

## Original Research Article

STUDIES ON THE PHYTOCHEMICAL COMPOUNDS IN THE ETHANOLIC LEAF EXTRACT (ELE), ETHANOLIC BARK EXTRACT (EBE) AND ETHANOLIC ROOT EXTRACT (ERE) OF *Bridelia ferruginea* BENTH (EUPHABIACEAE).

Comment [P1]: EUPHORBIACEAE

### ABSTRACT

The phytochemical compounds of *Bridelia ferruginea* plant parts was carried out using qualitative method to determine the bioactive compounds present in the plant leave, stem bark and root extracts. The samples was weighed, of which 100g each of the powder were extracted in solvents (ethanol) 1000 ml macerated and stand for 72 hours. The solvents contained in the maceration bottle was decanted and filtered using a filter paper, the filtration was aided using a suction pump. The filtrate was concentrated using a rotary evaporator and then transferred into thermostatic water cabinet (temperature was set at 45°C), allowed to dry completely. The plant parts extracts were separately kept in a screw capped bottle for further research. The bioactive compound in the plant parts were detected. The result revealed that Carbohydrates, Saponins, Flavonoids, Tannins, Cardiac Glycosides, fats and oils were present. Alkaloid present in Dragendoff's test in all plant parts extract but absent in Mayer's test in only leaf extract. Terpenoids/Steroids present in Liebermann-Burchard's test in all plant parts extract but absent in Salkowski's test in only leaf extract. Anthraquinones were absent in all plant parts extracts using Bontrager's test. Therefore, the presence of these phyto-pharmacological compounds is an indicative that the plant is medicinal and it can be used for the treatment of bacterial and other microbial infections. Further study can be done to separate the individual metabolites to test their antimicrobial activity against some pathogenic bacteria like bacterial meningitis, tuberculosis and syphilis to determine their potency.

Comment [P2]: There is no need to put the extraction details in the abstract

Keywords: *Bridelia ferruginea*, phyto-pharmacological compound, antimicrobial, medicinal plants, ethanolic extracts.

## INTRODUCTION

30 *Bridelia ferruginea* is a common savannah, deciduous tree of genus *Bridelia*. It is usually a  
31 gnarled shrub which sometimes reaches the sizes of tree in suitable condition. *Bridelia*  
32 *ferruginea* Benth (Guinea Fula-Pulaar) in English and their local common names are kizni, kirni  
33 (Hausa), Mirehi (Fulani), Iroladan or “*Epo Ira*” (Yoruba), Ola (Igbo), Awuya (Ebira), (Akuodor  
34 *et al.*, 2012). Its habitat is the savannah, especially in the moister region extending from Guinea  
35 to Zaire and Angola. *Bridelia ferruginea* grows up to 3-4 m high and may be 27.5 cm in width  
36 (Olatunji *et al.*, 2010; Ezike *et al.*, 2011). The stem is often crooked with branches occurring at  
37 the lower regions. The bark is gray, rough and often scaly (Rashid *et al.*, 2000). The plant of ten  
38 bears spines and may be crimson coloured. The leaves may be small to medium sized, *Bridelia*  
39 species belong to the family Euphorbiaceae and comprise approximately 60-70 species found in  
40 Asia, Africa and Australia (Rashid *et al.*, 2000; Kathriarachchi *et al.*, 2005; Nguem *et al.*,  
41 2009). *Bridelia ferruginea* is utilized in traditional African Medicine in treating disease  
42 conditions such as arthritis, bruises, boils, dislocation, burns, fever, headaches, stiffness,  
43 rheumatic pains and oedema (Olumayokun *et al.*, 2012). Other uses include intestinal disorders,  
44 diabetes, thrush, epilepsy, cough, gonorrhea, infectious diseases including sexually transmitted  
45 diseases, skin diseases and eruption, skin cancers, roundworm (Cimanga *et al.*, 2001). It is also  
46 an antidote for arrow poison (Nguem *et al.*, 2009), and used as anti-inflammatory (Olajide *et*  
47 *al.*, 2003) and antitumor agent (Rhashid *et al.*, 2000).

