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Isolation and Characterization of *Bauhinia Variegata* for The Prevention & Treatment of Skin Infection

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Abstract

Aim - *Identification and characterization of Bauhinia variegata are the goals of This study to evaluate the antioxidant activity of the species and its potential applications in treating and preventing skin infections. Additionally, it contains anti-ulcer, nephroprotective, hepatoprotective, antioxidant, immunomodulating, hypolipidemic, antibacterial, anti-inflammatory, molluscicidal, and wound-healing properties.* ***Materials & Methods -*** *The bark and flowers of the ethnobotanically important plant Bauhinia variegata L. were undertaken for the present study. Botanists from the Department of Botany, Safia Science College, validated the plant material taken from Botanical, the cultivated farm, and the open field in Bhopal.* ***Methodology -*** *The methodology for the isolation and characterization of Bauhinia variegata includes collecting plant material, extracting suitable solvents, evaluating antimicrobial properties, determining antioxidant activity through various assays, and characterization of bioactive compounds using spectroscopic techniques.* ***Result and Discussion -*** *Bark and flower extracts/fractions from Bauhinia variegata showed strong antioxidant activity, blocking DPPH free radicals. Particularly REB and MEB demonstrated notable scavenging activities in the bark extract/fractions. The greatest level of DPPH radical inhibition was seen in the MEF extract from flowers. Column chromatography's isolated fractions (BV1, BV3, and BV4) also demonstrated potent radical-scavenging abilities. Additionally, different levels of superoxide anion scavenging activity were seen in the extracts/fractions, with the polar fractions exhibiting a notable reducing power. Overall, the antioxidant activity of Bauhinia variegata extracts appears encouraging.*

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Keywords: *Antioxidants, Antimicrobial activity, Column chromatography, Gram-positive, Gram-negative bacteria, etc.*

1. Introduction

The orchid tree, also known as Bauhinia variegata, is a plant species noted for its antioxidant capabilities. It has been used as a natural antioxidant in traditional medicine for ages because of its bright blossoms and extensive medical history. The plant includes bioactive substances that prevent oxidative stress and aid in defending cells against injury from dangerous free radicals[1]. Including Bauhinia variegata in our diets or taking it as a supplement may have positive effects on our health by enhancing our general well-being and bolstering our body's natural antioxidant defences[2].

Rats with test liver tumors caused by N-nitroso diethylamine were shown to be protected against them by an ethanolic concentration of B. variegata stem[3]. The inclusion of an ethanolic extract aided in the prevention of liver tumors caused by N-nitroso diethylamine. When administered orally at doses of 100 and 200 mg/kg, the ethanolic concentrate of B. variegata's stem bark showed Rats' liver toxicity was induced by hepatotoxic movements against carbon tetrachloride, and their levels of AST, ALT, ALP, and GGTP were reduced as a result[4].

For their hostility to helminth activity against Pheretima posthuman and Ascardia galli, watery and chloroform concentrates of B. variegata bark were tested. All extractions demonstrated subordinate measures (25, 50, and 100 mg/ml), restriction of unrestricted motility (loss of motion), and the timing of worm death[5]. Both the Pheretima posthuma and the Ascaridia galli were killed by Remove that came from the bark. When piperazine citrate, a common drug, was centrally administered, and distilled water served as the control, the impressions were identical[6]. At a centralization of 100 mg/ml, both concentrates showed their most intense vermicide effect. According to the results of the test, *B. variegata* bark which has been concentrated in water and chloroform may have anthelmintic properties[7].

2. Materials And Methods

Plant Collection and Authentication

For this study, the bark and flowers of the Bauhinia variegata L. plant was examined. Botanists from the Department of Botany at Safia Science College collected the plant material from Bhopal's botanical gardens, cultivated farms, and open fields and validated it[8].

Isolation and Fractionation of Phytochemical from Bauhinia Bark

The 4.5-kilogram stem bark was dried in an oven at 402°C after being cleaned with distilled water and weighed. The bark was first dried and ground then percolated with 80% methanol and rotary evaporated to remove any remaining moisture[9]. To create the methanol extract known as MEB extract, the extract underwent further lyophilization. A succession of organic solvents in ascending order of polarity were used to fractionate the MEB extract after it had been rendered aqueous by dissolving in distilled water. Hexane (3x1000 ml) was used to extract MEB first, then the HEB fraction was obtained using a separating funnel[10].

The residual fraction was fractionated with hexane, and the CFB fraction was obtained by extracting it three times with 1000 cc of chloroform. Then, extraction with ethyl acetate (3x1000 ml) was used to acquire the EAB fraction, and extraction with n-butanol (3x1000 ml) was used to obtain the NBB fraction[11]. The REB fraction is the last fraction obtained following fractionation with n-butanol. Utilizing a rotary evaporator, all of the fractions were concentrated, and after being lyophilized to obtain the desired fractions, they were all stored in a desiccator. The number of different extracts and fractions that may be produced from bark powder (4500g) of B. variegata L[12].

