of the antibacterial fraction.

The predominance of aqueous-phase activity is also consistent with the broader herbal tea literature. A study of wild herbal teas from Poonch, Azad Jammu and Kashmir, found that species with the largest (for inhibition zones) total phenolic and flavonoid content in their aqueous infusions displayed the most pronounced antibacterial and antioxidant effects, reinforcing the view that water-extractable phenolics are principal drivers of bioactivity in traditional tea-based preparations of this region (Ethnobotanical importance and bioactivity of wild herbal teas, Scientific Reports).

Because green tea forms the major component of most Kahwa formulations, comparison with the antibacterial activity of green tea catechins is particularly relevant. Green tea is rich in catechins, especially epigallocatechin gallate (EGCG), which have been reported to exert antibacterial activity by interacting with bacterial cell membranes, increasing membrane permeability, disrupting membrane integrity, and interfering with essential cellular functions (Taylor et al., 2005; Daglia, 2012). These mechanisms are broadly consistent with the membrane-disruptive properties proposed for the phenolic compounds and saponins detected in the aqueous Kahwa extract in the present study.

Several studies have also reported that Gram-negative bacteria such as *Escherichia coli* are generally less susceptible to tea catechins than Gram-positive bacteria because the outer lipopolysaccharide membrane limits the penetration of these compounds (Taylor et al., 2005). Interestingly, despite this reported resistance, the aqueous Kahwa extract produced comparatively large inhibition zones against *E. coli*. This observation suggests that the antibacterial activity observed in the present study may not be attributable solely to green tea catechins but may instead result from the combined or synergistic effects of multiple phytochemicals, including alkaloids, phenolic compounds, coumarins, and saponins present in the polyherbal formulation (Khameneh et al., 2021).

The antibacterial activity observed in the aqueous extract may also be influenced by the combined contribution of different ingredients present in the Kahwa formulation. Since

Kahwa is a polyherbal preparation containing green tea, spices, and medicinal herbs, the overall antibacterial activity is likely to result from the collective action of multiple phytochemicals rather than a single constituent. Previous studies have reported that extraction efficiency and antimicrobial activity are strongly influenced by solvent polarity, plant material, and extraction conditions (Cowan, 1999; Vaou et al., 2021). Therefore, the aqueous-selective antibacterial activity observed in the present study may reflect the extraction of water-soluble bioactive compounds from multiple ingredients acting together, rather than the activity of any individual component alone (Khameneh et al., 2021).

The antibacterial activity observed in the aqueous extract is likely to reflect the combined effects of multiple phytochemicals extracted from the polyherbal Kahwa formulation rather than the activity of any single ingredient. Solvent polarity plays an important role in determining the extraction efficiency of bioactive compounds, and previous studies have reported that water is particularly effective for extracting several polar phytochemicals with antimicrobial potential (Cowan, 1999; Vaou et al., 2021). Therefore, the aqueous-selective antibacterial activity observed in the present study may result from the combined extraction of water-soluble bioactive constituents present in the formulation.

Taken together, the present findings are consistent with previous reports demonstrating that aqueous plant extracts rich in phenolic compounds and other polar phytochemicals frequently exhibit antibacterial activity (Cowan, 1999; Daglia, 2012; Vaou et al., 2021). The phytochemical screening performed in this study detected alkaloids, phenolic compounds, coumarins, saponins, carbohydrates, and oils and resins in the aqueous extract, all of which have previously been reported to possess antimicrobial properties and may collectively contribute to the observed antibacterial activity (Bouarab-Chibane et al., 2019; Cushnie et al., 2014; Souza et al., 2020). Because polyherbal formulations contain multiple bioactive constituents, the antibacterial activity observed in the present study may result from synergistic or additive interactions among these phytochemicals rather than the action of a single compound (Khameneh et al., 2021). Nevertheless, further investigations involving bioassay-guided fractionation, determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), and chromatographic characterization of the active constituents are required to identify the specific compounds responsible for the antibacterial activity of the Kahwa formulation.

