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## Research Article

# Pharmaceutical Characterization of Aqueous Stem Bark Extract of *Bridelia ferruginea* Benth (Euphorbiaceae)

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## Abstract

***Purpose**: To characterize some preformulation properties of aqueous stem bark extract of Bridelia ferruginea Benth (Euphorbiaceae) (BF).*
***Methods:** The stem bark was extracted by maceration in hot distilled water. Two batches of granules containing the extract were prepared by wet granulation using maize starch, polyvinylpyrrolidone (PVP), Avicel® PH-101, magnesium stearate, acacia and lactose as excipients. Some physicochemical and micromeritic properties of the powdered extract and granules were determined following standard procedures.*
***Results:** The pH of the aqueous BF extract was 5.4 ± 0.1 while the moisture content of the dry extract was 12.0 %. Total ash value of the extract was 7.91 ± 0.03. Particle size increased after granulation from 228 to 531 µm in the order: granules 1 > granules 2 > powder. The bulk and tapped densities decreased significantly ($p < 0.05$) from 0.40 ± 0.04 to 0.77 ± 0.09 and 0.49 ± 0.05 to 0.85 ± 0.09 g/ml, respectively in the order: granules 2 < granules 1 < powder. Similarly, the angle of repose increased after granulation from 24.0 ± 0.5 to 25.4 ± 0.9 $^{o}$ in the order: granules 2 < granules 1 < powder ($p < 0.05$). The flow rate and compressibility of the granules (2.45 ± 0.08 g/min and 0.17, respectively) improved significantly ($p < 0.05$) over those of the powdered BF extract (2.34 ± 0.05 g/min and 0.26, respectively).*
***Conclusion:** The results of this preformulation study indicates that the powdered extract of Bridelia ferruginea possesses properties that make it suitable for its formulation into standard solid dosage forms.*

***Keywords**: Herbal medicines, Bridelia ferruginea, Preformulation studies, Micromeritic properties, Wet granulation.*

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## INTRODUCTION

The term, herbal medicines, has been defined as medicinal products whose active ingredients are derived from aerial or underground parts of plants or other plant material or the combination of them, whether in the crude state or as a plant preparation [1-3]. Plant material includes such substances as juices, gums, and oils. Herbal medicines may contain plant materials other than the active ingredients and may even contain other non-plant organic or inorganic active ingredients [1-3].

Medicinal plants have played a key role in world health. They are distributed worldwide, but are most abundant in tropical countries. It has been estimated that about 25% of all modern medicines are directly or indirectly derived from plants [4]. Herbal drugs range from parts of plant, of which leaves, bark, roots, flowers and seeds are the most common, to isolated and purified active constituents. For use as medicines, they are usually eaten, swallowed, drunk, inhaled, or applied to the skin [5]. The plant materials are usually boiled in water to make decoctions [6].

Phytotherapeutic agents or phytomedicines are standardized herbal preparations that contain, as active ingredients, complex mixtures of plant materials in the crude or processed state. Phytopharmaceuticals are always mixtures of many constituents and are therefore very variable and difficult to characterize. Standardization of the preparation of herbal medicines still poses great challenges today. Therefore, critical steps have to be taken for their proper identification, screening and standardization to ensure quality [7].

*Bridelia ferruginea* Benth (Family: Euphorbiaceae) (BF) is a widely employed ethnomedicinal plant in several parts of Africa for the treatment of various ailments. It is usually used in the form of decoctions and extracts of parts of the plant and is about the most studied of the species *Bridelia* [8]. In Nigeria, it is commonly found in the Savannah and other more humid regions of Africa. The aqueous stem bark extract has been reported to demonstrate anti-inflammatory, antipyretic and analgesic activities *in vivo* and *in vitro* [9] as well as significant antiplasmodial activity against *Plasmodium berghei berghei* in mice [10].

Therefore, the objective of this study was to characterize the preformulation properties of *Bridelia ferruginea* stem bark extract in order to determine its suitability for formulation into solid dosage forms.

## EXPERIMENTAL

### Materials

Stem bark, leaves and fruits of BF, distilled water, and acacia gum (BDH, England) were used in the study, All other reagents were of analytical grade and used without further purification.

### Collection and identification of plant material

The stem of the plant, BF, with its leaves and fruits, was collected fresh from Odenigbo, Nkalagu-Obukpa, Nsukka, Enugu State, Nigeria on May 24, 2010 and identified by Jamilat Ibrahim of the Department of Medicinal Plant Research & Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria, and a voucher specimen (no. NIPRD/H/6414) kept for future reference at the NIPRD Herbarium.