Table 2.0. *The yield of various extracts/fractions from bark powder of B. variegata L.*

S. No.	Extract/fraction	Yield (g)	Yield (%)
1.	MEB extract	108.97 2.421	2.421
2.	HEB fraction	3.127 0.069	0.069
3.	CFB fraction	4.76 0.105	0.105
4.	EAB fraction	10.946 0.243	0.243

Bauhinia variegata bark was extracted using the Soxhlation technique with petroleum ether at 60°C. The marc was dried before being extracted with ethanol. The absence of residue indicated complete extraction. At 40°C, the extracts were evaporated in a revolving vacuum evaporator[13]. The % yield for each dried extract was calculated using the following formula:

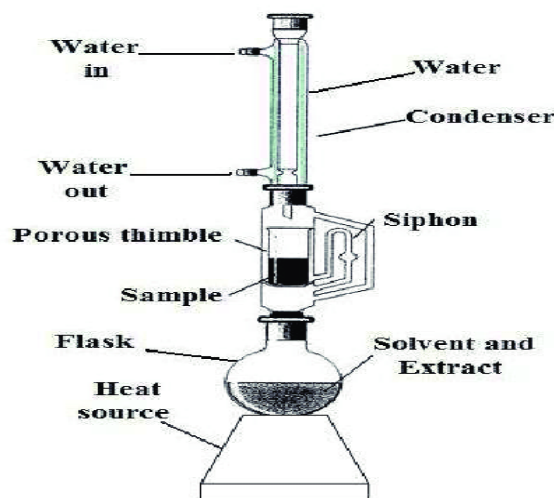

Figure 1.0. *Soxhlet apparatus for extraction of plants*

Methodology and Experimental Works

Physical Characteristics

We examined the solubility of the extract in ethanol, water, acetone, chloroform, ethyl acetate, and DMSO[14].

Phytochemical investigation

To determine the presence or absence of various phytoconstituents, thorough phytochemical testing was carried out.

Tests for Carbohydrates

Molisch Test

Ethanolic extract mixed with naphthol and sulphuric acid confirms the presence of carbohydrates with violet rings[15].

Fehling's Test

Mix extract, Fehling's A and B, and heat for 10 mins in the test tube. Red precipitate shows decreasing sugar[16].

Benedict's Test

To detect reducing sugar, heat Benedict's reagent and ethanolic extract in equal parts using a water bath for 5-10 minutes. Observe the color of the test solution[17].

Barfoed's Test

A mixture of Barfoed's reagent and 1 ml of extract was briefly heated in a test tube using a water bath. The presence of monosaccharide is shown by the crimson hue that results from the production of cupric oxide[18].

Tests for Protein and Amino Acids Biuret's Test

Heat the extract with 10% sodium hydroxide solution and add 0.7% copper sulfate solution. Look for a pink or violet color to identify proteins[19].

Tests for Glycosides

Sulphuric acid that had been diluted was added to 3 ml of the test solution, the solution was heated for 5 minutes and subsequently filtered[20]. The cool filtrate was mixed with an equal amount of benzene or chloroform before being shaken well. Ammonia was added after the layer of organic solvent was separated. Alkaloids are present when the ammoniacal layer develops a pink to red color, which is a sign of anthraquinone glycosides[21].

Tests for Saponins

One millilitre of the test solution was dissolved in pyridine. To make it alkaline, a 10% sodium hydroxide solution was employed after adding 1 ml of sodium nitroprusside solution. The emergence of a pink-to-blood-red tint can be used to identify cardiac glycosides[22].

In a test tube containing the other ingredients, add 3 ml of glacial acetic acid and one drop of 5% ferric chloride. Fill the test tube with 0.5 cc of concentrated sulfuric acid. The acetic acid layer's blue colouring is a sign that cardiac glycosides are present[23].

Test Tests for Alkaloids

Hydrochloric acid was added after thoroughly shaking the extract. The filtrate was used in the subsequent studies[24].

Mayer's Test

2-3 ml of filtrate was added to a small amount of Mayer's reagent that was placed around the tube's edges. When a creamy or white precipitate develops, alkaloids are present[25].

Hager's Test

A few drops of Hager's reagent were applied & added to a test tube that contained 1-2 ml of filtrate; the presence of alkaloids turned the precipitate yellow[26].

Wagner's Test

In a test tube that contained 1-2 ml of filtrate, we added a few drops of Wagner's reagent solution, so that it turned reddish-brown[27].

Tests for Saponins

The extract was stirred for 15 minutes in a graduated cylinder after being diluted with distilled water. The development of a foamy layer is a sign that saponins are present[28].

Tests for Flavonoids

Lead Acetate Test

In this test, we took a test tube that contained 1-2ml of the filtrate in which we added or mixed a few drops of lead acetate solution and mixed them well, the turning of solution color to yellow indicated the presence of flavonoid in it[29].

Alkaline Reagent Test

Another procedure involved treating the extract in a test tube with a few drops of sodium hydroxide[30]. The intense yellow hue that occurs when flavonoids are present is removed when a few drops of diluted acid are introduced[31].

Tests for Triterpenoids and Steroids

Salkowski's Test

Chlorination and filtration were applied to the extract. The filter was treated with a strong sulfuric acid solution before being let to stand. If the bottom layers become red, steroids are present. Because of the bottom layer's golden yellow color, triterpenes could be present[32].

Liebermann Burchard's Test

A chemical test called the Liebermann-Burchard's test is used to find cholesterol and associated substances. It involves the chemical reacting with concentrated sulfuric acid and acetic anhydride to produce a green or blue color which means cholesterol is present in it[33].

Tests for Tannin and Phenolic compounds

Test for Ferric Chloride

It was dissolved in distil. water together with a small amount of extract. This solution was diluted by 2 cc and then mixed with a 5% ferric chloride solution. Phenolic compounds are present when blue, green, or violet color production occurs[34].

Test for Lead Acetate

A small proportion of the filtrate or extract was dissolved in the distilled water & the lead acetate was added to it repeatedly. The appearance of a white precipitate is a sign that phenolic chemicals are present[35].