48 Reports on the plant have shown that aqueous leaf and root extracts of the plant possesses  
49 hypoglycemic activities. Ethnopharmacological reports have shown that the stem bark extract  
50 possesses antiulcerative properties (Ezike *et al.*, 2011) and anti-inflammatory and antibacterial  
51 (Olajide *et al.*, 2003) properties. Research further indicates that extract of the stem bark  
52 possesses antioxidant properties (Adetutu *et al.*, 2011), antipyretic and analgesic activities  
53 (Akuodor *et al.*, 2011). The aqueous stem bark extract possesses antihypertensive, diuretic and  
54 sedative actions (Nene-Bi *et al.*, 2010; Nene-Bi *et al.*, 2012). The stem bark extracts possess  
55 antioxidative and neuroprotective activities (Omotade, 2012). Furthermore, studies have shown  
56 that the aqueous extract of *B. ferruginea* stem bark reduces vascular permeability in both  
57 cyclophosphamide-induced hemorrhagic cystitis and acetic acid induced vascular permeability in  
58 rats and mice (Olajide *et al.*, 2003). *B. ferruginea* have shown that the stem bark extracts exhibit  
59 anti-inflammatory properties, which were attributed to the suppression of up-regulation of

Comment [P3]: Review the formulation

60 tumour necrosis factor alpha (TNF $\alpha$ ) (Olajide *et al.*, 2003). *B. ferruginea* stem bark extract  
61 inhibits xanthine oxidase and possesses superoxide scavenging activity due to the presence of 3-  
62 O-methylquercetin, myricetin, ferrugin and quercetin 3-O-glucoside (Cimanga *et al.*, 2001).The  
63 stem bark and leaf extracts have contractile effects on the smooth muscle of the bladder  
64 (Onoruvwe *et al.*, 2001). The extracts of *B. ferruginea* possess anti-thrombotic effects Chemical  
65 and pharmacological studies of *Bridelia species* have shown the presence of flavonoids,  
66 sesquiterpenes, triterpenoids, and phenolic compounds (Ngueyem *et al.*, 2009). *Bridelia species*  
67 possess variety of biological activities including antiamebic, antianemic, antibacterial,  
68 anticonvulsant, anti-diabetic, antidiarrhoeal, antihelminthic, anti-inflammatory, antineuro-  
69 inflammatory, antimalarial, antinociceptive, antiviral, and hypoglycemic (Ngueyem *et al.*, 2009).  
70 Traditional medicine constitutes an important source of drugs for ethnopharmacological  
71 relevance and investigation. Various medicinal food-plants and animal products-supplements are  
72 available for use in certain immune deficiency disease conditions related to malnutrition such as  
73 infectious disease and hemorrhagic sepsis (Kokori *et al.*, 2019) Purpose?

**Comment [P4]:** What is the purpose and scope of the work? It should be clearly announced at the end of the introduction.



76  
77 Plate 1: *Bridellia ferruginea* Stem Bark  
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Plate 2: *Bridellia ferruginea* Root

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Plate 3: *Bridellia ferruginea* Leaf

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## METHODOLOGY

### 87 **Collection and Identification:**

88 The fresh leave, bark and root samples of *Bridelia ferruginea* were collected at Baba Wali Street,  
89 NTA Community, Behind Kogi State University, Anyigba, Kogi State, Nigeria, April, 2019. The  
90 plant parts were identified and authenticated by U.S Gallah at the Department of Biological  
91 Sciences (Botany option), Kaduna State University. A voucher samples of the plant deposited in  
92 the herbarium unit and the voucher number KASU/BS/1323 was deposited in the herbarium.

