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^{o}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.

INTRODUCTION

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

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

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

Plate 1: *Bridelliaferruginea* Stem Bark

Plate 2: *Bridellia ferruginea* Root

Plate 3**:** *Bridellia ferruginea* Leave

METHODOLOGY

Collection and Identification:

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

Processing of the plant samples:

The plant parts were washed thoroughly with distilled water, shade dried for 3-4 weeks at room temperature in the Histology laboratory of the Anatomy Department, Kogi State University, Anyigba. The plant parts were pulverized in a mortar and pestle and was grounded into fine powder of 40mm mesh size. The samples were stored in an air-tight container for further use. 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^{o}C), allowed to dry completely. The extracts obtained was scrapped using a clean spatula and grounded using small laboratory mortar and pestle, the extracts were weighed using weighing balance.

The percentage yield were calculated as follows:

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

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

Determination of Phytochemical Compounds in *Bridelia Ferruginea Parts Extracts*

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

Test for Carbohydrate

Molisch's test

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

Test for Saponins

Frothing test

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

Test for Flavonoids

Shinoda test

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

Sodium hydroxide test

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

Ferric chloride test

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

Test for tannins

Lead sub-acetate test

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

Test for Terpenoids/Steroids

Salkowski's test

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

Liebermann-Burchard's test

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

Test for Alkaloids

Dragendoff's test

The extracts was dissolved in 2ml of 5% H_2SO_4 in 50 ethanol with continuous stirring in water bath. The mixture was filtered and few drops of Dragendoff's reagent was added, rose red precipitate indicates the presence of alkaloids (Trease and Evans, 2002; Sofowora, 2008).

Mayer's test

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

Test of Anthraquinones

Bontrager's test

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

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

Test for Cardiac Glycosides

Keller-Kiliani test

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

Test for Fats and Oils

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

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 *et al.*, 2016).
Saponins	Antitumor, antiviral, antifungal, anti-inflammatory, immunostimulant, antihypoglycemic, antihepatotoxic and hepatoprotective, anticoagulant, neuroprotective, antioxidant (Negi *et al.*, 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).

RESULT

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

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

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

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

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

Weight of plant= 100g.

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

= 24.91%, 24.25% and 16.37%.

The percentage yield of the extracts obtained is calculated above.

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

The Results of Phytochemical Compounds.

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

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		+	+	+

Key: + = Present

\- = Absent

DISCUSSION

The table 1, shown Biologic activity of main groups of natural compounds. Table 2, percentage yield of the plant parts of *Bridelia ferruginea Samples* and table 3, shown the Phytochemical Compounds of ELE, EBE and ERE of *Bridelia ferruginea* which 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. Temitayo *et al*., (2017) also carried out the same research and detected the presence of alkaloids, tannins, flavonoid, cardiac glycosides, saponins and using ethanol. This result indicates that the parts of the plants have active ingredients responsible for the antimicrobial activity. The presence of these secondary compounds makes the plants fits or good for the treatment of bacterial and other microbial infections because most therapeutic effects of medicinal plants are traced to the plant constituents and the medicinal actions of these plant parts extract are unique to particular species or family (Yunana *et al*., 2018). This plant may have high antimicrobial activity due to the presence of these metabolites. 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.

Conclusion and Recommendation

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

Competing Interests

Authors have declared that no competing interests exist.

REFERENCES

Adetutu, A., Morgan, W.A. and Corcoran, O. (2011): Antibacterial, antioxidant and fibroblast growth stimulation activity of crude extract of *Bridelia ferruginea* leaf, a wound-healing plant of Nigeria. *Journal of Ethnopharmacology* **133**(1): 116-119.

Akudor, G.C, Mbah, C.C., Anyalewechi, N.A., Idris-Usman, M., Iwuanyanwu, T.C. and Osunkwo, U.A. (2011): Pharmacological profile of aqueous extract of *Brideliaferruginea* stem bark in the relief of pain and fever. *Journal of Medical and Plants Research*. **5(**22): 5366- 5369.

Akuodor GC, Mbah CC, Essien AD, Akpan JL, Ezeokpo BC, Iwuanyanwu TC, Osunkwo UA (2012). Ulcer-protective and Antidiarrhoeal Effects of the Aqueous Stem Bark Extract of Bridelia ferruginea in Rodents. Pharmacology 3: 591–597

Anulika NP, Ignatius EO, Raymond ES, Osasere OI, Abiola AH. The chemistry of natural product: Plant secondary metabolites. International Journal of Technology Enhancements and Emerging Engineering Research. 2016; 4(8):1–8. ISSN 2347-4289

Chikezie PC, Ibegbulem CO, Mbagwu FN. Bioactive principles from medicinal plants. Research Journal of Phytochemistry. 2015; 9(3):88–115

Cimanga, K., Ying, L., de Bruyne, T., Appers S., Cos, P., Hermanis, N., Bakana, P., Tona, L., Kambu, K. Kalenda, D.T., Pieters, L., Vanden, B. D. and Vlietina, A. J. (2001): Radical Scavending and Xanthine Oxidase inhibitory activity of phenolic compounds from *Bridelia ferruginea* stem bark. *Journal of Pharmacy and Pharmacology* **53** (5): 757-761.