Test for Gelatin
When added to distilled water, the extract dissolved partially. After adding 2 cc of 1% gelatin solution combined with 10% sodium chloride, a white precipitate with phenolic constituents was generated[36].

Tests for Fats and Oils
A few quantities of extract were pressed between two filter papers. Persistent spot oil on the paper is a sign that fixed oils are present[37].

Solubility test
To determine the extract's solubility, we mixed 1-2 mL of its alcoholic solution with a small amount of chloroform. We also used 1-2 mL of the extract's alcoholic solution and a small amount of 90% ethanol to assess its solubility[38].

Pharmacological Activity

Table.3.0. Pharmacological Action performed on experimental animals

Strain	Albino Wistar rats
Sex	Either
Body weight	200 ± 50 gm
Housing Condition	As per CPCSEA Guidelines

Animals from the PBRI's animal sanctuary in Bhopal, India, were chosen at random and assigned to different treatment groups. They were kept in propylene cages with sterilized husk bedding. The animals lived in an environment with a 12:12 light-dark cycle, 30.7% humidity, and a temperature of 22.20°C. They were given normal pellets from Golden Feeds in New Delhi, India, and had full access to water, with supplementary components provided according to procedure. The Bhopal Institutional Animal Ethics Committee approved the use of experimental animals[39].

Acute oral toxicity
An oral acute toxicity test was carried out by OECD 423 principles, following standards established by the Organisation for Economic Co-operation and Development, a non-profit organization devoted to minimizing animal suffering in acute toxicity studies. The Wister albino rats were separated into groups and given single doses of bauhinia variegata extract. These rodents were fed regular rat pellet meals and had full access to tap water. The dosages used in the trial were 2000 mg/kg, 300 mg/kg, 50 mg/kg, and 5 mg/kg. The researchers observed animal mortality over 72 hours after the samples were administered (Balakrishnan et al., 2014). Based on the acute toxicity research and the maximum tolerated dosage limit (MTD), two tests were selected for pharmacological screening[40]. Finally, a dosage of 2000 mg/kg was determined to be appropriate for additional pharmacological testing.

Determination of total phenolic contents
The total phenolic content (TPC) was assessed using Singleton and Rossi's method from 1965. A blue complex is formed when the polyphenols in plant extracts react with the Folin-Ciocalteu reagent (a particular redox reagent), and this complex's size may be measured using visible light spectrophotometry. The proportion of phenolic compounds in the reagent combination and the alkaline solution both affect how much of the blue chromophore is absorbed at its maximum (Blainski et al., 2013). The blue chromophore is made up of a phosphotungstic-phosphomolybdenum complex[41].

Preparations and their Procedures

Determination of total phenolic contents
TPC was calculated using Singleton and Rossi's (1965) methodology. When the Folin-Ciocalteu reagent, a specific redox reagent, and polyphenols in plant extracts interact, a blue complex form can be recognized by visible-light spectrophotometry[42]. The alkaline solution as well as the concentration of phenolic compounds in the reagent combination determine the blue chromophore's maximal

absorption (Blainski et al., 2013). The blue chromophore is a phosphotungstic-phosphomolybdenum complex.

The Folin-Ciocalteu reagent (0.5 mL) was dissolved into 100l of extract/fraction and stirred for 6 minutes. The solution was then appropriately diluted to a final volume of 5 ml using deionized water[42]. After incubation for two hours with the produced blank, the absorbance at 765 nm was measured using a UV-visible spectrophotometer. The common phenolic component was gallic acid.

Antioxidant Activity

ABTS Radical Cation Scavenging Assay

Before the addition of extract, ABTS is first oxidized with potassium persulfate to produce stable $ABTS^{\bullet+}$ with blue-green chromophore absorption for this test. The radical cation reduction, which measures the attenuation of absorbance at 734 nm in percentage terms, is used to determine or assess the antioxidant activity of filtrated extracts and fractions[43].

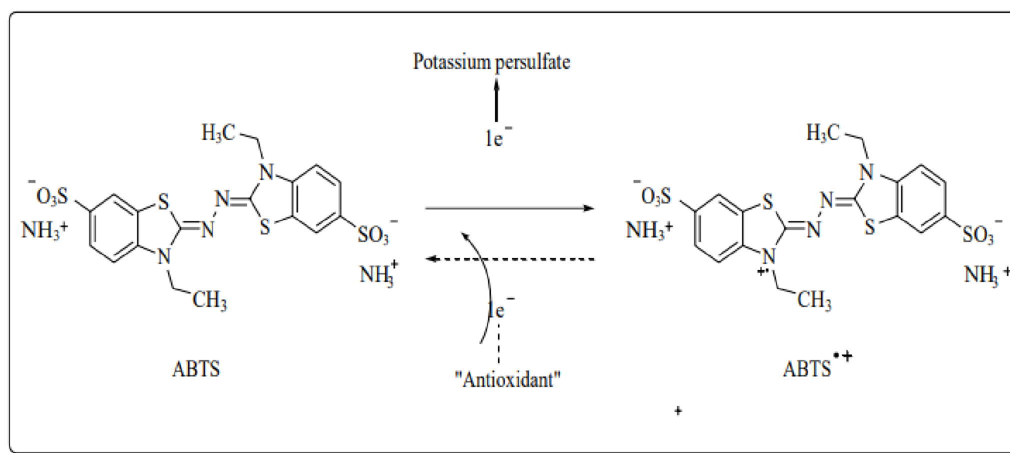

Figure.2. The formation of $ABTS^{\bullet+}$ as a result of reaction between ABTS and potassium persulfate and the scavenging of radical cation so formed by an antioxidant compound.

