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Effect of Extraction Solvents on the Yield and Phytochemical Composition of Bridelia Ferruginea Obtained by Cold Maceration

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Abstract
Bridelia ferruginea is an important medicinal plant widely used in African traditional medicine due to its rich phytochemical composition and diverse pharmacological activities. This study investigated the effect of solvent polarity on the extraction yield and phytochemical constituents of *Bridelia ferruginea* using successive cold maceration with n-hexane, ethyl acetate, ethanol, methanol, and water. The percentage yield, qualitative phytochemical profile and quantitative phytochemical composition of the various extracts were evaluated using standard analytical procedure.
The extraction yield varied significantly among the solvents, with ethanol producing the highest percentage yield (16.91%), followed by methanol (2.36%), ethyl acetate (1.78%), water (1.61%) and n-hexane (0.32%). Qualitative phytochemical screening revealed the presence of several bioactive compounds, including alkaloids, saponins, glycosides, cardiac glycosides, flavonoids, phenolics, tannins, terpenoids, anthraquinones, coumarins and quinones, with their distribution varying according to the extraction solvent. Polar solvents, particularly ethanol, methanol, and water, extracted a broader spectrum of phytochemicals compared with the non-polar solvent (n-hexane).
Quantitative phytochemical analysis further demonstrated solvent- dependent variations in metabolite concentration. The aqueous extract exhibited the highest levels of saponins (4.53± 0.21%), flavonoids (46.32± 0.14 QE/mg/g), phenol (285.50 ±0.49 GAE/mg/g). and tannis (88.12 ± 0.97 GAE/mg/g), while ethanol recorded the highest alkaloid content (9.52±0.14%). These findings indicate that solvent polarity strongly influence the extraction efficiency and phytochemical composition of *Bridelia ferruginea*. The abundance of phenolic, flavonoids, tannins, saponins and alkaloids supports the therapeutic potential of the plant and provides scientific evidence for its continued application in traditional medicine and future pharmaceutical development

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Keywords: Bridelia ferruginea, cold maceration, successive extraction, phytochemicals, extraction yield, solvent polarity, bioactive metabolites

Introduction

Bridelia ferruginea Benth is a medicinal plant classified under the family of phyllanthacea and it has a broad distribution across tropical Africa. It is a woody shrub or small to medium sized tree found in several West African Countries, Nigeria inclusive. The plant has drawn significant scientific attention due to its extensive traditional medicinal use and various biological activities have been reported for its leaves, stem bark, roots and other plants parts. Recent studies have placed special emphasis on its phytochemical constituents and its antioxidants, antidiabetic and gastrointestinal pharmacology (Luka, *et al*., 2020) [5].
The therapeutic value of *Bridelia ferruginea* is largely linked to its rich content of diverse secondary metabolites, including

phenolic compounds, flavonoids, tannins, saponins, alkaloids, triterpenes and polysterols. Recent phytochemical studies have revealed that plant extracts contain numerous bioactive constituents, spectroscopic and chromatographic analyses have further identified compounds belonging to various chemical classes. The antioxidant, antimicrobial and other pharmacological activities are largely attributed to these phytochemicals (Mahomoodally, *et al.*, 2021) [6].

Different parts of *Bridelia ferruginea* have long been employed in traditional medicine for managing various diseases, recent reviews have documented its ethnomedicinal uses, including for conditions like dysentery, diarrhea, diabetes, arthritis, skin disorders, oral infections and other inflammatory or infectious conditions. Several scientific investigations have experimentally validated these traditional applications. For instance, a 2021 attributed antidiarrhoeal activity to the bark methanolic extract, possibly through ATPase modulation and interactions with gastrointestinal receptors (Oyebode, *et al*., 2022) [11].

Of particular interest is the antidiabetic potential of the plant. Research conducted since 2020 has demonstrated that *Bridelia ferruginea* extracts possess inhibitory activity against important carbohydrate-digesting enzymes, including α-amylase and α-glucosidase, and many of these compounds also influence intestinal glucose absorption and glucose metabolism. Studies in diabetic animal models have also indicated that *Bridelia ferruginea* can attenuate oxidative imbalance and lipid-induced toxicity, with concurrent modulation of insulin- related signaling pathways, including increased GLUT4 expression in hepatic tissues. These findings provide experimental evidence supporting some of its traditional use in diabetes management, although further studies are required to establish clinical efficacy and safety in humans (Omolaso, *et al* 2023) [12].