93

### 94 **Processing of the plant samples:**

95 The plant parts were washed thoroughly with distilled water, shade dried for 3-4 weeks at room  
96 temperature in the Histology laboratory of the Anatomy Department, Kogi State University,  
97 Anyigba. The plant parts were pulverized in a mortar and pestle and was grounded into fine  
98 powder of 40mm mesh size. The samples were stored in an air-tight container for further use.  
99 The samples was weighed, of which 100g each of the powder were extracted in solvents  
100 (ethanol) 1000 ml macerated and stand for 72 hours. The solvents contained in the maceration  
101 bottle was decanted and filtered using a filter paper, the filtration was aided using a suction  
102 pump. The filtrate was concentrated using a rotary evaporator and then transferred into  
103 thermostatic water cabinet (temperature was set at 45°C), allowed to dry completely. The  
104 extracts obtained was scrapped using a clean spatula and grounded using small laboratory mortar  
105 and pestle, the extracts were weighed using weighing balance.

106 The percentage yield were calculated as follows:

$$107 \quad \% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of plant material}} \times 100$$

108  
109 The extract was stored air-tight in a refrigerator prior to use.

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### 111 **Determination of Phytochemical Compounds in *Bridelia Ferruginea* Parts Extracts**

112 The methods of Trease and Evans (2002) and Sofowora (2008) were used, the phytochemical  
113 compound was carried out in the Ethanolic Leaf Extract (ELE), Ethanolic Bark Extract (EBE)  
114 and Ethanolic Root Extract (ERE) of *B. ferruginea* according to standard procedures as follows:

115

116 **Test for Carbohydrate**

117 **Molisch's test**

118 To a small portion of the ELE, EBE AND ERE in a test tube, 3 drops of molisch's reagent was  
119 added followed by concentrated sulfuric acid. The formation of a reddish colored ring at the  
120 interface indicates the presence of carbohydrates (Trease and Evans, 2002; Sofowora, 2008).

121 **Test for Saponins**

122 **Frothing test**

123 About 10ml of distilled water was added to a portion of the leave, bark and root extract and was  
124 shaken vigorously for 30seconds. The solution was allowed to stand for 5 minutes, the formation  
125 of a persistent froth indicates the presence of saponins (Trease and Evans, 2002; Sofowora,  
126 2008).

127 **Test for Flavonoids**

128 **Shinoda test**

129 The extracts was dissolved in 2ml of methanol and pieces of metallic magnesium chips were  
130 added followed by few drops of concentrated hydrochloric acid, the formation of a pink, orange  
131 or red to purple coloration indicates the presence of flavonoids (Trease and Evans, 2002;  
132 Sofowora, 2008).

133 **Sodium hydroxide test**

134 Two drops of 10% Sodium hydroxide was added to the solution of the extracts, yellow coloration  
135 indicates the presence of flavonoids (Trease and Evans, 2002; Sofowora, 2008).

136 **Ferric chloride test**

137 An amount of 2 to 3 drops of ferric chloride solution were added to the solution of the extracts.  
138 Green colour was observed (Trease and Evans, 2002; Sofowora, 2008).

139 **Test for tannins**

140 **Lead sub-acetate test**

141 To a small portion of the extracts, 4 drops of lead sub-acetate solution was added, the formation  
142 of a cream coloured precipitate indicates the presence of tannins (Trease and Evans, 2002;  
143 Sofowora, 2008).

#### 144 **Test for Terpenoids/Steroids**

##### 145 **Salkowski's test**

146 A small portion of the extracts was dissolved in 2ml of chloroform, 3 drops of concentrated  
147 sulphuric acid was added at the side of the test tube. A red brown coloration at the interface  
148 indicates the presence of terpenoids.

##### 149 **Liebermann-Burchard's test**

150 To the small portion of the extracts equal volume of acetic anhydride was added and mixed  
151 gently. 1ml of concentrated sulphuric acid was added down the test tube. This was observed for  
152 instant colour changes and over a period of one hour. Blue to blue-green colour in the upper  
153 layer and reddish, pink or purple colour at the junction of the two layers indicates the presence of  
154 triterpene (Trease and Evans, 2002; Sofowora, 2008).