DPPH Free Radical Scavenging Assay

The DPPH test offers a reliable and straightforward method for evaluating antioxidant capacity due to the comparatively quick analytical time. With a peak absorbance of 517 nm, the DPPH methanolic solution is a stable radical[44]. The 2, 2'-Diphenyl-1-picrylhydrazyl radical, which is present in the purple solution, is reduced to 2, 2'-Diphenyl-1-picrylhydrazine, which is present in the yellow solution, rendering the absorbance inert.

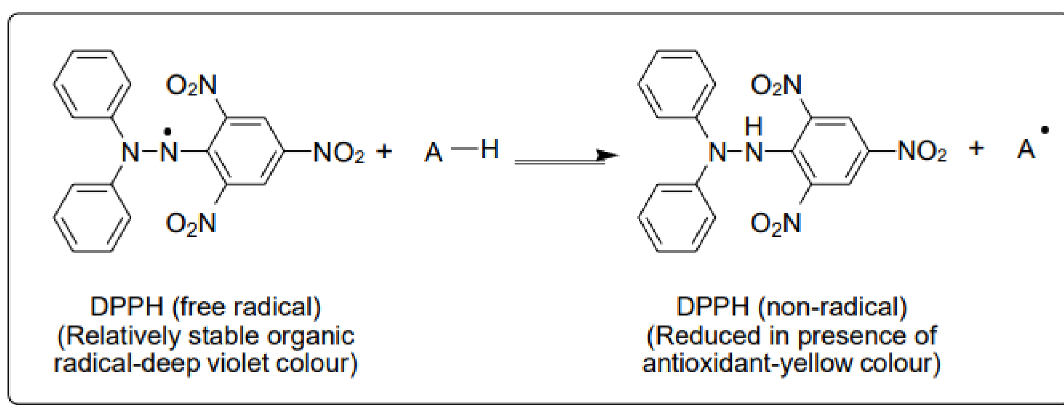

Figure 3.0. *The mechanism of action of antioxidant compound (A) in DPPH assay*

Superoxide Radical Scavenging Assay

We tested *B. variegate* bark and flower extracts/fractions using a non-enzymatic reaction with NADH, PMS, and NBT. The superoxide anion reduces NBT in the PMS/NADH-NBT system. The PMS/NADH coupling pathway generates superoxide anion from dissolved oxygen. The reduction in absorbance at 560 nm in the presence of extracts or fractions indicates superoxide anion consumption in the reaction mixture.

Ferric Ion Reducing Power (FRAP) Assay

The reducing power of various extracts of *B. variegata* was determined using the Oyaizu (1986) technique. The reduction potential of fractions was assessed using the reducing power test because it takes into account the reduction of the ferricyanide ion [Fe (CN)6]3- to the ferrocyanide ion [Fe (CN)6]4- by electron donation from an antioxidant chemical[43]. Fe4 [Fe (CN)6]3, a Prussian blue complex whose intensity is measured spectrophotometrically at 700 nm, is created when ferrocyanide ions interact with Fe (III) in an acidic solution[45]. The intensity of the colored complex grows as the antioxidant compound's ability to donate electrons or H ions increases.

The extract/fraction's reducing activity was determined by comparing its results to that of BHT, the standard reference chemical, and interpreting the rise in absorbance as an increase.

3. Results and Discussion

Plant Extraction

The soxhlation process was used for the extraction of the extract using a continuous hot percolation of the plant materials, resulting in a yield percentage of 2.43% (by ethanol) as calculated by the formula below.

Solubility Determination

Table 4.0. *Determination of Extract Solubility*

S. No.	Used Solvent	Solubility of AS Pet. Ether Extract	Solubility of ASE extract
1.	Water	Insoluble	Soluble
2.	Ethanol	Partial soluble	Soluble
3.	Petroleum ether	Soluble	Partial soluble
4.	DMSO	Soluble	Soluble

The AS Pet. ether extract is soluble in a variety of solvents, including water, ethanol, petroleum ether, and DMSO. In contrast, ASEE is soluble in all of these solvents, including water, ethanol, petroleum ether, and DMSO. In essence, AS Pet. ether extract has specific solubility patterns, but ASEE is versatile, dissolving successfully in a wide range of solvents for varied purposes.

Phytochemical Investigations

Table 5.0. *Phytochemical Investigation of B. variegata Extracts*

S. No.	*Experiment*	*Analysis of Phytochemical test*	
		Ethanolic Extract	*Pet. Ether extract*
1	***Alkaloids Test***		
1.1	*Mayer's reagent test*	√	-
1.2	*Wagner's reagent test*	√	-
1.3	*Hager's reagent test*	-	-
2	***Carbohydrates Test***		
2.1	*Molish's test*	√	√
2.2	*Fehling's test*	√	-
2.3	*Benedict's test*	-	-
2.4	*Barfoed's test*	-	-
3	***Proteins and Amino Acids Test***		
3.1	*Biuret test*	√	-
4	*Flavonoids Test*		
4.1	*Shinoda test*	√	-
4.2	*Lead Acetate test*	-	-

5	*Glycoside Test*		
5.1	*Borntrager test*	-	-
5.2	*Legal's test*	-	-
5.3	*Killer-Killiani test*	-	-
6	***Tannin and Phenolic Compounds Test***		
6.1	*Ferric Chloride test*	√	-
6.2	*Lead Acetate test*	√	√
6.3	*Gelatin test*	√	-
7	***Saponin Test***		
7.1	*Foam test*	√	-
8	***Triterpenoids and Steroids Test***		
8.1	*Salkowski's test*	√	-

An analysis of a *Bauhinia variegata* extract showed the presence of carbohydrates, proteins, alkaloids, flavonoids, tannin, and phenolic compounds, as well as saponin, triterpenoids, and steroids, but no glycosides were found.

