



Comparative Phytochemical and Antioxidant Evaluation of *Bridelia ferruginea* Leaf and Stem Bark

Singh Kumar A^{1*} and Alagbe John O²

¹Department of Animal Nutrition and Biochemistry, Gandhi College of Agriculture, Rajasthan, India

²Department of Animal Science, Center for Distance Learning and Continuing Education, University of Abuja, Gwagwalada, Nigeria



Abstract

This study evaluated the quantitative phytochemical composition and antioxidant potential of *Bridelia ferruginea* leaf and stem bark extracts. Quantitative analysis revealed that the leaf tissue accumulated significantly ($p < 0.05$) higher bioactive secondary metabolites than the stem bark: phenols (895.20 vs. 307.00 mg/g), flavonoids (609.60 vs. 200.60 mg/g), terpenoids (345.70 vs. 112.80 mg/g), saponins (203.50 vs. 87.55 mg/g), tannins (197.20 vs. 78.14 mg/g), steroids (112.70 vs. 100.60 mg/g), and alkaloids (102.10 vs. 90.40 mg/g). Radical-scavenging and reducing capacity assays demonstrated parallel superiority in leaf extracts: 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) (89.23 vs. 40.15 mg/g), Vitamin C (61.15 vs. 38.66 mg/g), and Ferric Reducing Antioxidant Power (FRAP) (43.18 vs. 25.61 mg/g Fe^{2+} Eq.). These findings establish *B. ferruginea* leaves as an exceptional, sustainable source of polyphenolic antioxidants, outperforming stem bark and offering a non-destructive alternative for therapeutic, veterinary, and nutraceutical exploitation.

Keywords: Phytochemical; Antioxidants; *Bridelia ferruginea*; Extracts; Vitamins; Nutraceuticals

Introduction

Bridelia ferruginea Benth. (family Euphorbiaceae) is a small-to-medium-sized tropical tree widely distributed across the savanna regions of tropical Africa [1, 2]. Traditionally, various anatomical parts of *B. ferruginea* including leaves, stem bark, and roots have been utilized in ethnomedicine to treat gastrointestinal disorders, arthritis, diabetes mellitus, cardiovascular ailments, and microbial infections [3]. Decoctions of the stem bark are commonly employed for their astringent and antimicrobial properties, whereas foliar preparations are traditionally consumed to manage inflammatory disorders and systemic fevers [4, 5].

Despite its extensive ethno-medicinal application, scientific optimization of *B. ferruginea* harvesting requires understanding the morphological distribution of its bioactive constituents [6, 7]. Secondary metabolites such as polyphenols, flavonoids, alkaloids, saponins, tannins, terpenoids, and steroids are not uniformly distributed throughout plant organs [8]. Variations in tissue-specific biosynthesis, vascular transport mechanisms, physiological demand, and environmental factors create distinct chemical profiles across vegetative structures [9]. Moreover, the destructive harvesting of stem bark poses severe ecological risks to wild stands, leading to ring-barking, desiccation, and tree death [10].

The pharmacological screening of the plant extract has revealed its anti-helminthic, anti-cancer, anti-malarial, anti-hyperglycemic, anti-inflammatory, anti-rheumatic, anti-measles and anti-pyretic activities [10]. Its anti-diuretic and anti-bacterial [8], anticonvulsant, anti-anaphylactic, anti-diarrheal and anti-viral [9] activities have also been reported. Additionally, its anti-asthmatic [7], antiamoebic and anti-estrogenic activities [6] as well as its nematocidal and abortifacient [11-13] activities have been documented. Hepatoprotective and antioxidant properties of *Bridelia ferruginea* have also been reported [13]. The phytochemical analysis of the plant shows that it contains saponins, alkaloids, flavonoids, triterpenoids, diterpenoids, tannins and steroids [13].

Quantifying the bioactive components and radical-scavenging capacities of *B. ferruginea* leaves compared to stem bark provides critical insight into tissue partitioning. Therefore, this study presents a comparative quantitative analysis of the phytochemical and antioxidant components of *B. ferruginea* leaf and stem bark extracts, evaluating their therapeutic potential and ecological utility.