### Preparation of crude extract

The stem bark was extracted by maceration in hot distilled water as previously reported [10]. The bark was cleaned and scales removed. The clean bark was then cut into pieces, air-dried at room temperature for 7 days and ground to powder using mortar and pestle. The powder was soaked in boiled

distilled water overnight and filtered. The filtrate was heated over a hot water bath to recover the dry extract which was pulverized and kept in an air-tight container pending further tests.

**Determination of organoleptic properties**

The colour and taste of the extract were assessed in line with the hedonic scale

**Determination of pH**

A 1 % w/v solution of BF extract in distilled water was prepared, filtered and the pH read on a pH meter (Accumet Research, model AR10, Fisher Scientific, Singapore), and the mean of triplicate readings taken.

**Moisture content determination**

Moisture content of the aqueous BF extract was determined by slight modification of the requirement in the British Pharmacopoeia (BP) for loss on drying of extracts [11]. A clean dry Petri dish was weighed and 1.0 g of the extract, accurately weighed with an analytical balance (AB54, Mettler Toledo, Switzerland), was placed on it. The Petri dish was then placed in a hot air oven (N30C, Genlab, England) set at 60 $^{o}$C and the extract dried to constant weight (approx. 3 h). It was cooled over silica gel in a desiccator and re-weighed. The per cent loss in weight was taken as moisture content. Triplicate measurements were made and mean recorded.

**Ash value determination**

The ash value of the extract was determined following method 1 of BP 2004 [11]. A silica crucible was dried in an oven for 10 min and weighed on an analytical balance (AB54, Mettler Toledo, Switzerland). A 2.0 g quantity of the powdered extract was weighed and spread in the crucible with a clean, dry spatula. The crucible and its content were then placed in an oven (Nabertherm, Karl Kolb Scientific Technical Supplies, D 6072, Dreleich, West Germany), heated to 450 $^{o}$C, gradually cooled and then re-weighed immediately. Ash value was calculated as in Eq 1. Triplicate determinations were made and the mean taken.

Ash value (%) = $\{(W_2 - W_1)/M\}$ …… (1)

where $W_1$ = weight of the crucible, $W_2$ = final weight of the crucible + ash, and M = initial weight of sample.

**Preparation and evaluation of granules**

Two batches of granules of the powdered extract were prepared by wet granulation. Using a 3% acacia mucilage, the mixture of the ingredients was wetted and the wet mass passed through a standard granulating sieve with the aid of a spatula. The granules were then dried in an oven at 60 $^{o}$C for 1 h. Batch 1 contained (as per hypothetical compressing weight of tablet) the extract (100 mg), maize starch (30 mg) as disintegrant and 3 % acacia mucilage (9 mg dry weight) as binder. Batch 2 was similar to Batch 1 in composition and quantities except that Avicel® PH-101 and polyvinylpyrrolidone were used as disintegrant and binder, respectively. Magnesium stearate (lubricant) and D-lactose monohydrate, (bulking agent) were added to the granules for both batches prior to compression into tablets. The disintegrants were incorporated in the formulations 80 % intragranularly and 20 % extragranularly.

**Micromeritic properties**

The following micromeritic properties were assessed (in triplicate) for the powdered extract and granules.

**Bulk density ($D_B$)**

A mass of the sample was screened through sieve of aperture size 850 µm, 16.37 g of which was poured at an angle of about 45$^{o}$ into a clean, dry, graduated 50 mL measuring cylinder. The volume occupied was noted and the mean $D_B$ calculated from Eq 2 [12].

$D_B = M/V_B$ …… (2)

where M = weight of the sample and $V_B$ = mean bulk volume of the sample.

**Tapped density ($D_T$)**

A 16.37 g quantity of the screened sample was poured at an angle of about 45$^o$ into a clean dry 50 mL measuring cylinder, placed on a Stampfvolumeter (JEL STAV, 2003 model) and tapped until no further change in volume occurred. The constant volume was noted. Tapped density ($D_T$) was calculated from Eq 3 [12].

$D_T = M/V_T$ …….. (3)

where M = weight of the sample and $V_T$ = tapped volume of the sample.

**Hausner ratio (HR) and Compressibility index (CI)**

The Hausner ratio (HR) and Compressibility index (CI) of the samples were derived from Eqs 4 and 5, respectively.

