

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).

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.

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

Comment [U1]: Keywords are usually arranged in alphabetical. In this study, no antimicrobial tests were carried out, the word antimicrobial should be deleted from the keyword

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

60 tumour necrosis factor alpha (TNF α) (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, antihelmintic, 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)

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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.

110

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).

Comment [U2]: the nominal amount must be clear not a portion

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).

Comment [U3]: the nominal amount must be clear not pieces and few drops

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).

Comment [U4]: the nominal amount of the solution extract must be clearly

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).

Comment [U5]: the nominal amount of the solution extract must be clearly

139 **Test for tannins**

Comment [U6]: Title should be moved to the next page so that it is not separate from its contents

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).

Comment [U7]: the nominal amount of the extract must be clearly

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.

Comment [U8]: the nominal amount of the extract must be clearly

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% H₂SO₄ 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).

Comment [U9]: the nominal amount of the extract must be clearly

Comment [U10]: 50 mL ethanol or 50 % ???

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

Comment [U11]: the nominal amount of the extract must be clearly

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).

Comment [U12]: the nominal amount of the extract must be clearly

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.**

Comment [U13]: We recommend that tables on one page. The letters in the table must be smaller than the text.

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,

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:

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

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

190 Weight of plant= 100g.

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

193 = 24.91%, 24.25% 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

196 **The Results of Phytochemical Compounds.**

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

200

201 **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		+	+	+

202 Key: + = Present

203 - = Absent

204

205 **DISCUSSION**

206 The table 1, shown Biologic activity of main groups of natural compounds. Table 2, percentage
207 yield of the plant parts of *Bridelia ferruginea* Samples and table 3, shown the Phytochemical
208 Compounds of ELE, EBE and ERE of *Bridelia ferruginea* which revealed that Carbohydrates,

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

223 **Conclusion and Recommendation**

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

230 **Competing Interests**

231 Authors have declared that no competing interests exist.

232

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Comment [U14]: There are 7 from 26 references not up to date (more than 10 years)

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Comment [U15]: ???

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