The antibacterial and antifungal potential of *Bridelia ferruginea* has similarly been the subject of investigation. A 2020 study analyzed aqueous and ethanolic extracts of the root and stem bark of *Bridelia ferruginea*, documenting their phytochemical composition and antibacterial activity against selected bacterial strains. Research published in 2020 also explored the plant's activity against oral pathogens and its phytochemicals makeup, further supporting its potential for antimicrobial applications (Luka, *et al*., 2020) [5]

A 2020 review also highlighted that *Bridelia ferruginea* exhibits considerable potential in herbal medicine, phytochemistry, and pharmacological, but that further toxicological and clinical evaluation are warranted. Therefore, the plant constitutes a potentially valuable source of bioactive natural products for therapeutic drug development. Nonetheless, while laboratory evidence is encouraging, the majority of studies remain at the preclinical stage, and the therapeutic potential of *Bridelia ferruginea* has not yet been confirmed in clinical settings.

Collectively, its traditional medicinal use, diverse phytochemical composition, and demonstrated biological effects underscore the importance of continued research on *Bridelia ferruginea* in pharmacognosy, phytochemical and pharmacological. Further investigations focusing on the solvent dependent variations in bioactive metabolite extraction of active compounds could contribute to the scientific validation and possible development of *Bridelia ferruginea* -derived therapeutic products. This study aims to investigate the effect of solvent polarity via cold maceration on *Bridelia ferruginea* stem bark and to provide insights into its qualitative and quantitative phytochemicals

Materials and methods

Plant collection

The stem bark of *Bridelis ferruginea were* collected from Oyo State college of Agricultural and Technology Igboora, Oyo State, Nigeria.

Extraction of Plant Materials

The stem bark was washed, gently with cleaned water and then with distilled water to remove debris and air dried at room temperature for 60 days. The dried barks were then grounded into a fine powder. The extraction followed the procedures described by Ojo *et al*. (2017) with modification. Successive maceration was performed using n-hexane, ethyl-acetate, ethanol, methanol and water. 500 g of powdered sample was soaked in 2 L of each solvent for 72 hours at room temperature with intermittent shaking, followed by filteration using Whatman No. 1 filter paper, the residue was rewashed before proceeding to fresh solvents under similar conditions. Filtrates would be concentrated under reduced pressure at 40 °C using a rotary evaporator. The obtained extracts would be stored in a desiccator at 4°C, and percentage yield would subsequently be determined.

$$\text{Yield (\%)} = \frac{\text{Weight of extract}}{\text{Weight of powdered sample}} \times 100$$

Phytochemicals screening

Qualitative phytochemicals screening

Standard qualitative test methods were used to check the extract for the presence or absence of major phytochemicals groups (Adepoju *et al*., 2024) [1]. Screening includes alkaloids, saponnins, glycosides, cardiac, flavonoids, phenols, tannins, phlobatannins, terpenoids, anthraquinones, coumarins and quinones.

Quantitative phytochemical screening

Estimation of alkaloids

A standard precipitation-based method was used to measure the total alkaloid. In a 250 mL beaker, 5g of the sample was weighed. 200 mL acetic acid in ethanol (10%) was added and left to stand for 4 hours. The extract was then filtered and concentrated to one quarter of its original volume in a water bath. To produce precipitation, concentrated ammonium hydroxide was applied to the extract drop by drop. The entire solution was allowed to settle, and the precipitate was collected and filtered after being washed with diluted ammonium hydroxide. The residual is the dried and weighed alkaloid.

Formula = B - A × 100 / S

Where B = Weight of Whatman filter paper.

A = Weight of Whatman filter paper, after drying.

S = Sample weight.

Estimation of total flavonoids

The volume was made up to 100 ml with distilled water after 100 mg of tannic acid has been dissolved in a little amount of distilled water. By diluting the standard with distilled water, different concentrations of the standard were achieved. The

solution's concentration was 100 mg/ml. At zero-time, 0.5 ml of aqueous extract sample was diluted with 3.5 ml distilled water. The tubes were filled with 0.3 ml of 5% sodium nitrate. After five minutes, all of the tubes received 0.3 ml of 10% aluminum chloride. 2 ml of 1M sodium hydroxide was added to the mixture at the 6th minute. The contents of the reaction mixture were immediately diluted with 2.4 mL distilled water and properly stirred. The mixture's absorbance was immediately measured at 510 nm in comparison to a prepared blank. Total flavonoids were measured in mg per 100g of edible part using tannic acid as a reference ingredient.

