International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Research Article **CODEN: IJPNL6**

SCREENING OF IN-VITRO ANTIOXIDANT, BRINE SHRIMP LETHALITY BIOASSAY AND ANTIMICROBIAL ACTIVITIES OF EXTRACTS OF *BRIDELIA RETUSA* (L.) SPRENG. FRUIT

Tufikul Islam[1]*, Mohammad Raquibul Hasan[1], Aumit Roy[1], Md. Shafiqul Islam[1], Md. Afaz Uddin[1], Md. Ariful Islam[2], Md. Nuruzzaman Neon[1], Md. Sohel Rana[1]

[1]Department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh.
[2]Department of Botany, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh.

***Corresponding author e-mail:** tufikulislam@gmail.com

ABSTRACT

The objective of present study was to investigate the antioxidant, brine shrimp lethality and antimicrobial activities of *Bridelia retusa* (L.) Spreng. fruit using n-hexane, ethyl acetate and methanol extracts. Several methods were used for assessing antioxidant activity. In total phenol content determination methanolic extract showed highest value of 134.5 mg/g as Gallic Acid Equivalent (GAE). N-hexane extract showed highest total flavonoid content with 60.50±4.95 mg/gm as Quarcetin Equivalent (QE). Methanolic extract showed highest tannin content with 652.50±3.53 mg/gm as Tannic Acid Equivalent (TAE) and total anti-oxidant capacity with 236.00±36.78 mg/gm as Ascorbic Acid Equivalent (AAE). Methanolic extracts showed good DPPH free radical scavenging activity with IC_{50} of 168.757 µg/mL. Ethyl acetate extract showed highest reducing capacity in CUPRAC test. Methanolic extracts showed good cytotoxic activity with IC_{50} value of 20 µg/ml in brine shrimp lethality bioassay. In antimicrobial assay extracts showed poor activity. Further studies are needed to isolate active compounds.

Key Words: Antioxidant Activity, Free Radical Scavenging, Tannic Acid, CUPRAC, Flavonoids Equivalent Content and Brine Shrimp Lethality Bioassay.

INTRODUCTION

Although the modern medicine has achieved tremendous development, plants are still playing an important role in healthcare. Several plants have been investigated for their antioxidant potential [1]. *Bridelia retusa* (L.) Spreng. is a medium-sized to large deciduous tree that commonly grows in forests and open land [2]. This plant belongs to the euphorbiaceae family and the Bengali name is Kantakoi. It is found at the forests of Chittagong and Chittagong Hill Tracts, Cox's Bazar, Mymensingh, Sylhet and Dhaka in Bangladesh [2]. The plant is also found in China, India, Nepal, Sri Lanka and Sumatra [3]. The fruit is fleshy drupe, size of a pea, purple-black [2]. The fruits of the plant are edible [3]. Different parts of the plant are used for their medicinal activities. The leaf is useful in treating urinary tract infection, wound healing and as antibacterial agent [4, 5, 6]. The bark is used to treat dysentery, rheumatism and as astringent and contraceptive agents [4, 7, 8]. So the present study was designed to assess the antioxidant, cytotoxic and antimicrobial activities which were previously explored very little.

Antioxidants are substances that are used to deactivate or stabilize the free radicals like Reactive Oxygen Species (ROS) or other oxidants. These are involved in food and chemical material degradation and damage nucleic acids, proteins and lipids. Eventually they will cause oxidative stress and leading to degenerative diseases such as cancer, Alzheimer's disease, Parkinson's diseases and some cardiovascular diseases. In recent years, prevalence of the diseases result from oxidative stress has been increased [9]. In human body, an imbalance between ROS and the inherent antioxidant capacity

directed the use of dietary and /or medicinal supplements. Investigation on medicinal plants, vegetables, and fruits has indicated the presence of antioxidants such as phenolics, flavonoids, tannins, and proanthocyanidins. The antioxidant contents of medicinal plants may contribute to the protection against disease. The relationship between morbidity and mortality from degenerative disorders and the ingestion of natural antioxidants is inversely proportional [10].

Cytotoxicity of a substance is the quality of being toxic to cells. Research interest on screening of medicinal plants has intensified in recent years with a view to finding out potential cytotoxic compounds for cancer chemotherapy [11, 12, 13, 14]. As a result, several potential lead compounds such as vincristine, vinblastine, taxol, camptothecin, podophyllotoxin, combretastatins, etc have been isolated from plants and are currently successfully employed in cancer treatment [15]. In developing countries, synthetic drugs are expensive and inadequate for the treatment of disease and need to search for new infection-fighting agents to control microbial infections [16]. The antimicrobial activities were determined by disc diffusion method.