OPEN ACCESS

*Correspondence:

Singh Kumar A, Department of Animal Nutrition and Biochemistry, Gandhi College of Agriculture, Rajasthan, India, E-mail: demsonfarms00@gmail.com

Received Date: 20 Sep 2026

Accepted Date: 14 Oct 2026

Published Date: 16 Oct 2026

Citation:

Singh Kumar A, Alagbe John O. Comparative Phytochemical and Antioxidant Evaluation of *Bridelia ferruginea* Leaf and Stem Bark. WebLog J Toxicol. wjt.2026. j1603. <https://doi.org/10.5281/zenodo.23572475>

Copyright © 2026 Singh Kumar A. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Materials and Methods

Description of experimental location

The study was carried out at the Department of Animal Nutrition and Biochemistry, Gandhi College of Agriculture, Jaipur, Rajasthan India between the months of October - December, 2025. The experimental site lies between latitude 26°54'21.36" N and longitude 75°44'06.52" E (Elevation: 435 m above sea level) [14].

Apparatus and instruments used with their technical specifications

Spectrophotometric measurements were recorded using a Thermo Scientific Evolution 220 UV-Vis Spectrophotometer (dual-beam geometry, 190-1100 nm spectral range, ± 0.8 nm wavelength accuracy).

Centrifugation was executed using an Eppendorf 5810 R Refrigerated Centrifuge equipped with an A-4-81 swinging-bucket rotor (14,000 rpm/20,854 $\times$ g).

Precision mass determinations were performed on a Mettler Toledo ME204E Analytical Balance (0.1 mg readability).

Büchi R-300 Rotavapor System coupled with a V-300 Vacuum Pump and I-300 Pro Interface. Ultra-pure water (18.2 M Ω -cm resistivity at 25°C) was generated *via* a Millipore Milli-Q IQ 7000 Water Purification System.

DPPH Radical Scavenging Kit: Sigma-Aldrich (Cat. No. NJO01-1MK); microplate-compatible radical indicator solution.

Ferric Reducing Antioxidant Power (FRAP) Assay Kit: Abcam (Cat. No. d236YYO); containing TPTZ reagent, Ferric Chloride Color Controller, and FeSO₄ standard solutions.

Ascorbic Acid Quantitation Kit: BioVision / Abcam (Cat. No. cHc01921); colorimetric ascorbic acid assay system.

Whatman No 1 filter paper, buckner flask funnel, volumetric flask beaker, brown bottle, dropper, glass rod, funnel, weighing balance, test tube, filter paper, desiccator and kjehdals flask.

Analytical grade reagents

Trioxonitrate V acid (HNO₃), sodium hydroxide, hydrochloric acid (HCl), distilled water ethanol, kjehdals tab, picric acid solution, ferric chloride (FeCl₃) solution, sulphuric acid (H₂SO₄), aqueous ammonia solution (NH₄OH), sodium hydroxide (NaOH), Boric acid, copper sulphate (CuSO₄), sodium sulphate (Na₂SO₄), selenium tablet, anhydrous diethyl ether, chloroform, Fehling's solution, Mayer's reagent, Wagner's reagent, dragendorff's reagent and Hager's reagent.

Plant collection and processing

Fresh, disease-free leaves and stem bark samples of *Bridelia ferruginea* were harvested from verified botanical stands at Gandhi College of Agriculture in Rajasthan India in October, 2025. Botanical authentication was carried out at the institutional herbarium and assigned a reference number GH09A/2025/H111. Plant samples were washed thoroughly under running tap water to remove surface debris and air-dried at ambient room temperature (25°C) for 14 days until constant weight was achieved. The shade-dried tissues were pulverized into a uniform coarse powder using a commercial mechanical grinder.

Sample extraction

100 g of leaf and stem bark powders were extracted separately

with 500 mL of 80% ethanol (v/v) at 65°C for 24 hours. The crude extracts were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure at 40°C using the Büchi R-300 Rotavapor System coupled with a V-300 Vacuum Pump and I-300 Pro Interface. Ultra-pure water (18.2 M Ω -cm resistivity at 25°C) was generated *via* a Millipore Milli-Q IQ 7000 Water Purification System. Concentrated extracts were stored in airtight amber glass vials at -20°C until analytical quantification.