Acute Oral Toxicity

The research on acute oral toxicity followed the OECD 423 standards. Toxicology tests were performed using four different dosage levels: 5mg/kg, 50mg/kg, 300mg/kg, and 2000mg/kg[44]. Each animal was observed for 4 hours after receiving the dosage for signs of mortality, and then again 72 hours later.

Table.6.0. Study of acute oral toxicity over different body weights

S. No.	Groups (Bodyweight)	Observations/ Mortality
1.	5 mg / kg	0/3
2.	50 mg / kg	0/3
3.	300 mg / kg	0/3
4.	2000 mg / kg	0/3

During an acute oral toxicity experiment, mice were administered doses of 5, 50, 300, and 2000 mg/kg b.w. for 72 hours. No deaths or changes in behavior were observed, leading to the selection of doses of 200 and 400 mg/kg b.w. as per LD507.

Quantitative Analysis

Determination of Total Phenolic Content

Table 7.0. *Standard Table of Gallic Acid*

S. No.	Concentration (μg/ml)	Absorbance
1.	10.00	0.1097
2.	20.00	0.1766
3.	30.00	0.2467
4.	40.00	0.2985
5.	50.00	0.3257

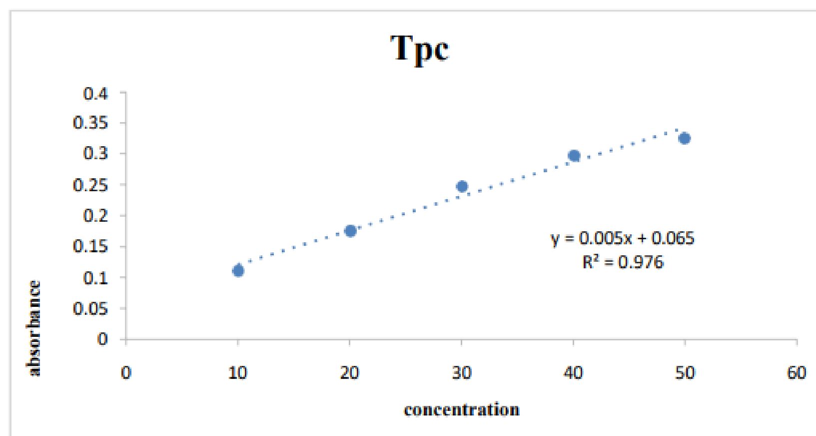

Figure 4.0. *Standard Graph of Gallic Acid*

Total Phenolic Content

Table.8.0. Total Phenolic Content in bauhinia variegata.

S. No.	Concentration (mg/ml)	Absorbance	TPC in mg/g equivalent of Gallic acid
1	1	0.083	3.6
2	1	0.087	4.4
3	1	0.096	6.2
		MEAN±S.D	4.733±1.0873

The phenolic content of bauhinia variegata ethanolic extract was discovered to be 4.733 mg/g, comparable to Gallic acid. In layman's terms, the extract has a particular number of phenolic chemicals, with a concentration of 4.733 mg of Gallic acid per gram of extract.

Total Flavonoid Content

Table.9.0. Standard Reading of Rutin

S. No.	Concentration (µg/ml)	Absorbance
1	10	0.135
2	30	0.165
3	40	0.177
4	50	0.201

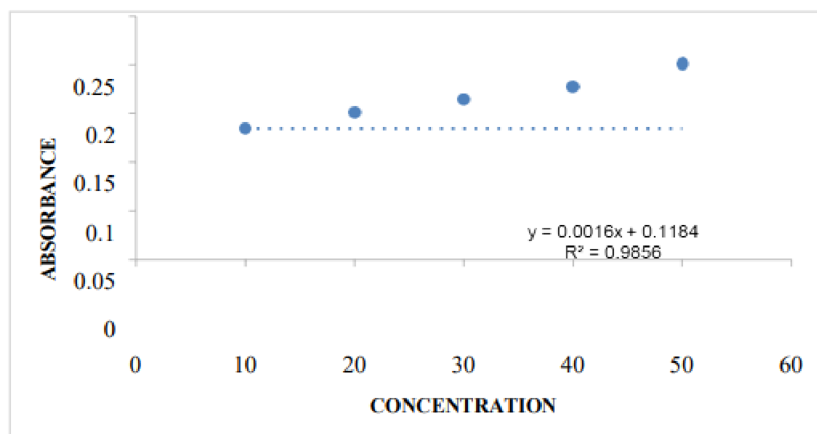

Figure.5.0. Standard Graph of Rutin

Table.10.0. Total Flavonoid Content in Bauhinia Variegata.

S. No.	Concentration (mg/ml)	Absorbance	TFC in (mg/g) equivalent of Rutin
1	1	0.119	0.375
2	1	0.121	1.625
3	1	0.122	2.25
		MEAN±S.D	1.416±0.7793

The Flavonoid Content of the ethanolic extract of *bauhinia variegata* concerning Rutin was found to be 1.416 mg/g equivalent to Rutin.

Antioxidant Activity of extracts isolated from Bauhinia variegata L.