MATERIALS AND METHODS

Chemicals: Ammonium molybdate, Methanol, Sodium Phosphate (Na_3PO_4) and Folin-Ciocalteu reagent were purchased from Merck, Germany. Sodium carbonate (Na_2CO_3), Potassium Acetate and H_2SO_4 (98%) were purchased from Merck (India) Limited. Gallic acid, Quercetin and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma Chemicals, USA. Aluminium Chloride and Ascorbic acid were purchased from SD Fine Chem. Ltd., Biosar, India. Vincristine sulphate was collected from Techno Drugs Ltd., Bangladesh. All chemicals and reagents used were of analytical grade.

Collection of Plant Material: The fruit of the plant was collected from Gazipur, Bangladesh and identified by the taxonomist of the Department of Botany, Jahangirnagar University, Savar, Dhaka, Bangladesh.

Preparation of Plant material & Extraction procedure: Fruits of the plant were first washed with water to remove adhering dirt and then cut into small pieces and sun-dried for few days and then dried in a hot air oven (Size 1, Gallenkamp) at reduced temperature (not more than 50°C). Dried fruits were grinded into coarse powder using high capacity grinding mill. The powdered fruits were used for serial extraction by Soxhlet apparatus at elevated temperature (65°C) using n-Hexane, Ethyl Acetate and Methanol consecutively (500 mL of each solvent). After each extraction the plant material was dried and used again for the next extraction. Extraction was considered to be completed when the fruit materials become exhausted of their constituents that were confirmed from cycles of colorless liquid siphoning in the Soxhlet apparatus. The filtrates obtained were dried at temperature of 40±2°C to have gummy concentrate of the crude extract. The extract was kept in a suitable container with proper labeling and then stored in cold and dry place for further use [17].

Phytochemical Screening: The freshly prepared crude extract was qualitatively tested for the presence of chemical constituents i.e. carbohydrates through molisch's test and fehling's test, flavonoids, glucosides through general test for glycoside and glucoside, steroids through liebermann-burchard's test, saponins through frothing test, tannins through Ferric chloride and Potassium dichromate test, alkaloids through mayer's test, hager's test, wagner test and dragendorff's test. These phytochemicals were identified from their respective characteristic color changes as stated in the standard procedures [18].

Antioxidant Activity Evaluation

Total phenol content determination: Total phenolic content of the prepared n-hexane, ethyl acetate and methanol extracts was determined by using Folin–Ciocalteu Reagent (FCR) [19].One (1.0) mL of fruit extract (200 µg/mL) and the standard (gallic acid) of different concentrations (6.25, 12.5, 25, 50, 100 and 200 µg/mL) were taken into marked six test tubes. All test tubes were marked accordingly. Five (5) mL of Folin–Ciocalteu reagent solution (diluted to 10 fold) were taken in the test tubes followed by the addition of 4 mL of 7.5% sodium carbonate solution in each. The test tubes were incubated at 20°C (30 minutes for standard solutions, and 1 hour for extract solution). Absorbance at 765 nm was measured using a UV-Vis spectrophotometer (Shimadzu UV PC-1600) against a blank. Total phenol contents of the fractions were expressed as Gallic acid equivalents (GAE) [20, 21].

Determination of Total Flavonoids Content: Total Flavonoid was determined using the Aluminum chloride colorimetric method described by Wang and Jiao [22]. One (1.0) mL of fruit extract (200µg/mL) and standard (Quercetin) of six different concentrations (6.25, 12.5, 25, 50, 100 and 200 µg/mL) were taken into different marked test tubes.

Then 3 mL of methanol was added to each of the test tubes followed by 200 µL of 10% aluminium chloride solution and 200 µL of 1 M potassium acetate solution. Finally, 5.6 mL of distilled water was added to the test tubes. After this the test tubes were incubated for 30 minutes at room temperature to complete the reaction. Absorbance of the solution was measured at 415 nm using UV-Vis spectrophotometer (Shimadzu UV PC-1600) against a blank. Total Flavonoid content of the extract was expressed as Quercetin equivalents (QE).

Determination of Total Tannin Content: The tannins were determined by slightly modified Folin and Ciocalteu method [19]. The standard (Tannic Acid) solution of six different concentrations (6.25, 12.5, 25, 50, 100 and 200 µg/mL) and the fruit extract (200µg/mL) of 0.1 mL were taken in different marked test tubes. Then 7.5mL of distilled water, 0.5 mL of Folin Phenol reagent, 1 mL of 35% sodium carbonate solution were added and the volume was finally adjusted upto 10 mL with distilled water. The mixture was shaken well, kept at room temperature for 30 minutes and absorbance was measured using UV-Vis spectrophotometer (Shimadzu UV PC-1600) at 725 nm against a blank. Total Tannin content of the extracts was expressed as Tannic Acid Equivalent (TAE).