Analytical procedures for phytochemical quantification

Total phenolic content: Determined through the Folin-Ciocalteu colorimetric method. Extract aliquots (0.5 mL) were mixed with 2.5 mL of 10% Folin-Ciocalteu reagent and 2.0 mL of 7.5% Na₂CO₃. Absorbance was read at 765 nm against a blank after 30 min incubation at 40°C. Calibration curves were constructed using Gallic acid (0-500 mg/L); results were expressed as mg Gallic Acid Equivalents per gram dry extract (mg GAE/g).

Total flavonoids: Quantified using the Aluminum Chloride colorimetric assay. 1.0 mL extract was combined with 0.3 mL 5% NaNO₂, followed by 0.3 mL 10% AlCl₃ at five minutes, and 2.0 mL 1.0 M NaOH at 10 minutes. Absorbance was measured at an optical density of 510 nm. Quercetin served as standard; results expressed as mg QE/g.

Terpenoids: Assayed *via* vanillin-perchloric acid colorimetric reaction. Extracts were incubated with 0.5 mL 5% vanillin-acetic acid and 1.0 mL HClO₄ at 60°C for 15 minutes, cooled, diluted with ethyl acetate, and measured at an optical density of 548 nm against linalool standard controls.

Saponins and Tannins: Tannins were quantified *via* vanillin-HCl assay (500 nm) referenced against Catechin/Tannic acid standards. Saponins were extracted *via* 20% aqueous ethanol, precipitated with diethyl ether, and determined gravimetrically/colorimetrically against diosgenin standards.

Alkaloids and Steroids: Total alkaloids were isolated *via* acid-base extraction with 10% acetic acid in ethanol, precipitated with ammonium hydroxide, and quantified gravimetrically. Steroids were determined through the Liebermann-Burchard reaction (620 nm) using cholesterol calibration curves.

Determination of Antioxidant Components

DPPH free radical scavenging assay: DPPH was carried out using the Sigma-Aldrich DPPH Kit protocol. Extract dilutions (100 μ L) were mixed with 100 μ L 0.2 mM DPPH methanolic solution in 96-well microplates. The mixture was incubated in the dark at 25°C for 30 minutes, and absorbance was measured at 517 nm. Radical scavenging capacity was extrapolated against Trolox/standard calibration curves and reported as mg/g.

Vitamin C (Ascorbic Acid): Vitamin C concentration was assayed using the BioVision/Abcam Colorimetric Kit. Samples were deproteinized, reacted with the proprietary colorimetric probe, and measured at an optical density of 570 nm. Values were quantified against an L-ascorbic acid reference curve.

Ferric Reducing Antioxidant Power (FRAP): It was carried out using the Abcam FRAP Kit (ab234626). The FRAP reagent (acetate buffer pH 3.6, TPTZ solution, and FeCl₃·6H₂O) was prepared fresh and warmed to 37°C. 10 μ L of sample was reacted with 190 μ L FRAP reagent for 60 minutes in the dark. Absorbance was read at 593 nm and calculated against a ferrous sulfate (FeSO₄·7H₂O) standard curve

(mg Fe²⁺ Eq./g).

Statistical analysis

All experiments were done in triplicate (n=3), and quantitative data are presented as Mean ± Standard Deviation (SD). Statistical computations were performed using IBM SPSS Statistics (Version 28.0). Differences between leaf and stem bark mean values were analyzed using an Independent Samples *t*-test. Statistical significance was established at *p*<0.05.