Ezike, A. C., Akah, P. A., Nnamani, M. E., Okoli, C. O., Ojike, F. U., Eze, F. S., Chime, C. E. and Azosiri, I. J. (2011): Studies on the antiulcer and gastrointestinal effects of stem bark extract of *Bridelia ferruginea*. *Journal of Complementary and Integrated Medicine* **8** (1): Article 27. DOI. 10.2202/1553-3840.1507.

Kathriarachchi, H., Hoffmann, P., Samuel, R., Wurdack, K.J. and Chase, M.W. (2005): Molecular phylogenetics of Phyllanthaceae inferred from five genes (plastid atpB, matK, 3'ndhF, rbcL, nuclear PHYC). *Molecular Phylogenetic Evolution.* **36:** 112 -134.

Kokori B. T., Abdullahi A. A. and Abdullahi A. S. (2109). Studies on Phytochemical, Nutraceutical Profiles and Potential Medicinal Values of *Allium sativum* Linn (Lilliaceae) on Bacterial Meningitis. *International Neuropsychiatric Disease Journal* 13(2): 1-15; Article no.INDJ.51609, ISSN: 2321-7235, NLM ID: 101632319

Kumar S, Pandey AK. (2013). Chemistry and biological activities of flavonoids: An overview. The Scientific World Journal. 2013: Article ID 162750

Negi JS, Negi PS, Pant GJ, Rawat SM, Negi SK (2013). Naturally Occurring Saponins: Chemistry and Biology. Journal of Poisonous and Medicinal Plant Research. 2013; 1(1):001–006

Nene-Bi, S. A., Zahoui, O. S., Soro, T. Y. and Traore, F. (2012): Diuretic and natriuretic activity of an aqueous extract of *Bridelia ferruginea* Benth in normal rats. *J. Physio.Pharmacol.* **2**(10): 349-353.

Nene-Bi, S.A., Zahoui, O.S., Soro, T.Y. and Traore, F. (2010): Evaluation de leffet dun extrait aqueux de *Bridelia ferruginea* Benth (Euphorbiaceae) sur les activites spontanee et locomotrice chez le rat. *Intl. J. Biol. Chem. Sci*. **04(**1): 34-41.

Ngueyem, T.A., Brusotti, G., Caccialanza, G. and Vitafinzi, P. (2009): The genus *Bridelia*: A phytochemical and ethnopharmacological review. *Journal of Ethnopharmacology*. **124**: 339-349.

Olajide, O.A., Okpako, D.T. and Makinde, J. M. (2003): Anti-inflammatory properties of *Bridelia ferruginea* bark. Inhibition of lipopolysaccharide–induced septic shock and vascular permeability. *Journal of Ethnopharmacology* **88** (2-3): 221-224.

Olatunji, M. K. and Taofik, O. S. (2010): Effect of Waste Water Treated with Methanolic Bark Extract of Bridelia ferruginea Benth on Rat Kidney and Liver. *Journal of Applied Science and Environmental Sanitation* **7:**14-23.

Olumayokun, A.O., Mutalib, A.A., Uchechukwu, P. O. and Bernd, L. F. (2012): *Brideliaferruginea* produces antineuroinflammatory activity through inhibition of nuclear factor- KappaB and P38 MAPK signaling. *Evidence and Complementary Alternative Medicine* (**10)**:1155-1163.

Omotade, I. O and Seun, O. B. (2012): *In vitro* Antioxidant Activity of Ethanolic Extract of *B. ferruginea* (stem bark). *Academic Research International* **2**: (2) 246-251.

Onoruvwe, O., Olayinka, A. O., Lot, T. Y. and Udo, F. V. (2001): Effects of the stem bark and leaf extracts of *Bridelia Ferruginea* on rat bladder smooth muscle. *Fitoterap*. **72**(3): 230-235(6).

Rashid, M. A., Gustafson, K. R., Cardellina, J. H. and Boyd, M. R. (2000): A new podophyllotoxin derivatitive from *Bridelia ferruginea*. *Nat. Prod. Let*. **14:** 285-292.

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

Shakya A. K. Medicinal plants: Future source of new drugs. *International Journal of Herbal Medicine.* 2016; 4(4):59-64

Sofowora, E. A. (2008). Medicinal Plants and Traditional Medicine in Africa. (3rd edition), Spectrum Books Ltd, Ibadan, Nigeria. Pg 199-205. *Journal of Advances in Medical and Pharmaceutical Sciences.* 14(3): 1-13.

Temitayo Omotola Akinsete, Bukola Christianah Adebayo-Tayo and Aimbola Olumide Adekanmbi (2017). The phytochemical and Antimicrobial Potentials of the Crude Extract of Bridelia ferruginea and the Extracellular Biosynthesized Silver Nanoparticles. *Journal of Advances in Medical and Pharmaceutical Sciences.* 14(3): 1-13.

Trease, G. E. and Evans, W. C, (2002). *Textbook of Pharmacognosy* 15th edition, Elsevier Publication Ltd, pg 6-9.

Yunana B. T., Guiyi J. C. and Bukar B. B (2018). In vitro and in vivo evaluation of antibacterial activity of *Bridelia ferrugine*a extracts on some clinical isolates. *The Journal of Phytopharmacology*. 2018; 7(4): 392-398 Online at: www.phytopharmajournal.com