#### 155 **Test for Alkaloids**

##### 156 **Dragendoff's test**

157 The extracts was dissolved in 2ml of 5% H<sub>2</sub>SO<sub>4</sub> in 50 ethanol with continuous stirring in water  
158 bath. The mixture was filtered and few drops of Dragendoff's reagent was added, rose red  
159 precipitate indicates the presence of alkaloids (Trease and Evans, 2002; Sofowora, 2008).

##### 160 **Mayer's test**

161 To 2ml acidic solution of the extracts in a test tube, few drops of Mayer's reagent were added, a  
162 cream precipitate indicate the presence of alkaloids (Trease and Evans, 2002; Sofowora, 2008).

#### 163 **Test of Anthraquinones**

##### 164 **Bontrager's test**

165 A small portion of the extract was dissolved in 5ml chloroform, shaken and filtered. To the  
166 filtrate, an equal volume of 10% ammonium solution was added with continuous shaking, bright

167 pink colour in the aqueous upper layer indicates the presence of anthraquinones (Trease and  
168 Evans, 2002; Sofowora, 2008).

### 169 **Test for Cardiac Glycosides**

#### 170 **Keller-Kiliani test**

171 A small portion of the extracts was dissolved in 1ml glacial acetic acid containing traces of ferric  
172 chloride solution. The solution was then transferred into a dry test tube to which an equal volume  
173 of sulphuric acid was added, a brown ring obtained at the interface will indicate the presence of  
174 deoxy sugar (Trease and Evans, 2002; Sofowora, 2008).

### 175 **Test for Fats and Oils**

176 Filter paper soaked in the extracts solution or impregnated with extracts was allowed to dry and  
177 checked for translucence film, which indicates the presence of fats and oils.

178 **Table 1. Biologic activity of main groups of natural compounds.**

Compound type	Pharmacological properties
Terpenoid/steroid	Antimicrobial, antiviral, anthelmintic, antibacterial, anticancer, antimalarial, anti-inflammatory, antineuroinflammatory (Shakya, 2016).
Phenolics acids	Anticarcinogenic and antimutagenic, anti-inflammation and anti-allergic (Anulika <i>et al.</i> , 2016).
Saponins	Antitumor, antiviral, antifungal, anti-inflammatory, immunostimulant, antihypoglycemic, antihepatotoxic and hepatoprotective, anticoagulant, neuroprotective, antioxidant (Negi <i>et al.</i> , 2013).
Flavonoids	Antioxidant activity, cardiovascular protective, anti-inflammatory, hepatoprotective, antiviral, antibacterial (Kumar and Pandey, 2013).
Alkaloids	Antispasmodic, antimalarial, analgesic, diuretic activities, local anesthetic, antihypertensive, antiasthma, antimalarials, diuretic,

**Comment [P5]:** This information should be provided above. They are not part of the methodology.



bactericidal (Chikezie *et al.*, 2015).

Tannins Antioxidant, anti-carcinogenic, diuretics, hemostatic, anti-mutagenic,  
metal ion-chelators, antiseptic, (Saxena *et al.*, 2013).

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180

## 181 RESULT

182 The results obtained during the course of this experiment/ projects are presented below.

### 183 Percentage Yield of Ethanolic Leave Extract, Ethanolic Bark Extract and Ethanolic Root 184 Extract of *Bridelia ferruginea*

185 The percentage yield of the extracts (plant parts of *Bridelia ferruginea*) samples is calculated  
186 below:

$$187 \text{ \% yield} = \frac{\text{Weight of extracts}}{\text{Weight of plant}} \times 100\%$$

189 Weight of ELE= 24.91g, EBE=24.25 and ERE=16.37.

190 Weight of plant= 100g.

$$191 \text{ Therefore, \% yield} = \frac{24.91\text{g}}{100\text{g}} \times 100\%, \quad \frac{24.25\text{g}}{100\text{g}} \times 100\% \quad \text{and} \quad \frac{16.37\text{g}}{100\text{g}} \times 100\%$$

$$193 = 24.91\%, 24.25\% \text{ and } 16.37\%.$$

194 The percentage yield of the extracts obtained is calculated above.

195 **Table 2: percentage yield of the plant parts of *Bridelia ferruginea* Samples**