ABTS radical cation scavenging activity

The polar extracts and fractions from the bark of *B. variegata L.* exhibit significant potential in scavenging ABTS radical cations. At a concentration of 200 g/ml, EAB fraction inhibited 93.51 percent, whereas NBB fraction and MEB extract inhibited 97.42 and 97.25 percent, respectively, with EC50 values of 48.15 and 47.50 g/ml. The REB extract also showed a high capacity to scavenge ABTS radical cations (84.27%; EC50 52.19 g/ml). The non-polar solvent fractions of B. variegata bark, namely HEB and CFB, scavenged ABTS radical cation by 23.76% and 57.44%, respectively. BHT (the standard reference chemical) inhibited ABTS radical cations with an EC50 of 58.48 g/ml (**Table 11.0, Figure 6.0**).

Table 11.0. *The ABTS radical cation scavenging activity of bark extraction*

Concentration (μg/ml)	HEB	CFB	EAB	NBB	REB
20	5.63 ± 0.25	10.89 ± 1.79	17.83 ± 2.25	23.93 ± 0.61	15.88 ± 1.01
40	5.57 ± 0.62	16.14 ± 0.43	30.87 ± 0.75	43.06 ± 0.47	38.08 ± 1.07
60	8.95 ± 0.69	21.83 ± 0.30	48.71 ± 6.54	60.63 ± 3.18	57.76 ± 1.64
80	9.17 ± 0.40	24.56 ± 2.57	55.07 ± 0.49	74.90 ± 3.96	64.38 ± 0.35
100	10.22 ± 1.13	30.90 ± 0.14	68.53 ± 2.54	90.43 ± 0.14	70.84 ± 0.80
120	13.26 ± 1.48	32.39 ± 1.84	75.13 ± 3.58	92.25 ± 0.30	72.65 ± 0.48
140	13.75 ± 0.38	41.51 ± 1.77	85.79 ± 2.99	94.74 ± 0.14	76.40 ± 0.54
160	14.52 ± 0.71	46.42 ± 0.94	91.77 ± 0.96	96.10 ± 0.74	81.66 ± 0.69
180	15.42 ± 0.53	52.76 ± 0.48	93.39 ± 1.00	96.62 ± 0.11	83.56 ± 0.35

200	23.76 ± 0.40	57.44 ± 1.94	93.51 ± 0.51	97.42 ± 0.58	84.27 ± 0.76
F-ratio (df= 9,20)	53.728*	106.654*	92.975*	253.493*	662.337*
HSD	3.967	7.37	14.097	8.239	4.306
EC_{50} (µg/ml)	N. D	172.14	66.66	48.15	52.19

[#]Standard reference compound; SE = Standard error; Significant at p ≤ 0.05; N.D.= Not determined

Table 12.0. *The ABTS radical cation scavenging activity of different fractions obtained from column chromatography of MEB*

Concentration (µg/ml)	Percentage Inhibition (Mean ± SE)		
	BV1	**BV3**	**BV4**
2	2.53 ± 0.36	14.77 ± 2.90	5.87 ± 0.09
4	7.94 ± 1.27	25.79 ± 1.99	22.76 ± 1.95
6	14.9 ± 2.47	33.61 ± 1.65	36.49 ± 1.25
8	22.13 ± 0.36	45.1 ± 2.45	42.54 ± 1.18
10	34.75 ± 0.45	57.53 ± 6.60	51.12 ± 2.66
12	46.03 ± 1.36	65.53 ± 2.68	67.74 ± 1.36
14	51.64 ± 0.09	78.33 ±2.29	71.18 ± 0.18
16	56.71 ± 1.48	84.74 ± 1.39	78.31 ± 3.08
18	68.26 ± 0.15	86.90 ± 0.18	81.56 ± 4.47
20	69.73 ± 0.60	87.38 ± 0.49	83.82 ± 0.59
F-ratio (df= 9,20)	481.188*	91.773*	157.067*
HSD	5.634	14.109	10.712
EC_{50} (µg/ml)	13.45	8.76	9.39

Fraction BV2 did not show any noticeable activity at the above-mentioned concentrations; SE = Standard error concentrations; SE = Standard error