Determination of Total Antioxidant Capacity: Total antioxidant capacity of the fruit extracts was determined by following the method described by Prieto P et al., 1999 [23]. Three hundred micro-liters (300µL) of extract (200µg/mL) and standard (ascorbic acid) of different concentrations (6.25, 12.5, 25, 50, 100 and 200 µg/mL) were taken in different marked test tubes and then 3 mL of reagent solution (a mixture of 3.3mL of concentrated 98 % H_2SO_4, 0.381gm sodium phosphate and 0.494gm of ammonium molybdate prepared in a 100mL volumetric flask adjusting the volume to 100 mL with distilled water) was added to each test tubes. These test tubes were then incubated at 95°C for 90 minutes to complete the reaction. Absorbance of each of the incubated solutions, after cooling to room temperature, was measured at 695 nm using a UV-Vis spectrophotometer (Shimadzu UV PC-1600) against a blank. Total antioxidant capacity of the extract was expressed as ascorbic acid equivalent (AAE).

DPPH Free Radical Scavenging Assay: DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging activity of the plant extract was determined following the method described by Braca *et al*. [24]. One (1.0) mL fruit extract of different concentrations (12.5, 25, 50, 100, 200, 400 and 800 µg/mL) and 1.0 mL standard (ascorbic acid) of different concentrations (2.5, 5, 10, 20, 40 and 80 µg/mL) were taken in different pre-marked test tubes. Then, 2 mL of 0.004% methanolic DPPH solution was added to each test tube. All the prepared test tubes with their contents were then incubated for 30 minutes at room temperature. Absorbance of each of the incubated solutions was determined at 517 nm using UV-Vis spectrophotometer (Shimadzu UV PC-1600) against a blank and IC_{50} value was calculated from the curve. (Fig.: 5).

Cupric Reducing Antioxidant Capacity: Cupric Reducing Antioxidant Capacity of the fruit extracts was determined following the method described by Resat A.et. al. [25]. Five hundred (500) µL solution of each plant extract and standard (Ascorbic Acid) having different concentrations (12.5, 25, 50, 100, 200, 400 and 800 µg/mL) were taken in different marked test tubes. One (1.0) mL of 0.01M $CuCl_2.2H_2O$ solution, 1.0 mL of ammonium acetate buffer (pH 7.0), 1.0 mL of 0.0075 mL of neocaproin solution and 600 µL of distilled water were added to each test tubes and the final volume of the mixture was adjusted to 4.1 mL. The total mixtures were incubated for 1 hour at room temperature and the absorbance of the solutions were measured at 450 nm using UV-Vis spectrophotometer (Shimadzu UV PC-1600) against a blank.

Brine shrimp lethality bioassay: Lethal activity of the fruit extracts of *Bridelia retusa* (L.) Spreng. was determined by Brine shrimp lethality bioassay described by Meyer *et al*. [26]. Brine shrimp eggs (*Artemia salina* leach) were hatched in simulated seawater (3.8% NaCl) with continuous oxygen supply for two days and got the nauplii. Stock solution of the sample was prepared by dissolving 20 mg of extract in 400 µL of pure dimethylsulfoxide (DMSO) and adding sea water to make the total volume 20 mL. Thus the stock solution gained concentration of the extract as 1µg/µL. Then specific volumes of stock solution was transferred into different test tubes so that the final concentration of the extract becomes 6.25, 12.5, 25, 50, 100, 200, 400 and 800µg/mL in the respective test tubes after volume adjustment to 5 mL with sea water. In the control tubes 75µL and 150µL DMSO were taken and volume was adjusted to 5mL with sea water (as in the sample tubes). Vincristine sulfate was used as positive control and evaluated at very low concentration (10, 5, 1, 0.5, 0.25, 0.125 and 0.06µg/mL). Using a Pasteur pipette 10 living nauplii were put to each of the test tubes. After 24 hours the test tubes were observed and the number of nauplii

survived in each test tube were counted.The mortality was corrected using Abott's formula [27].

$P_t = [(P_o - P_c)/ (100 - P_c)] \times 100$

Where, P_t = Corrected mortality, P_o= Observed mortality and P_c= Control mortality.

LC_{50} values of the test samples after 24 hours are obtained by regression analysis.