The present study represents a preliminary evaluation of the antibacterial potential of Kahwa extracts. Although the agar well diffusion assay demonstrated promising antibacterial activity, determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) was beyond the scope of this primary investigation. These analyses will be undertaken in future studies to provide a more comprehensive assessment of the antibacterial efficacy of the extracts.

4. Conclusion

The aqueous extract of the investigated polyherbal Kahwa formulation demonstrated antibacterial activity against *Escherichia coli*, producing a maximum zone of inhibition of 33 mm at a concentration of 100 µL. Phytochemical screening revealed the presence of alkaloids, saponins, carbohydrates, phenolic compounds, coumarins, and oils and resins, which may

collectively contribute to the observed antibacterial activity. In contrast, the ethanol, methanol, propanol, and chloroform extracts did not exhibit detectable antibacterial activity under the experimental conditions employed. These findings suggest that the aqueous extract of the investigated Kahwa formulation may represent a promising source of water-soluble bioactive compounds with antibacterial potential. However, further studies involving replicated experiments, determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), chromatographic characterization of the active constituents, and evaluation against additional microbial strains are required to validate and extend the present findings (Chassagne et al., 2021).

References:

1.Arabski M, Węgierek-Ciuk A, Czerwonka G, Lankoff A, Kaca W. Saponins as membrane-active compounds: biological implications. *Journal of Biomedicine and Biotechnology* 2012;2012:474624.

2.Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis.* 2016;6(2):71–79.

3.Bouarab-Chibane L, Forquet V, Lantéri P, et al. Antibacterial properties of polyphenols: characterization and QSAR models. *Frontiers in Microbiology*. 2019;10:829.

4.Chassagne F, Samarakoon T, Porras G, et al. A systematic review of plants with antibacterial activity. *Journal of Ethnopharmacology*. 2021;268:113656.

5.Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Susceptibility Testing*. 33rd ed. Wayne (PA): CLSI; 2023.

6.Cowan MM. Plant products as antimicrobial agents. *Clinical Microbiology Reviews*. 1999;12(4):564–582.

7.Cushnie TPT, Lamb AJ. Recent advances in understanding the antibacterial properties of flavonoids. *International Journal of Antimicrobial Agents*. 2011;38(2):99–107.

8.Cushnie TPT, Cushnie B, Lamb AJ. Alkaloids: an overview of their antibacterial, antibiotic-enhancing and antivirulence activities. *Natural Product Reports*. 2014;31(11):1479–1492.

9.Daglia M. Polyphenols as antimicrobial agents. *Current Opinion in Biotechnology*. 2012;23(2):174–181.

Harborne JB. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. London: Springer; 1998.

10.Khameneh B, Iranshahy M, Soheili V, Bazzaz BSF. Synergistic interactions between plant extracts and antibiotics against infectious diseases. *Phytomedicine*. 2021;85:153543.

11.Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. *The Scientific World Journal*. 2013;2013:162750.

12.Parekh J, Chanda S. Screening of aqueous and alcoholic extracts of some Indian medicinal plants for antibacterial activity. *Pharmaceutical Biology.* 2007;45(5):392–395.

13.Saxena M, Saxena J, Nema R, Singh D, Gupta A. Phytochemistry of medicinal plants. *Journal of Pharmacognosy and Phytochemistry*. 2013;1(6):168–182.

14.Sofowora A. *Medicinal Plants and Traditional Medicine in Africa*. 3rd ed. Ibadan: Spectrum Books Ltd.; 2008.

15.Souza SM, Delle Monache F, Smânia A Jr, Smânia EF. Coumarins as antimicrobial agents: A review. *Molecules*. 2020;25(14):3296.

16.Taylor PW, Hamilton-Miller JMT, Stapleton PD. Antimicrobial properties of green tea catechins. *Food Science and Technology Bulletin*. 2005;2:71–81.

17.Trease GE, Evans WC. *Pharmacognosy*. 16th ed. Edinburgh: Saunders Elsevier; 2009.

18.Vaou N, Stavropoulou E, Voidarou C, et al. Towards advances in medicinal plant antimicrobial activity: A review. *Microorganisms*. 2021;9(9):1991.

19.World Health Organization. *Global Antimicrobial Resistance and Use Surveillance System (GLASS) Report*. Geneva: World Health Organization; 2024.