$HR = D_T/D_B$ …… (4)

$CI = \{(D_T – D_B)/D_T\} \times 100\ \%$ …… (5)

**Kawakita test**

A weighed amount of the sample was poured at an angle of about 45$^o$ into a clean dry graduated measuring cylinder and the initial volume ($V_0$) noted. The cylinder was positioned on a tapping machine (Stampfvolumeter, JEL STAV 2003 model) and tapped intermittently, noting the tapped volume (V) after a specific number of taps (N). Tapping was continued until no further change in volume of the sample occurred. The degree of volume reduction during tapping (C) and the ratio of number of taps (N) to this degree were calculated from Eqs 6 - 8 [13].

$C = (V_0 – V)/V_0$ …….. (6)

$R = N/C$ …….. (7)

$N/C = N/a + 1/ab$ …….. (8)

where R = ratio of number of taps to the degree of volume reduction, and *a* and *b* are constants; *a* describes the degree of volume reduction at the limit of tapping and is referred to as compressibility as it reflects the compactibility of the material; *b* relates to the cohesion and shear strength of the material.

Eq 8, referred to as Kawakita equation, is applied in tapping experiments to assess the cohesiveness and compactibility of powdered materials, and hence their flow properties.

A plot of N/C against number of taps, N, gives a graph of slope equal to 1/*a* and intercept equal to 1/*ab* from which numerical values for the constants *a* and *b* can be obtained. While *a* gives an indication of the maximum of volume reduction available, characteristic for the powder and also known as compressibility, *b* describes an inclination towards volume reduction [13].

**True density ($D_t$)**

The fluid displacement method was used for the determination of the true density of the powdered extract. A density bottle was washed, dried thoroughly in an oven and weighed. The bottle was then filled with xylene as the displacement fluid and weighed. Some of the xylene was removed from the bottle, and approximately 1.0 g quantity of the powdered extract, accurately weighed, and transferred into the bottle with sample and then weighed. Triplicate measurements were made and mean $D_t$ computed from Eq 9.

$D_t = w/\{(a + w) – b\} \times SG$ …… (9)

where w = weight of the powdered extract (1.0 g); a = weight of density bottle + xylene; b = weight of density bottle + xylene + powdered extract; and SG = specific gravity of the displacement fluid, xylene (0.863).

**Flow rate and Angle of repose**

The fixed-height cone method [14] was used. A glass funnel was fixed at a 10 cm height over a flat horizontal surface. The exit/orifice of the funnel was temporarily blocked with a

flat ruler. A clean white paper was spread beneath the funnel on the horizontal surface for collection of the powder. A 16.35 g quantity of the sample was then weighed and poured into the funnel. The exit of the funnel was then sharply unblocked and the time (seconds) taken for the sample to completely flow out onto the horizontal surface was measured with a stop clock. The height and radius of the cone formed were measured with a ruler. Flow rate and angle of repose of the sample were calculated as in Eqs 10 and 11:

Flow rate = $M/t$ …….. (10)

Angle of repose = tan $A = h/r$ …..…(11)

where M = mass (g) of the sample, t = time (seconds) taken for the sample to completely flow out of the funnel, A = angle ($^o$) formed by the sample cone, and h = height (cm) of the sample cone formed after flowing from the funnel.

### Particle size analysis

A sieve-shaker assembly was used for this determination. A set of testing sieves (Standard Testing Sieves, ASTM E-11 Specification, VWR Scientific, West Chester, USA) of sizes 150, 250, 500, 600, 710, 850 µm and 1.00 mm were arranged in descending order on the shaker (Retsch 2002, Germany). A 15.0 g quantity of the sample was poured on the top sieve (1.00 mm) and a plate placed at the bottom. The shaker was run for 20 min at an amplitude of 1.5 mm/g. At the end of the run, the amount of material retained on each sieve was weighed. The average particle size was computed as in Eq 12 [15]:

Mean diameter = [∑ (% retained) x (mean aperture size)]/100 …… (12)

### Statistical analysis

The results were analyzed for statistical significance by Student's *t*-test at 95 % confidence interval ($p < 0.05$).

## RESULTS

### Organoleptic properties

The extract was brown in colour but had neither odour nor taste.