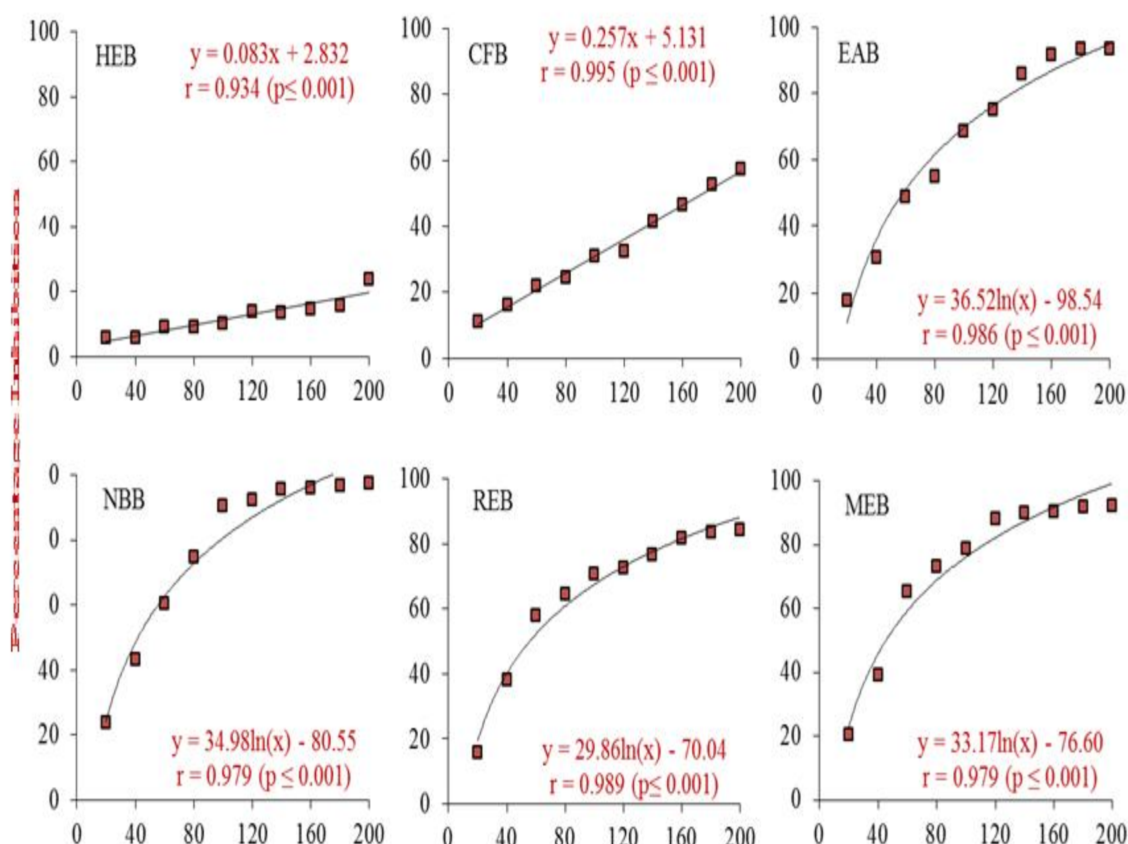

Figure 6.0. *The scavenging activity of different extracts/fractions from B. variegata bark on ABTS radical cations*

Figure *9.0. The scavenging activity of different fractions from column chromatography of MEB extract on ABTS radical cations*

Using column chromatography, the antioxidant activity of the sample recovered from MEB extract was determined. The BV3 fraction scavenged 87.38% ABTS radical cations (EC50 8.766 g/ml), followed by the BV4 fraction (83.82%) and BV1 fraction (69.73%) at conc. of 20g/ml. When compared to the other isolated fractions, the BV2 fraction exhibited no appreciable ABTS radical cation scavenging activity at doses ranging from 2 g/ml to 20 g/ml.

DPPH radical scavenging activity

The study of the capacity of bark and flower extracts/fractions to suppress DPPH free radicals indicated that REB had the highest DPPH radical scavenging activity in terms of EC50 value (38.59 g/ml), followed by MEB (44.16 g/ml). REB, MEB, and EAB all showed lower EC50 values than the typical antioxidant chemical BHT (76.16 g/ml). At the maximum measured dose of 200g/ml, HEB, and CFB fractions showed relatively minimal DPPH free radical scavenging efficacy, with 6.54% and 26.98% inhibition, respectively.

Among the extracts derived from B. variegata flowers, the MEF extract was shown to have the most activity. At the maximum studied dose of 1000 g/ml, it inhibited DPPH free radicals 81.42%, followed by EAF (62.58%), CFF (61.96%), and PEF (54.95%). After conducting column chromatography on the MEB extract, the resulting fractions were found to be effective in inhibiting DPPH free radicals. The BV1 fraction demonstrated a 50.51% inhibition rate at a concentration of 20 g/ml, while the BV3 fraction scavenged 66.45% of the radicals at the same concentration. However, the BV4 fraction did not exhibit any inhibitory activity.

Superoxide anion scavenging activity

MEB extract had the lowest EC50 (880.21 g/ml) in the superoxide anion scavenging experiment, followed by REB (929.28 g/ml) and EAB (979.11 g/ml). HEB, CFB, and NBB fractions were shown to be the least effective in scavenging superoxide anions from the sample extracts. At the maximum measured concentration of 1000g/ml, these fractions were inhibited by 21.18% (HEB), 25.12% (CFB), and 34.17% (NBB). When the activity of extracts/fractions was compared to that of the standard reference chemical BHT, it was discovered that BHT had a substantially greater superoxide anion scavenging activity, with an EC50 of 161.16 g/ml.

Ferric ion reducing antioxidant power (FRAP) assay

A rise in spectrophotometer absorbance was interpreted as indicative of the test sample's reducing power. At a concentration range of 40-200g/ml, the polar fractions showed a considerable rise in absorbance with increasing concentration. NBB fraction had the greatest range of growth. The absorbance of the HEB fraction did not increase with concentration. In the instance of CFB, there was a little rise in absorbance with increasing concentration in the reaction mixture. The reducing power of polar fractions was found to be equivalent to that of the typical antioxidant chemical BHT.

Molybdate ion reduction assay

Tables 12.0 and 13.0 show how different extracts/fractions of Variegata bark and flowers decrease molybdate ions. Figure 10 shows that extracts and fractions can decrease molybdate ions in the phosphomolybdenum complex, represented as Ascorbic Acid Equivalents (AAE) per gram of dry weight. The NBB fraction had the best reduction ability (2.14) as stated in terms of AAE among bark extract/fractions, followed by MEB (1.80), EAB (1.77), REB (1.62), CFB (1.27), and HEB (0.98) **(Table.13.0).** MEF had the best reduction ability of molybdate ions (1.31) among floral extracts. CFF had a larger reduction potential (1.07) than the more polar extract EAF (0.67). Among the floral extracts, PEF showed the lowest reduction power (0.59) **(Table 13.0).**