Results and Discussion

Quantitative extraction revealed marked variation in the concentrations of secondary metabolites between the leaf and stem bark of *B. ferruginea* (*p*<0.05). The leaf higher concentrations across all quantified chemical classes. The leaf extract reveals a high level of total phenols (895.20 mg/g) and flavonoids (609.60 mg/g), approximately three times the values recorded in the stem bark (307.00 mg/g and 200.60 mg/g, respectively). The concentration of flavonoid, phenolic compound in this study was lower than 287.1 mg/g; 185.8 mg/g and 309.2 mg/g and 126.3 mg/g reported by [15, 16] for *Sauropus androgynous* leaf extract and *Thaumatococcus daniellii* leaf extract respectively [17, 18], also reported a flavonoid concentration of 304.2 mg/g in *Kigellia africana* powder. This suggests that Leaf tissues of *B. ferruginea* serves as the metabolic epicenter of the plant, synthesizing phenylpropanoids and flavonols to protect light-harvesting complexes from ultra-violet induced photo-oxidation and oxidative stress [18]. Compared with other prominent medicinal species *B. ferruginea* leaves thus represent an extraordinarily concentrated source of natural polyphenols. Flavonoids and phenolic compounds play an important role in neutralizing the activities of free radicals, gastro-protective, immuno-protective and antimicrobial properties [19, 20]. Leaf extracts of *B. ferruginea* accumulated significantly higher levels of terpenoids (345.70 vs. 112.80 mg/g), saponins (203.50 vs. 87.55 mg/g), and tannins (197.20 vs. 78.14 mg/g). Terpenoids and saponins function as primary chemical defenses, acting as anti-feedants against phytophagous insects and micro-organisms [20]. Tannins prevent leaf grazing by binding salivary proteins and digestive enzymes, reducing nutrient digestibility [21, 22]. While many woody tropical trees (e.g., *Acacia nilotica*) concentrate condensed tannins in their bark for structural defense, *B. ferruginea* displays a reversed allocation strategy, prioritizing leaf protection [22]. This distribution contrasts with species like *Azadirachta indica* (Neem), where bitter terpenoids (e.g., azadirachtin) accumulate heavily within bark tissue [21, 23]. Unlike phenolic compounds, steroids (112.70 vs. 100.60 mg/g) and alkaloids (102.10 vs. 90.40 mg/g) exhibited nearly uniform distribution between leaf and stem bark tissues. Pharmacologically, steroids have been reported to exhibit antifungal, antibacterial, antiviral hypolipidemic, hormonal and anti-inflammatory activities [20]. They are used as contraceptives, androgenic and anabolic agents [19]. Alkaloids show several pharmacological activities on human health such as anticancer, anti-inflammatory, antimicrobial, antimalarial, antidiabetic and antioxidant [23, 24]. The concentration of alkaloids and steroids recorded in this study was higher than those reported by [25] for *Prosopis africana* leaf and stem bark (98.12 mg/g and 86.11 mg/g).

Assays evaluating free radical scavenging (DPPH), non-enzymatic reducing agents (Vitamin C), and ferric-reducing capacity (FRAP) confirmed significantly greater antioxidant potency in the leaf extract of *B. ferruginea* (*p*<0.05). The superior DPPH radical scavenging capacity (89.23 mg/g) and Ferric Reducing Antioxidant Power (43.18

Table 1: Phytochemical composition of *Bridelia ferruginea* leaf and stem bark extract.

Phytochemical Parameter	Leaf Extract (mg/g)	Stem Bark Extract (mg/g)
Total Phenols	895.20±4.12	307.00±2.85
Flavonoids	609.60±3.15	200.60±1.94
Terpenoids	345.70±2.80	112.80±1.25
Saponins	203.50±1.95	87.55±0.92
Tannins	197.20±1.82	78.14±0.88
Steroids	112.70±1.10	100.60±1.05
Alkaloids	102.10±0.95	90.40±0.82

Table 2: Antioxidant potential and reducing capacity of *Bridelia ferruginea* leaf and stem bark extract.

Antioxidant Parameter	Leaf Extract	Stem Bark Extract
DPPH Scavenging (mg/g)	89.23±0.85	40.15±0.42
Vitamin C (mg/g)	61.15±0.54	38.66±0.38
FRAP (mg Fe ²⁺ Eq./g)	43.18±0.48	25.61±0.22

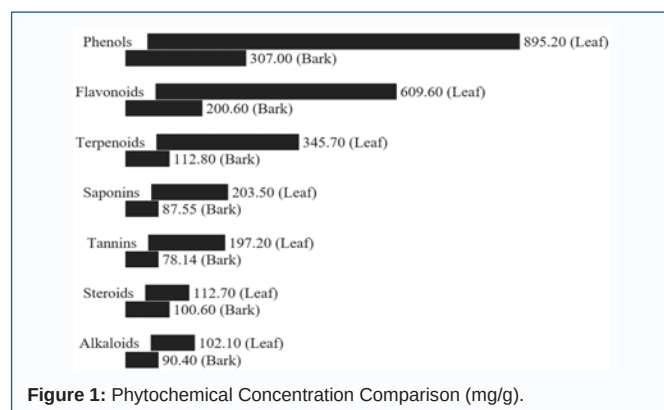


Figure 1: Phytochemical Concentration Comparison (mg/g).

mg Fe²⁺ mg/g) of *B. ferruginea* leaf extract directly reflect its rich polyphenolic content [26, 27]. Hydroxyl (–OH) groups on aromatic phenolic rings readily donate hydrogen atoms and single electrons to neutralize DPPH free radicals and convert ferric (Fe³⁺) complexes to their stable ferrous (Fe²⁺) state. Furthermore, high leaf ascorbic acid content (61.15 mg/g) provides hydrophilic reactive oxygen species neutralizing and acts synergistically to regenerate oxidized lipophilic antioxidants enhancing overall cellular protection [28-30].