### Micromeritic properties

Table 1 shows a summary of the micromeritic properties and other physicochemical parameters of the preparations. The values generally indicate good flow properties for the powdered extract and granules. Significant variation in particle size distribution was observed with a preponderance (38.5 %) of fines over other sizes. Angle of repose, bulk and tapped densities decreased in the rank order: powder > Batch 1 > Batch 2. Expectedly, flow rate increased following granulation. The moisture content obtained (12.0 %) was high when compared to the normal limit of not more than 10.0 % as specified in the European Pharmacopoeia [16]. The ash value obtained (7.91 ± 0.03 %) was within pharmacopoeial limits of not more than 14.0 % [16]. Bulk density of the powder (0.77 ± 0.09 g/ml) was significantly higher than that obtained for granules of Batch 1 (0.60 ± 0.08 g/ml) and this was in turn higher than that of Batch 2 (0.40 ± 0.04 g/ml) (Table 1). Compressibility index increased significantly after granulation in the rank order: Batch 1 > Batch 2. The pH of the aqueous solution of the extract was slightly acidic. The Kawakita plots were linear with regression coefficient ($R^2$) values close to 1.

## DISCUSSION

The bland taste of the extract is a good attribute as a formulator will not be bordered about taste masking. Also, cases of non-compliance due to taste would be minimal. The pH of the extract is slightly acidic. According to the pH-partition hypothesis, weakly acidic drugs, which exist predominantly in the unionized form at gastric pH, are well absorbed from the stomach [17]. Although the chemistry of the extract is yet to

**Table 1:** Physicochemical characteristics of *Bridelia ferruginea* extract

| Parameter | Powder | Granules | |
|---|---|---|---|
| | | ***Batch 1*** | ***Batch 2*** |
| Colour | Darkbrown | - | - |
| Odour | Nil | - | - |
| Taste | Nil | - | - |
| pH | 5.4±0.1 | - | - |
| Moisture content (%) | 12.0±0.0 | - | - |
| Ash value (%) | 7.91±0.03 | - | - |
| Bulk density ($D_B$, g/ml) | 0.77±0.09 | 0.60±0.08 | 0.40±0.04 |
| Tapped density ($D_T$, g/ml) | 0.85±0.09 | 0.75±0.01 | 0.49±0.05 |
| Hausner ratio (HR) | 1.20 | 1.25 | 1.21 |
| Compressibility index (CI, %) | 10.6 | 20.2 | 17.1 |
| True density ($D_t$, g/ml) | 1.39 | - | - |
| Flow rate (g/s) | 2.34 ± 0.05 | 2.45±0.08 | 2.45±0.08 |
| Angle of repose ($^0$) | 25.4±0.9 | 24.2±0.4 | 24.0±0.5 |
| Compressibility | 0.26 | 0.17 | 0.19 |
| Cohesiveness | 0.27 | 1.62 | 0.92 |
| Kawakita regr Coefficient | 0.996 | 0.955 | 0.985 |
| Mean particle diameter (µm) | 228 | 531 | 458 |

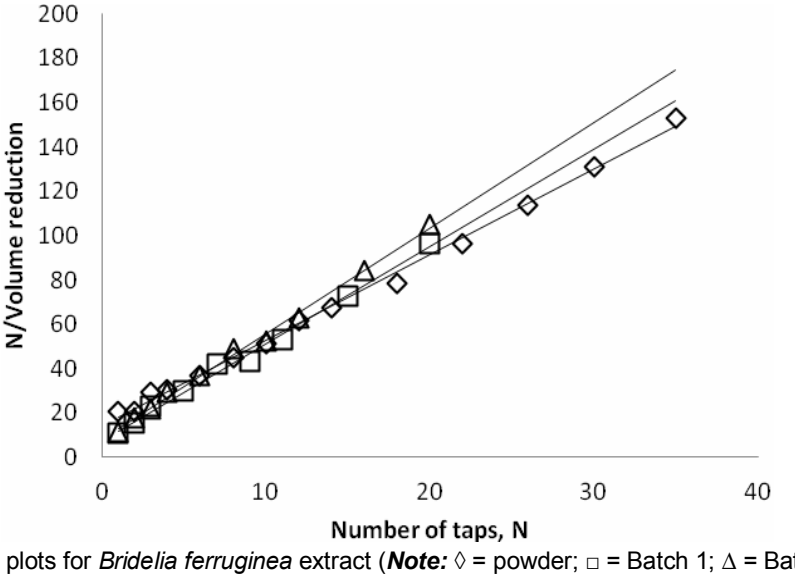


**Fig 1:** Kawakita plots for *Bridelia ferruginea* extract (***Note:*** ◊ = powder; □ = Batch 1; ∆ = Batch 2)

be elucidated, it is probable that it is likely to be well absorbed from oral dosage forms in the stomach due to the weakly acidic nature.