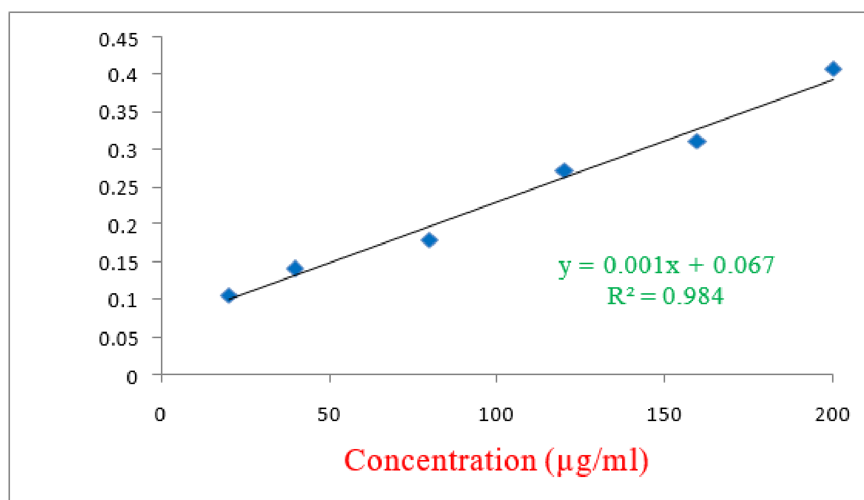

Figure.10.0. Calibration Curve for Molybdate ion reduction assay using Ascorbic acid as standard.

Table 13.0. Molybdate ion reduction ability of different extract/fractions of B. variegata bark.

Extract/Fraction	**Molybdate ion reduction ability (mg ascorbic acid equivalents/g dry weight of extract/fraction)**
HEB	0.98
CFB	1.27
EAB	1.77
NBB	2.14
REB	1.62
MEB	1.80

When there are too many free radicals in the body, it can lead to oxidative stress. The brain is a very important organ and if it experiences any form of stress or injury, it can have serious consequences for the entire body. The brain's low antioxidant capacity makes it especially vulnerable to oxidative stress, as it consumes 20% of the body's oxygen supply. Although it has some protection against reactive oxygen species, it is limited. Antioxidants are the best natural defences against oxidative stress, and flavonoids are particularly effective. Flavonoids exhibit a variety of benefits such as anti-inflammatory, antioxidant, anti-allergic, neuroprotective, antithrombotic, and anti-carcinogenic properties.

The study of the capacity of bark and flower extracts to suppress DPPH free radicals indicated that REB had the highest DPPH radical scavenging activity in terms of EC50 value (38.59 g/ml), followed by MEB (44.16 g/ml). REB, MEB, and EAB all showed lower EC50 values than the typical antioxidant chemical BHT (76.16 g/ml). At the maximum measured dose of 200g/ml, HEB, and CFB fractions showed relatively minimal DPPH free radical scavenging efficacy, with 6.54% and 26.98% inhibition, respectively.

Among the extracts derived from B. variegata flowers, the MEF extract was shown to have the most activity. At the maximum studied dose of 1000 g/ml, it inhibited DPPH free radicals 81.42%, followed by EAF (62.58%), CFF (61.96%), and PEF (54.95%).

Column chromatography was used to separate the MEB extract into fractions, and these fractions were also shown to be potent DPPH free radical inhibitors, with the BV1 fraction suppressing DPPH radicals by 50.51% at a concentration of 20 g/ml. The BV4 fraction was unable to scavenge the DPPH radicals at the same concentration as the BV3 fraction, which was able to do so.

The superoxide anion scavenging experiment revealed that MEB extract had the lowest EC50 (880.21 g/ml), followed by REB (929.28 g/ml) and EAB (979.11 g/ml). The fractions extracted from the bark of *B. variegata*, HEB, CFB, and NBB were shown to be the poorest scavengers of superoxide anions. At the maximum measured concentration of 1000g/ml, these fractions demonstrated a percentage inhibition of 21.18% (HEB), 25.12% (CFB), and 34.17% (NBB). When the activity of extracts/fractions was compared to that of the standard reference chemical BHT, it was discovered that the superoxide anion scavenging activity of BHT was substantially greater, with an observed EC50 of 161.16 g/ml.

A rise in spectrophotometer absorbance was interpreted as indicative of the test sample's reducing power. At a concentration range of 40-200g/ml, the polar fractions showed a considerable rise in absorbance with increasing concentration. NBB fraction had the greatest range of growth. The absorbance of the HEB fraction did not increase with concentration. In the instance of CFB, there was a little rise in absorbance with increasing concentration in the reaction mixture.

The presence of moly date ions in the phosphomolybdenum complex has been measured in AAE (Ascorbic Acid Equivalents) per mg/g of dry weight of extract/fractions. This was determined by referring to the ascorbic acid standard curve. It has been found that the reducing power of polar fractions is similar to that of the standard antioxidant compound BHT.

The NBB percentage had the highest reduction ability (2.14) as stated in terms of AAE, followed by MEB (1.80), EAB (1.77), REB (1.62), CFB (1.27), and HEB (0.98) **(Table.12.0).** In the case of floral extracts, MEF had the maximum reduction ability of molybdate ions (1.31). CFF had a larger reduction potential (1.07) than the more polar extract EAF (0.67). Among the floral extracts, PEF showed the lowest reduction power (0.59) **(Table 13.0).**

4. Conclusion

The current investigation revealed that the plant extract of *Bauhinia variegata Linn* administration was capable of inducing antioxidant activity. As a result of investigating many biochemical characteristics, it is obvious that the extract is altered, albeit in diverse ways. Thus, the study suggests that a plant is a promising option for future investigation as an antioxidant to reduce stress-induced oxidative damage. Purification of bioactive compounds from the ethanolic extract for bioassay-guided investigations requires further research.

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