Conclusion

This study confirms that the leaves of *Bridelia ferruginea* accumulate significantly higher concentrations of secondary metabolites - particularly phenols (895.20 mg/g), flavonoids (609.60 mg/g), terpenoids (345.70 mg/g), saponins (203.50 mg/g), and tannins (197.20 mg/g) - than the stem bark. This rich phytochemical profile translates directly to enhanced DPPH radical scavenging, ferric reducing power, and ascorbic acid activity. Pharmacologically, *B. ferruginea* leaves represent a potent source of natural antioxidants for human therapeutics, veterinary formulations, and phyto-genic feed additives in livestock production.

References

- Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, Linder T, Wawrosch C, Uhrin P, et al. Discovery and resupply of pharmacologically active plant-derived natural products: A review. *Biotechnol Adv.* 2015; 33(8): 1582-1614.

2. Awouafack MD, McGaw LJ, Gottfried S, Mbouangouere R, Tane P, Spiteller M, et al. Antimicrobial Activity and Cytotoxicity of the Ethanol Extract, Fractions and Eight Compounds Isolated from *Eriosema Robustum* (Fabaceae). BMC Complement Altern Med. 2013; 13(1): 289.
3. Carranza MG, Seigny MB, Banerjee D, Fox-Cubley L. Antibacterial Activity of Native California Medicinal Plant Extracts Isolated from *Rhamnus Californica* and *Umbellularia Californica*. Ann Clin Microbiol Antimicrob. 2015; 14(1): 29.
4. Saidu AN, Mann A, Onuegbu CD. Phytochemical screening and hypoglycaemic effect of aqueous *Blighia sapid* a root bark extract on normoglycemic albino rats. Brit J Pharmaceutic Res. 2012; 2: 89-97.
5. Olajide OA, Makinde JM, Okpako DT, Awe SO. Studies on the anti-inflammatory and related pharmacological properties of the aqueous extract of *Bridelia ferruginea* stem bark. J Ethnopharmacol. 2000; 71: 153-160.
6. Olajide OA, Okpako DT, Makinde JM. Anti-inflammatory properties of *Bridelia ferruginea* stem bark Inhibition of lipopolysaccharide-induced septic shock and vascular permeability. J Ethnopharmacol. 2003; 88(2-3): 221-224.
7. Nene-Bi SA, Zahoui OS, Soro TY, Traore F. Diuretic and natriuretic activity of an aqueous extract of *Bridelia Ferruginea* Benth (Euphorbiaceae) in normal rats. J Physiol Pharmacol Adv. 2012; 2(10): 349-353.
8. Nene-Bi SA, Ramachandran V, Vengal RP, Gopalakrishnan R, Dhanabal SP, Traore F. Subchronic toxicity studies of the aqueous stem bark extract of *Bridelia ferruginea* in Wistar rats. Bull Env Pharmacol Life Sci. 2016; 5(10): 14-21.
9. Nene-Bi S, Traore F, Soro T, Souza A. Etudes phytochimique et pharmacologique de *Bridelia ferruginea* benth (euphorbiaceae) sur la motricite du Taenia coli de cobaye. Afr Sci Rev Int Sci Technol. 2009; 5(2): 305-320.
10. Ngueyem TA, Brusotti G, Caccialanza G, Finzi PV. The genus *Bridelia*: a phytochemical and ethnopharmacological review. J Ethnopharmacol. 2009; 124(3): 339-349.
11. Owoseni AA, Ayanbamiji TA, Ajayi YO, Ewegbenro IB. Antimicrobial and phytochemical analysis of leaves and bark extracts from *Bridelia ferruginea*. Afr J Biotechnol. 2010; 9(7): 1031-1036.
12. Owoseni AA, Ayanbamiji TA, Ajayi YO, Ewegbenro IB. Antimicrobial and phytochemical analysis of leaves and bark extracts from *Bridelia ferruginea*. Afr J Biotechnol. 2010; 9(7): 1031-1036.