Estimation of saponins

Ethanol extraction and partitioning method was used to measure the amount of saponins. Conical flask containing 100 ml of 20% aqueous ethanol was filled with 20 g of sample. At roughly 55°C, the sample was heated for four hours in a hot water bath with constant stirring. The residue was re-extracted with another 200 mL of 20% ethanol after the mixture was filtered. Over a water bath at roughly 90°C, the combined extract was reduced to 40mL. The concentrated solution was poured into a 250mL separator funnel, along with 20mL of diethyl ether, and rapidly shaken. The aqueous layer was saved, while the ether layer was discarded, and the purification procedure was repeated. The extract of n-butanol was then added in 60mL. The extracted n-butanol was rinsed twice with 10mL aqueous sodium chloride. In a water bath, the rest of the solution was heated. The sample was dried to a consistent weight in the oven after evaporation. The percentage of saponins was determined. Formula = B – A × 100 / S Where B = Weight of Whatmann filter paper.
A = Weight of Whatmann filter paper with sample.
S = Sample weight.

Estimation of tannins

In 100 mL of distilled water, 100 milligrams of tannic acid were dissolved. With distilled water, 5 mL of stock solution was diluted to 100 mL. 1mL contains 50g of tannic acid. Tannin Extraction: The powdered substance was weighed and placed to a 250 mL conical flask, along with 75 mL water. The flask was gently heated and cooked for 30 minutes before centrifuging for 20 minutes at 2,000 rpm and collecting the supernatant in a 100ml volumetric flask to make up the volume. 1mL of the sample extract was placed in a 100mL volumetric flask with 75 mL of water. 5mL folin denis reagent, 10 mL sodium carbonate solution, and 100mL water were mixed together. Shake well. After 30 minutes, the absorbance was measured at 700 nm. Make a 1 + 4 dilution of]the sample if the absorbance is more than 0.7. Instead of the sample, water was used to make a blank. With 100 mg of tannic acid, a typical graph was created (Robert, 1971). From the standard graph, the tannin content of the sample was estimated as tannic acid equivalents.

Phenol determination

Total phenol was determined spectrophotometrically. The dried pulverized sample was boiled with 50ml of ether for 15 min to extract the phenols. 5 ml of the extract was pipette into 50 ml volumetric flask to which 10 ml of distilled water, 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl-alcohol were also added. The solution was made up to mark and left to react for 30 min for colour development. The absorbance was measured at 505 nm

Results and Discussion

The percentage yield of each extract

Table 1: Percentage yield Result of *Bridelis ferruginea*

Solvent	Yield	% Yield
n-hexane	1.938	0.32
Ethyl-acetate	10.698	1.78
Ethanol	101.436	16.91
Methanol	14.184	2.36
Water	9.66	1.61

The use of different solvents in successive maceration of *Bridelis ferruginea* led to differences in extraction yield. Ethanol gave the highest extraction yield (16.91%) followed in descending oder by methanol (2.36%), ethyl acetate (1.78%, water (1.61%) and n- hexane (0.32%),resulting in a total extraction yield of 22.98%. The dominance of the ethanol extract implies a relatively high content of polar, ethanol-soluble compounds in the plant materials. This could be attributed to ethanol capacity to dissolve a wide range of polar and moderately polar phytochemicals and it may likely due to successive extraction that as remove most polar constituents in earlier stages, the result is in accordance with Olawoore, 2026. Prevoius studies have reported that *Bridelis ferruginea* contain a wide range of phytochemicals constituents, including flavonoids, phenolics, saponins, alkaloids, polysterols and triterpenes, which could be responsible for its therapeutic potential (Yeboak *et al*., 2022). The comparatively low yield from n-hexane implies limited extraction of non-polar compounds in this study. The study demonstrates that solvent properties markedly affect extraction efficiency, and ethanol proved to be the most effective solvent for crude extract recovery from *Bridelis ferruginea*