***Determination of Antibacterial Activity by Disc Diffusion Method*:** The antimicrobial activity of different extracts was determined by the disc diffusion method [28]. The bacterial strains used for the experiment were collected as pure cultures from Department of Microbiology of Jahangirnagar University. Both Gram positive and gram negative organisms were taken for the test and they are listed in the following Table 1. Solutions of known concentration (40µg/mL) of the test samples were made by dissolving measured amount of the samples in calculated volume of solvents. Dried and sterilized filter paper discs were then impregnated with 10µL of the test samples (400µg/ Disc) using micropipette. Discs containing the test material were placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic discs (Chloramphenicol 50 µg/disc) and blank discs (impregnated with respective solvents 10µL) were used as a positive and negative control. These plates were then incubated at 37ºC for 24 h allowing maximum growth of the organisms. The test materials having antibacterial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the medium. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in millimeter.

RESULTS

Phytochemical screening: In the present study, various qualitative tests were done to detect the presence of different phytochemical compounds in the n-hexane, ethyl acetate and methanolic extracts of the fruits of *Beidelia retusa* (L.) Spreng. Phytochemical constituents in the plants are known to be biologically active compounds and they are responsible for different activities against diseases [29]. The results of the phytochemical testing are given in Table 2.

Antioxidant activity evaluation

Total phenol content determination: Phenolic compounds of plants have been said to account for most of the antioxidant activities of plant extracts [30]. They show antioxidant activities by preventing decomposition of hydroperoxides into free radicals or by inactivating the lipid free radicals [31]. The total phenolic compounds content of the test solutions were calculated using the calibration curve of the standard (Fig. 1) of Gallic acid ($y = 0.0106x + 0.0507$, $R^2 = 0.9998$)**.** The results were expressed as gallic acid equivalents (GAE) per gram of the extract. Methanolic extract of fruit of *Bridelia retusa* (L.) Spreng was found to contain the highest amount of phenols 134.50±0.00 mg/gm GAE (Table 3). Phenol contents of the extracts were found to the following order: methanol> ethyl acetate> n-hexane (Table 3).

Determination of Total Flavonoids Content: The total Flavonoid content of the extracts were calculated using the standard line (Fig. 2) of Quercetin ($y=0.0055x+0.0142$, $R^2 = 0.9973$). Flavonoid content of the extracts was expressed as mg/gm Quercetin equivalent (QE). The n-hexane extract was found to have the highest total flavonoid content 60.5±4.950 mg QE/gm of the extract and the methanolic extract was lowest 17.00±2.82 mg QE/gm (Table 3). The flavonoid content in plant extracts depend on the polarity of solvent used in extract preparation[32]. The flavonoid contents of the extracts were found to the following order: n-hexane > ethyl acetate> methanol (Table 3).

Determination of Total Tannin Content: Total tannin content of the different extracts fruit of *Bridelia retusa* (L.) Spreng. was evaluated by the Folin method and was expressed as tannic acid equivalents (TAE) per gram of plant extract. Total tannin capacity of the test samples was calculated using the standard curve of tannic acid ($y = 0.0014x + 0.023$; $R^2 = 0.9987$) (Fig. 3). Methanolic extract was found to possess the highest Total tannin content (652.50±3.53 mg/gm TAE); ethyl acetate extract also possesses good 650.00±7.07 mg/gm TAE (table 3). Total tannin content of the extracts was found to the following order: Methanol> Ethyl Acetate > n-Hexane (Table 3).

Total antioxidant capacity assessment: Total antioxidant capacity of *Bridelia retusa* (L.) Spreng. fruit extracts was evaluated by the phosphomolybdenum method and was expressed as ascorbic acid equivalents (AAE) per gram of fruit extracts. Total antioxidant capacity of the test samples was calculated using the standard line (Fig. 4) of ascorbic acid ($y=0.0052x+0.0164$, $R^2 = 0.9908$). Methanolic extract of fruit of *Bridelia retusa* (L.) Spreng. was found to possess the highest total antioxidant capacity with 236.00±36.78mg/gm AAE (Table 3). Total antioxidant capacity of the extracts was found to decrease in the following order: Methanol> Ethyl Acetate > n-Hexane (Table 3).

The total antioxidant activity of fruit extracts of *Bridelia retusa* (L.) Spreng. was evaluated in the present study from its ability to reduce Phosphate/Mo (VI) complex to Phosphate/Mo (V). Recently it is reported that a highly positive relationship between total phenols and antioxidant activity appears to be the trend in many plant species [33]. The statement has been justified in the current study where the methanol and ethyl acetate extracts of fruit of *Bridelia retusa* (L.) Spreng. showed significant total antioxidant capacity (in term of ascorbic acid equivalent) (Table 3) with maximum phenol content (Table 3).