13. Ajiboye A, Agboola D, Fadimu O, Afolabi A. Antibacterial, Phytochemical and Proximate Analysis of *Prosopis africana* (Linn) Seed and Pod Extract. FUTA J Res Sci. 2013; 9(1): 101-109.
14. Encyclopedia Britannica. Rajasthan Location. Geography and Climate. 2026.
15. Alagbe JO, Anorue DN. Quantitative screening of bioactive fractions in three tropical medicinal plants (*Ageratum conyzoides*, *Acmella oleracea* and *Aegle marmelos*) from Gujarat India. Pharm Drug Development. 2026; 5(1): 047.
16. Alagbe JO, Anorue DN. Bioactive compounds in ethanol stem bark extracts of *Newbouldia* leaves adopting GC-MS analysis. Pharmacy Drug Development. 2026; 5(1): 1-6.
17. Alagbe JO. Evaluation of bioactive compounds and antimicrobial analysis of essential oil from the leaves of *Sauropus androgynous* in Rajasthan India. Am J Biol Nat Sci. 2026; 3(1): 124-131.
18. Alagbe JO. Accessing the bioactive compounds of *Thaumatococcus daniellii* leaf extract by GC-MS. Int J Glob Sustain Res. 2025; 3(9): 617-626.
19. Alagbe JO. Accessing the Qualitative and Bioactive Compounds in the leaves of *Withania somnifera* from Rajasthan India. J Clin Lab Res. 2025; 8(4): 1-8.
20. Ojediran TK, Emiola IA, Durojaye V, Alagbe JO. Proximate, vitamin and GC-MS profiling of *Kigella africana* powder. Cerrado: Agric Biol Res. 2024; 1(1): 13-20.
21. Ojediran TK, Emiola IA, Durojaye V, Alagbe JO. Analysis of *Kigellia africana* fruit's powder antioxidant and phytochemical properties. Braz J Sci. 2024; 3(7): 38-49.
22. Alagbe JO, Bamigboye S, Nwosu GC, Agbonika DA, Kadiri MC. Characterization of bioactive compounds in *Luffa aegyptiaca* leaf ethanolic extracts using gas chromatography and mass spectrometry (GC-MS). Drug Discover. 2023; 17: e10dd1011.
23. Alagbe JO. Bioactive compounds in ethanolic extract of *Strychnos innocua* root using gas chromatography and mass spectrometry (GC-MS). Drug Discover. 2023; 17: e4dd1005.
24. Singh S, Alagbe OJ, Liu X, Sharma R, Kumar A. Comparative analysis of ethanolic *Juniperus thurifera* leaf, stem bark and root extract using gas chromatography and mass spectroemetry. Int J Agric Anim Product. 2022; 2(6): 18-27.
25. Agubosi OCP, Oluwafemi RA, Alagbe JO. Preliminary study on GC-MS analysis of *Prosopis africana* seed (*African mesquite*) oil. J Ethics Divers Int Commun. 2021; 1(4): 18-20.
26. Alagbe JO, Shittu MD, Ushie FT. GC-MS analysis of methanolic stem bark extract of *Indigofera zollingeriana*. Asian J Adv Res. 2021; 11(4): 144-146.
27. Alagbe JO. Proximate, mineral and phytochemical analysis of *Piliostigma thonningii* stems bark and roots. Int J Biol Phys Chem Stud. 2019; 1(1): 1-7.
28. Alagbe JO. Proximate, phytochemical and vitamin compositions of *Prosopis africana* stem bark. Eur J Agric Rural Educ. 2020; 1(4): 1-7.
29. Shittu MD, Alagbe JO. Phyto-nutritional profiles of broom weed (*Sida acuta*) leaf extract. Int J Integr Educ. 2020; 3(11): 119-124.
30. Pettit GR, Searcy JD, Tan R, Cragg GM, Melody N, Knight JC, et al. Antineoplastic agents. 585. Isolation of *Bridelia ferruginea* anticancer podophyllotoxins and synthesis of 4 - aza-podophyllotoxin structural modifications 1. J Nat Prod. 2016; 79(3): 507-518.