Qualitative Phytochemicals Screening

Table 2: Qualitative Phytochemicals screening result

Phytochemicals	A	B	C	D	E
Alkaloids	+	+	+	+	+
Saponins	+	+	-	+	+
Glycosides	+	+	-	+	+
Cardiac	+	+	-	+	+
Flavonoids	+	+	-	+	+
Phenols	+	+	-	+	+
Tannins	+	+	-	+	+
Phlobatannins	-	-	-	-	+
Terpenoids	+	+	-	+	+
Anthroquinones	+	-	-	-	+
Coumarins	-	-	-	+	+
Ouinones	-	-	-	-	+

Key: A- Aqueous extract, B- Methanol extract, C- n-Hexane extract, D- Ethyl-acetate extract, E-Ethanol extract

The table shows the qualitative phytochemical screening of *Bridelis ferruginea* in different solvents extract, the phytochemical composition differs according to solvent polarity. Polar solvents water, methanol and ethanol extracted more phytochemicals than the less polar solvent n-hexane, indicating that most bioactive constituents of *Bridelis ferruginea* are polar or moderately polar compounds.

The presence of alkaloids in each solvent extract proposes that alkaloids are among the main constituents of *Bridelis ferruginea*. Antimicrobial, antioxidant activities of alkaloids may account for the plant's traditional use in treating fever and infections. Recent phytochemical investigations of *Bridelis ferruginea* have also reported alkaloids among its important constituents (Ogbonnia, *et al*., 2021). Saponins were absent in n-hexane but present in water, methanol, ethyl acetate and ethanol extracts. The detection of saponins in *Bridelis ferruginea* is consistent with recent studies that identify saponins as one of the characteistics phytochemical groups occurring in the plants (Luka *et al* 2020) [5].

The presence of cardiac glycosides and glycosides in all extracts suggest that these compounds occur in high abundance and are easily extracted. Cardiac glycosides may contribute to the *Bridelia ferruginea* medicinal use in managing cardiovascular conditions, however, high intake may lead to toxicity. Flavonoids and phenolic compounds were not detected in the n-hexane but were present in extract obtained with more polar solvents. This observation aligns with their chemical structure, as phenolic compounds contain hydroxyl groups that enhance their polarity. These metabolites are potent antioxidants that can scavenge free radicals and protect tissues from oxidative stress. Their presence may account for the anti-inflammatory and hepatoprotective effects reported for the plant (Kuevi, *et al*., 2024) [4].

Tannins were detected in water, methanol, ethyl acetate and ethanol extracts but not in n-hexane. Tannins are polyphenolic compounds that can contribute to the astringent, antimicrobial, and antioxidant properties of medicinal plants. Their detection agrees with the recent of *Bridelis ferrugine*, which identified tannins, flavonoids, phenolic and saponnins among the phytochemical groups reported from different parts of the plant (Yeboah, *et al*., 2022) [14]. Phlobatannins was present only in ethanol extract; this suggests that condensed tannins were either absent or present at level below the detection limit in the plant materials analyzed. Similar variation in phlobatannin presence has been documented across different plant parts and extraction methods (Aminu, *et al*., 2022) [2]

Terpenoids were present in water, methanol, ethyl acetate and ethanol extracts but was not detected in n-hexane extracts. Terpenoids are recognized for antimicrobial, anti-inflammatory and anticancer activities and may substantially enhance the therapeutic potential of the plant. Anthraquinones were exclusively detected in the aqueous extract and methanol, indicating selective extraction by highly polar solvents. Anthraquinones are frequently linked to laxative and antimicrobial activities. Their limited presence may suggest low concentrations in the plant material (Arijeniwa, *et al*., 2022).

Quinones were identified exclusively in the ethanol extract, indicating that ethanol is the most effective solvent for extracting these compounds. Quinones are linked to antimicrobial and anticancer through their capacity to engage in redox reactions and disrupt microbial metabolism.

Table 3: Quantitative Phytochemical Analysis of *Bridelis ferruginea*

Parameter	Ethyl-acetate	Ethanol	Methanol	Water
Saponins (%)	2.19±0.06	3.36±0.11	4.06±0.11	4.53±0.21
Alkaloids (%)	8.78±0.12	9.57±0.14	8.54±0.16	8.79±0.02
Flavonoid (QE/mg/g)	15.750±0.13	11.734±0.06	21.86±0.22	46.32±0.19
Phenol (GAE/mg/g)	22.881±0.58	126.326±0.57	79.79±0.29	285.50±0.49
Tannins (GAE/mg/g)	7.812±0.12	29.981±0.13	24.96±0.09	88.10±0.97

From the table 3, the quantitative phytochemical composition of *Bridelis ferruginea* extracts acquired using different solvents (ethyl acetate, ethanol, methanol, and water) exhibited significant variation in bioactive compounds concentrations. This variation is primarily attributed to differences in solvent polarity and extraction efficiency.