DPPH Free Radical Scavenging Assay: The DPPH free radical scavenging assay is considered as a valid accurate, easy and economic method to evaluate radical scavenging activity of antioxidants, since the radical compound is stable and need not be generated [34]. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance at 517 nm. The IC_{50} values of the different extracts of fruit of *Bridelia retusa* (L.) Spreng. are presented in the Table 4. The ethyl acetate fraction exhibited highest antioxidant activity with an IC_{50} value of 141.954μg/mL compared to other fractions. The value is 16.654μg/mL for the standard ascorbic acid. DPPH radical scavenging capacity of the extracts was found to decrease in the following order: Ethyl Acetate > Methanol > n-Hexane (Fig. 5).

The lower the IC_{50} the higher the antioxidant property of a plant [35]. In the present study, extracts showed DPPH radical scavenging activity in a similar manner to that of the reference antioxidant ascorbic acid-increasing activity with the increase in concentration (Fig. 5). This free radical scavenging activity might be due to the presence of phenols and flavonoids in the extracts. Saurabh K et al., found the IC_{50} value of 48μg/mL and 69μg/mL for two samples of bark of methanolic extract of *Bridelia retusa* (L.) Spreng., which is much less than that of the finding of the present study [36]. This may suggest that the fruit part of the plant possesses higher antioxidant capacity than bark.

Cupric Reducing Antioxidant Capacity: Reduction of Cu^{2+} ion to Cu^{+} was found to rise with increasing concentrations of the different extracts. Reducing capacity of the component of plant extracts is a key indicator of antioxidant property [37]. Different studies have indicated that the antioxidant activity is associated with the electron donation capacity [38, 39].

Increase in the absorbance of the sample solution is an indicator of reducing power of the sample [40]. The standard ascorbic acid showed highest reducing capacity. Among the extracts the ethyl acetate extract of the fruits showed maximum reducing capacity that is comparable to ascorbic acid (Fig. 6). Cupric Reducing Antioxidant Capacity of the extracts was found to be decreased in the following order: Ethyl Acetate > Methanol > n- Hexane.

Brine shrimp lethality bioassay: All the extracts of fruit were subjected to Brine Shrimp lethality bioassay for possible cytotoxic action. In this study, methanol extract of fruits was found to be the most toxic to Brine Shrimp nauplii, with LC_{50} of 20.00µg/ml, whereas anticancer drug vincristine sulphate showed LC_{50} value 0.699µg/ml. On the other hand, ethyl acetate extracts showed good and n-hexane extract showed low toxicity (table 4).

For the determination of bioactivities of synthetic compound as well as plant derivatives the brine shrimp bioassay has been established as a safe, practical and economic method [41, 42]. According to the national Cancer Institute (NCI, USA) , the relationship between the brine shrimp bioassay and growth inhibition of human solid tumor cell lines (*In vitro*) is significant because it exhibits the importance as a pre-screening tool for anti-cancer drug research [43]. Extracts derived from natural resources which have $LC_{50} \leq$ 1000µg/mL in brine shrimp bioassay were claimed to contain bioactive principles [42]. Principle of brine shrimp toxicity for compound or plant extract above 1000µg/ml is non-toxic, between 500 & 1000µg/ml is weakly toxic, and below 500µg/ml is toxic which were established as LC_{50} values [44].

In this study methanol and ethyl-acetate extracts showed very good LC_{50} values of 20.00 µg/mL and 191.618 µg/mL respectively. According to the National Cancer Institute (NCI), the LC_{50} value of $\leq$ 20µg/mL is considered to be very cytotoxic [45]. So methanolic extract may be considered as very cytotoxic. It was reported that toxicity of plant extracts is attributed to the presence of different types of secondary metabolites such as saponins, terpenoids, steroids, tannins, alkaloids etc. [46].

Determination of Antibacterial Activity by Disc Diffusion Method: The result of antimicrobial screening of different extracts of fruit of *Bridelia retusa* (L.) Spreng. has been presented in Table 5. Among the extracts the ethyl acetate extracts of fruit showed a slight activity against *Escherichia coli* and *Pseudomonus aeruginosa*. The standard,

chloramphenicol, exhibited significant zone of inhibition against all the test organisms. The ethyl acetate extract of fruits exhibited slight antimicrobial activity and it is probably attributed to the presence of saponins, flavonoids and total tannin content [47, 48] which were detected in phytochemical screening (Table 2).