When a plant material is subjected to heat both water and volatile matter evaporate. The test for loss on drying therefore actually determines both water and volatile matter content. Excess of water content in herbal medicines promotes microbial growth, insect infestation, and deterioration by hydrolysis. It is, therefore, important to establish or set limits for water content for every plant material used in preparing phytomedicines, especially materials that will absorb moisture easily or deteriorate quickly in the presence of water. The desiccation method of determining water content is especially useful for materials that melt to a sticky mass at elevated temperatures while the azeotropic method gives a direct measurement of the water content of the material being examined [7]. The moisture content was rather high when compared to official requirement for herbal drug preparations of ≤ 10 % after drying for 2 h, unless otherwise justified [16].
Total ash value indicates the total amount of material remaining from the plant extract after the carbon content have been burnt away by ignition [7]. This includes both 'physiological ash', which is derived from the plant tissue itself, and 'non-physiological ash', which is residue of extraneous matter (e.g., soil) adhering to the plant surface. Ash value is a criterion for judging the identity and purity of crude drugs. This parameter is very useful in evaluating the purity of powdered herbal and conventional drugs such as determination of adulteration with other plant parts or extraneous matter. The result obtained implies that the extract evaluated showed a high degree of purity, and was relatively low in inherent inorganic salts, such as calcium oxalate, and inorganic matter from external sources [16].

The micromeritic properties (bulk and tapped densities, HR, CI, flow rate, angle of repose and average particle size) obtained for the powdered extract generally indicate good flow properties. Angle of repose is characteristic of the internal friction or cohesion of the particles. It is high if cohesive and other forces are high and vice versa. Generally, if the angle exceeds $50^{o}$, the powder will not flow satisfactorily while materials having values near the minimum, circa $25^{o}$, flow easily and well [14]. From the results, it can be inferred that both the powder and the granule formulations would flow well into the die of the tablet press.

It had been reported that HR values less than 1.25 indicate good flow, while values greater than 1.25 indicate poor flow. HR values between 1.25 and 1.5 require addition of a glidant to improve flow [17]. The granules exhibited satisfactory flow properties. Powder as well as granule flow had been described as excellent for values of CI within 5 – 15 %, good for 12 -16 % and fair to passable for 18 – 21 %, while between 23 – 35 % are said to be poor, 33 - 38 % are very poor and values > 40 % are extremely poor [17]. CI was excellent for the powdered BF extract, good for the Batch 2 granules and fair to passable for Batch 1 granules. HR and CI are useful indices for assessing drug powder and granule flow properties. An increase in particle size or a more uniform shape leads to a smaller angle of repose and smaller CI, and this is supported by the data obtained in the present study. However, CI is a one-point determination and therefore may not always reflect the ease or speed with which the powder or granules consolidates. Some materials may have high CI (indicating poor flow) yet may consolidate rapidly in the tableting machine die [17].

The inverse relationship of the slope of the Kawakita plot implies that the higher the slope, the less the value of *a,* and the better the compressibility of the granules or powder. As the tapping was increased, the ratio of the number of taps to the degree of volume reduction also increased gradually. But the slope of the linear plot is in the rank order: powder < Batch 1 < Batch 2. In the Kawakita plot, *a* describes the degree of volume

reduction at the limit of tapping [13]. Hence, the high value of *a* for the powder correlates with its tendency to form very poor or no compacts. Similarly, the values of *b* obtained were in the rank order: powdered extract < Batch 1 < Batch 2, indicating that the powdered particles of the extract were very cohesive, and hence had a greater tendency to form compacts than Batches 1 and 2 granules. Batch 2 granules were the least cohesive, meaning that interparticulate forces favouring compression were least, and therefore, would most probably result in the production of friable tablets. The $R^2$ values obtained from the plot show good linearity which was close to unity, indicating reliability of the estimated equation parameters.

## CONCLUSION

This study demonstrates the feasibility of developing standard solid dosage forms, such as tablets and capsules, of the aqueous stem bark extract of *B. ferruginea* Benth for oral use. Their excellent micromeritic properties also indicate that the powdered extract can be packaged in sachets. Further research is, however, required to establish these possibilities and ascertain the stability and release properties of the dosage forms of the extract as well as their *in vivo* behavior.

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