The saponin content varied from 2.19 ± 0.06% in ethyl acetate extract to 4.53 ± 0.21% in the aqueous extract, with

the value of 3.36 ± 0.11% and 4.06 ± 0.11% recorded for the ethanol and methanol extract respectively. This suggests that saponins in *Bridelis ferruginea* are predominantly polar compounds and therefore more efficiently extracted by polar solvents such as methanol and water. Saponins are known for their antimicrobial, antioxidant, anti-inflammatory and antidiabetic properties, which may contribute to the ethnomedicinal uses of the plant (Musa, *et al*., 2023). Alkaloid concentration varied from 8.54 ± 0.16% in methanol extract to 9.57 ± 0.14% in ethanol extract, with aqueous and ethyl acetate extracts containing 8.78 ± 0.12% and 8.79 ± 0.02%, respectively. The elevated alkaloid content in the ethanol extract suggests that ethanol was more effective at extracting nitrogenous compounds from the plant material. Alkaloids are pharmacologically important because they exhibit analgesic, antimicrobial, antimalarial, antihypertensive, and anticancer activities (Donhouedé, *et al*., 2022) [3]. Flavonoid levels varied considerably across solvents with concentrations of 11.73 ± 0.06 mg QE/g in ethanol extract, 15.75 ± 0.13 mg QE/g in ethyl acetate extract, 21.86 ± 0.22 mg QE/g in methanol extract, and 46.32 ± 0.19 mg QE/g in aqueous extract. Aqueous extracted the highest number of flavonoids, indicating that flavonoids in *Bridelia ferruginea* are predominantly polar compounds. Flavonoids are recognized for their strong antioxidant, anti-inflammatory, cardioprotective, and anticancer properties. High flavonoid content is generally associated with increased free radical scavenging activity and improved therapeutic potential (Oguejiofor *et al*., 2024) [7]

The phenolic content was highest in the ethanol extract (192.418 ± 0.59 mg GAE/g), followed by the methanol extract (72.36 ± 0.14 mg GAE/g), the aqueous extract (55.92 ± 0.24 mg GAE/g), and the ethyl acetate extract (46.861 ± 0.17 mg GAE/g).

Phenolic compounds are key contributors to antioxidant activity in medicinal plants due to their ability to donate electrons and neutralize reactive oxygen species. The marked variation was observed in phenolic content. The aqueous extract exhibited the highest value (285. 50 ± 0.49 GAE/mg/g) followed by ethanol (126.33 ± 0.57 GAE/mg/g), methanol (79.79 ± 0.29 GAE/mg/g) and ethyl acetate (22.88 ± 0.58 GAE/mg/g). This result indicates that water is particularly effective for extracting phenolic compounds from *Bridelia ferruginea* (Shahidi *et al*., 2023) [13].

Tannin content was highest in the aqueous extract 88.12 ± 0.97 mg GAE/g, followed by ethanol extract (29.98 ± 0.13 mg GAE/g), methanol extract(24.96 ± 0.09 mg GAE/g) and ethyl acetate (7.81 ± 0.12 mg GAE/g). The high tannin concentration in the aqueous extract indicates efficient extraction of these highly polar polyphenols compounds. Tannins possess antimicrobial, antioxidant, anti-diarrheal, and wound-healing activities and may contribute to the medicinal properties of *Bridelia ferruginea*

Conclusion

The results indicate that aqueous extract showed the highest levels of saponins, flavonoids, phenols and tannins while ethanol was most effective for alkaloids extraction. These findings suggest that methanol and water are the most suitable for recovering antioxidant phytochemicals from *Bridelia ferruginea*. The high levels of phenolic, flavonoids, tannins, saponins and alkaloids support the medicinal importance of the plant and provide scientific evidence for its traditional use in the management of inflammatory, microbial and oxidative stress related diseases

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