CONCLUSION

From the foregoing, it was observed that the methanolic and ethyl acetate extracts of *Bridelia retusa* (L.) Spreng fruits possess good antioxidant property. N-hexane extracts also demonstrated good total flavonoid content and DPPH free radical scavenging activity. The result suggests that the methanolic extract possess overall good antioxidant and cytotoxic potential. Further well-structured studies are needed to isolate the bioactive compounds responsible for these activities and to determine their underlying molecular mechanism to find out novel lead compounds.

Table 1: Microorganisms used in antimicrobial assay

Gram Positive Bacteria	Gram Negative Bacteria
Bacillus subtilis	*Escherichia coli*
	Salmonella typhi
	Pseudomonus aeroginusa

Table 2: Results of Phytochemical Screening of the extracts

Phytochemical Group Of Compounds	n-Hexane	Ethyl Acetate	Methanol
Alkaloid	+	+	+
Carbohydrate	–	+	+
Flavonoid	–	+	+
Glucoside	+	+	-
Glycoside	–	+	+
Saponin	–	+	+
Steroid	–	–	–
Tannin	–	+	+

['+' sign indicates presence of phytochemical group of compounds while the '-' sign indicates absence of phytochemical group of compounds tested for]

Table 3: Total phenolic, flavonoid contents, Total Tannin Contents (mean ± SD) of extracts from fruit extracts of *B. retusa*

Plant Extracts	Total Phenolic Content (mg/gm GAE)	Total Flavonoid Content (mg/gm QE)	Total Tannin Content (mg/gm TAE)	Total Antioxidant Capacity (mg/gm AAE)
Methanol	134.50±0.00	17.00±2.82	652.50±3.53	236.00±36.78
Ethyl Acetate	91.00±4.243	46.00±0.00	650.00±7.07	180.50±31.81
n-Hexane	3.50±1.414	60.50±4.95	42.50±3.53	24.50±16.26

Table 4: IC_{50} and LC_{50} values of the different extracts in DPPH scavenging assay and brine shrimp lethality bioassay

DPPH Scavenging Assay		Brine Shrimp Lethality Bioassay	
Sample	**IC_{50} (µg/mL)**	**Sample**	**LC_{50}(µg/mL)**
Methanol	168.757	Methanol	20.00
Ethyl Acetate	141.954	Ethyl Acetate	191.618
n-Hexane	381.609	n-Hexane	915.4545
Ascorbic Acid	16.654	Vincristine	0.699

Table 5: Anti-microbial screening of different extracts of fruit of *B. retusa*.

Test Organisms	Inhibition Zone Diameter (mm)			
	Chloranphenicol (50µg/Disc)	Methanol (400 µg/Disc)	Ethyl Acetate (400 µg/Disc)	n-Hexane (400 µg/Disc)
Bacillus subtilis	23	_	_	_
Escherichia coli	32	_	11	_
Salmonella typhi	39	_	_	_
Pseudomonus aeruginosa	23	_	9	_