Samples	Weight of Plant Parts (g)	% yield extract (g)	Observed Coloration
Leave extract	100	24.91	Light green
Stem bark extract	100	24.25	Reddish
Root extract	100	16.37	Brownish

**Comment [P6]:** No need. The above formula is sufficient.

196

197 **The Results of Phytochemical Compounds.**

198 The table below is a summary of the phytochemical compounds or secondary metabolites of the  
199 Ethanollic Leaf Extract (ELE), Ethanollic Bark Extract (EBE) and Ethanollic Root Extract (ERE)  
200 of *B. ferruginea* were tabulated below.

201

202 **Table 3: Phytochemical Compounds of ELE, EBE and ERE of *Bridelia ferruginea* Parts**

PHYTOCHEMICALS	TEST	INTERFERENCE		
		ELE	EBE	ERE
Carbohydrates	Molisch's test	+	+	+
Saponins	Frothing's test	+	+	+
Flavonoids	Shinoda's test	+	+	+
	Sodium Hydroxide's test	+	+	-
	Ferric Chloride's test	+	+	+
Tannins	Lead Sub-acetate's test	+	+	+
Terpenoids/Steroids	Salkowski's test	-	+	+
	Liebermann-Burchard's test	+	+	+
Alkaloids	Dragendoff's test	+	+	+
	Mayer's test	-	+	+
Anthraquinones	Bontrager's test	-	-	-
Cardiac Glycosides	Keller-kiliani's test	+	+	+
Fats and Oils		+	+	+

203 Key: + = Present

204 - = Absent

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208 **DISCUSSION**

209 The table 1, shown Biologic activity of main groups of natural compounds. Table 2, percentage  
210 yield of the plant parts of *Bridelia ferruginea* Samples and table 3, shown the Phytochemical  
211 Compounds of ELE, EBE and ERE of *Bridelia ferruginea* which revealed that Carbohydrates,  
212 Saponins, Flavonoids, Tannins, Cardiac Glycosides, fats and oils were present. Alkaloid present  
213 in Dragendoff's test in all plant parts extract but absent in Mayer's test in only leaf extract.  
214 Terpenoids/Steroids present in Liebermann-Burchard's test in all plant parts extract but absent in  
215 Salkowski's test in only leaf extract. Anthraquinones were absent in all plant parts extracts using  
216 Bontrager's test. Temitayo *et al.*, (2017) also carried out the same research and detected the  
217 presence of alkaloids, tannins, flavonoid, cardiac glycosides, saponins and using ethanol. This  
218 result indicates that the parts of the plants have active ingredients responsible for the  
219 antimicrobial activity. The presence of these secondary compounds makes the plants fits or good  
220 for the treatment of bacterial and other microbial infections because most therapeutic effects of  
221 medicinal plants are traced to the plant constituents and the medicinal actions of these plant parts  
222 extract are unique to particular species or family (Yunana *et al.*, 2018). This plant may have high  
223 antimicrobial activity due to the presence of these metabolites. Further study can be done to  
224 separate the individual metabolites to test their antimicrobial activity against some pathogenic  
225 bacteria like bacterial meningitis, tuberculosis and syphilis to determine their potency.

226 **Conclusion and Recommendation**

227 The phytochemical composition of the leaf, stem bark and root extracts of the *Bridelia*  
228 *ferruginea* indicate the presence of eight active constituents. The presence of these phyto-  
229 pharmacological compounds is an indicative that the plant has antibacterial property and it can  
230 be used for the treatment of bacterial and other microbial infections. Further investigation,  
231 purification and determination of these promising constituents can be done to assay their  
232 antimicrobial activity as alternative medicine.

233 **Competing Interests**

234 Authors have declared that no competing interests exist.

235

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