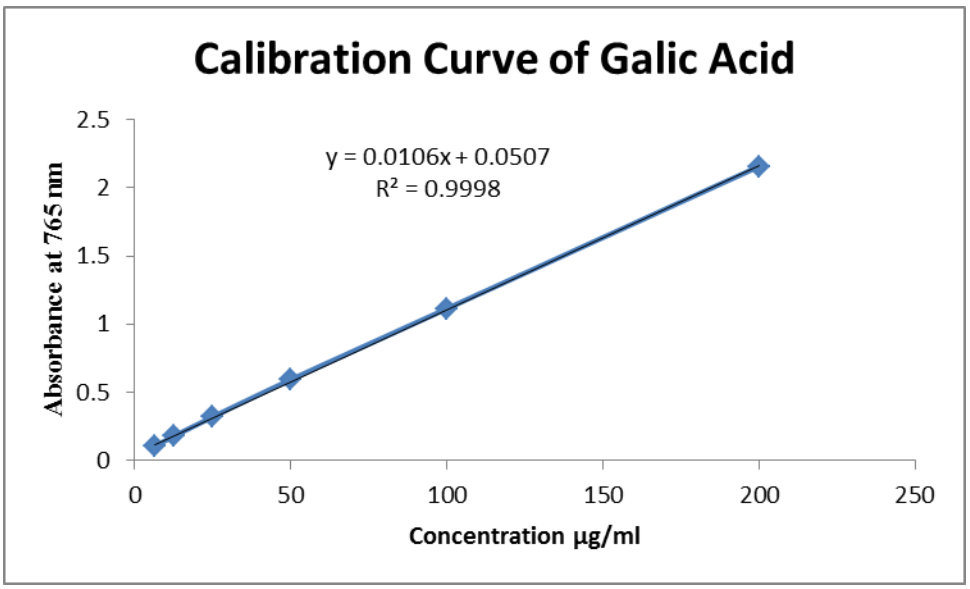

Figure 1: Calibration line of gallic acid standard

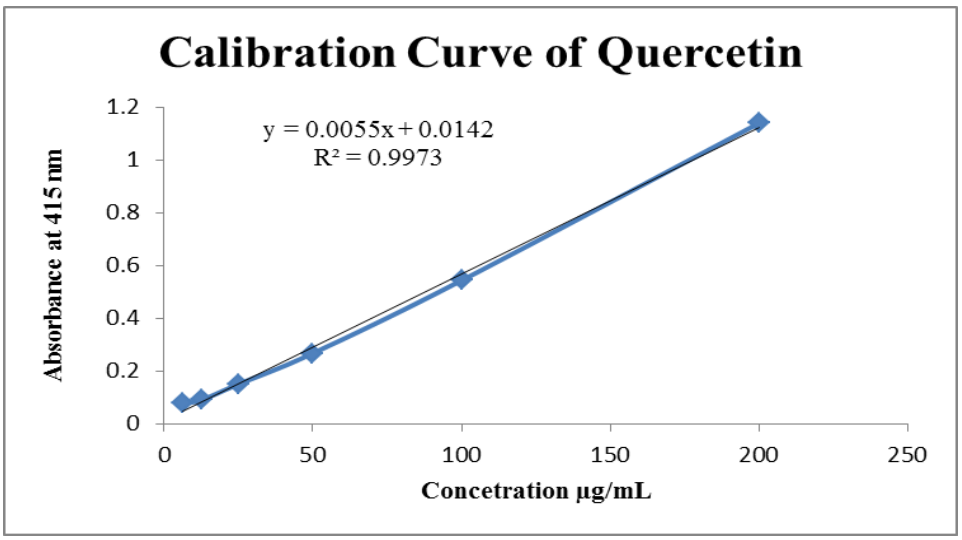

Figure 2: Calibration line of quercetin standard

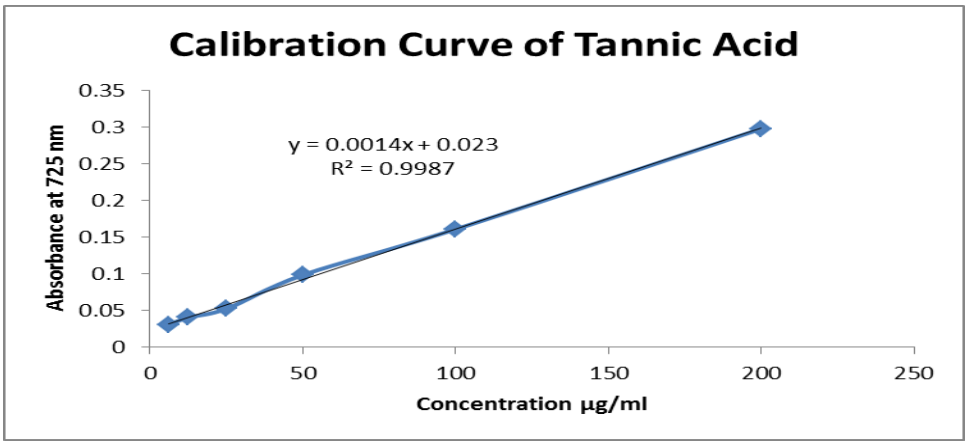

Figure 3: Calibration curve of tannic acid

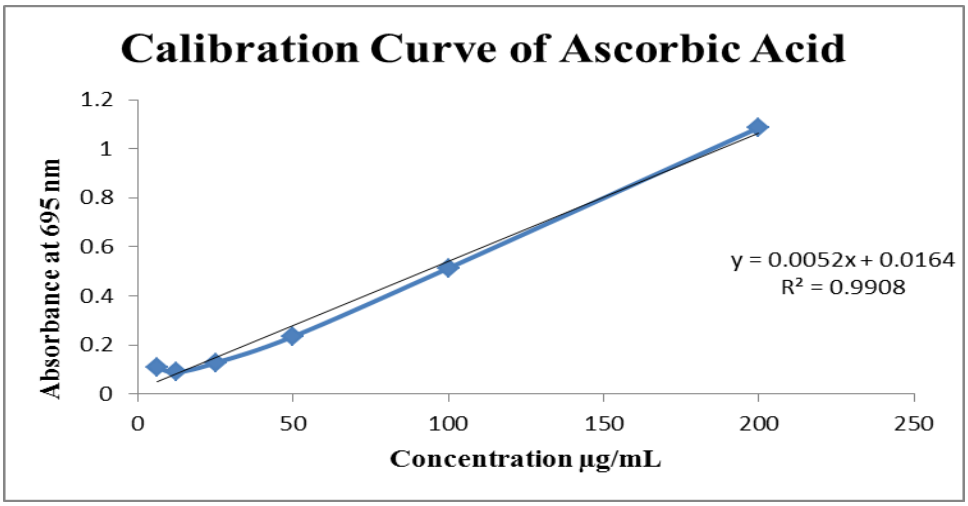

Figure 4: **Calibration curve of ascorbic acid**

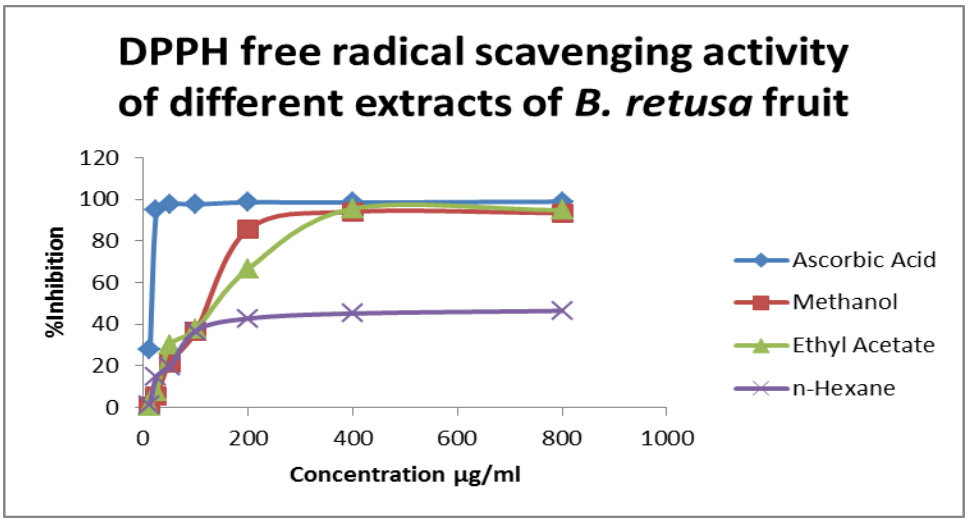

Figure 5: DPPH radical scavenging activity of the different extracts of fruit of *B. retusa.*

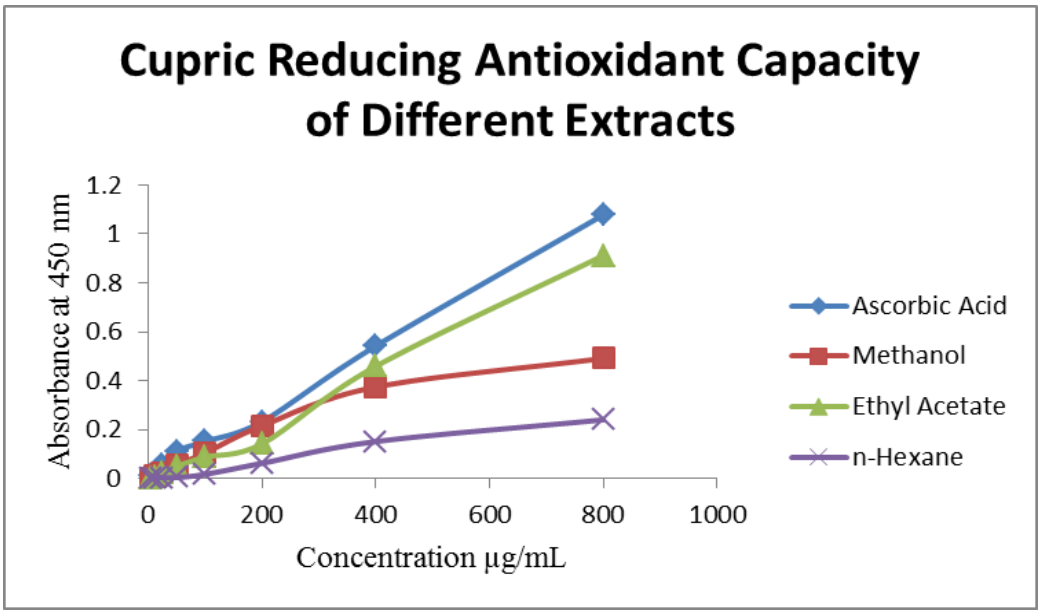

Figure 6: Cupric Reducing Antioxidant Capacity *Bridelia retusa* (L.) Spreng. fruit.

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