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HR-LCMS analysis of phytochemicals from *Bignonia magnifica* and evaluation of antioxidant activity using the FRAP method

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ABSTRACT

Background: *Bignonia magnifica*, known as glow vine or purple funnel vine, is a stunning evergreen climber from the Bignoniaceae family, native to humid forests in Colombia, Ecuador, Panama, and Venezuela. It has an aesthetic value and potential medicinal properties. **Objectives:** The present study was carried out to evaluate phytochemical compositions and antioxidant activity of *B. magnifica* leaf extracts. **Materials and Methods:** Sequential leaf extraction was performed by Soxhlet apparatus using Chloroform, Ethyl acetate, Ethanol and Methanol solvents. The qualitative and quantitatively estimation of phytochemical constituents by using standard procedure. High Resolution- Liquid Chromatography Mass Spectroscopy analysis to identify bioactive compounds. The antioxidant activity was evaluated by ferric reducing antioxidant power assay. **Results:** Preliminary phytochemical screening of leaf extracts revealed the presence of carbohydrates, tannins, phenols, flavonoids, terpenoids, proteins, alkaloids, saponins, glycosides, and cardiac glycosides. The Quantitative analysis indicates that the ethyl acetate solution has a high phenolic content (49.50±0.07 mg/g) and tannin (506.00±0.041TAE) content. In contrast, the chloroform infusion shows increased flavonoid levels (4.00±0.001mg/gQE), terpenoids (1.42±0.002mg-TPE/g) and alkaloids (16.50±0.58%). The FRAP assay measures the antioxidant task and demonstrates that ethyl acetate (2241.07±0.01mg/gAAE) infusion has a greater diminishing effect than chloroform (1062.50±0.01 mg/gAAE) infusion. The HR-LCMS analysis identified bioactive compounds such as Genistein, Hydroquinidine, 7',8'-Dihydro-8' hydroxycitraniaxanthin, 14,19-Dihydroaspidospermatine, Pyrrhoxanthinol, Notoginsenoside R10. **Conclusion:** These findings indicate that the *B. magnifica* leaf contain a significant amount of bioactive substances and natural antioxidants, which supports the idea that the leaf could be used as a curative agent in the treatment of oxidative stress disorders and medicinal properties.

Keywords: *Bignonia magnifica*, Phytochemical screening, Total phenolic content, Bioactive compounds, HR-LCMS analysis, Ferric reducing antioxidant power (FRAP).

INTRODUCTION

Medicinal plants play significant role in human life since ancient time, which are used for health and wellbeing's. In ancient India, Vedic period these medicinal plants are worshipped as a deity and Ayurvedic plants offers medications for prevention of several diseases and ailments. Traditional medicinal plants having a diverse group of secondary metabolites which acts as a defensive mechanism in plants but also cures various diseases of human and animals' pathogens. Due to lower cost, higher efficacy, less side effects than synthetic drugs, hence traditional medicinal plants got importance in this world [1]. The medicinal value of plants endowed various bioactive compounds such as alkaloids, flavonoids, phenols, steroids, glycosoids, terpenoids, tannins and saponins which are importance in antioxidant and anticancer activity [2].

These secondary metabolites can be attributed to the biological efficacy of medicinal plants, and their presence and quantity may be measured by qualitative and quantitative phytochemical screening of plant extracts [3].

Being byproducts of normal metabolic processes, living things constantly generate reactive oxygen species (ROS) and free radicals. Oxidative stress, caused by an overabundance of these reactive molecules, is linked to aging and a host of chronic illnesses, including diabetes, cancer, cardiovascular disease, and neurological disorders [4]. To protect the body from oxidative damage and free radical scavenging, antioxidants are necessary [5]. Since ROS are implicated in the development of numerous human diseases, the exploration of antioxidant substances from foods and medicinal plants has become a major focus in current research.

B. magnifica is an evergreen, beautiful ornamental climber from the Bignoniaceae family, renowned for its vibrant purple-pink trumpet-shaped flowers. Originating from northern South America, including Colombia, Ecuador, Panama, and Venezuela, it thrives in tropical and subtropical gardens. Its striking floral display, combined with its ability to climb robustly, makes it a favourite for arbours, trellises, and walls, where it can create a breathtaking canopy of colour and charm [6]. Phytochemical screening, which involves the detection and measurement of the chemical composition of *B. magnifica*'s components, is a serious primary measure for the examination of the functioning of medicinal plants. Quantitative study creates a focus on explicit lectures on compounds, while qualitative phytochemical screening reveals otherwise insufficient information on the class/type of compound present [7]. Together, these studies provide a complete picture of phytochemical elements in the *B. magnifica* plant and demonstrate the promise of the plant to remain curative or medicinal property [8]. The primary objective of this report is to conduct HR-LCMS profiling of bioactive compounds, estimate the qualitative and quantitative phytochemical screening of *B. magnifica* and evaluate its antioxidant activity using the FRAP assay.

MATERIAL AND METHODS

Collection of Plant Material

Leaves of *B. magnifica* were collected in January 2024 from botanical garden of the University of Mysore, Manasagangotri campus in Mysuru, Karnataka, India. The taxonomical identity of this remarkable plant species was confirmed by the esteemed botanist Dr. S. S. Hameed, Scientist 'F' and Chairman of the Botanical Survey of India. A voucher specimen (BSI/SRC/5/2024-25/Tech/964) has been carefully preserved at the Botanical Survey of India, Coimbatore, Tamil Nadu, India. The freshly collected leaves were washed with tap water, rinsed with distilled water, and then air-dried in a shaded area at room temperature for 15 days. After drying, the leaves were ground into a fine powder using an electric grinder. The powdered leaves were then securely stored in airtight containers, protected from light, to ensure they are ready for analysis.

Preparation of Plant extracts

Finely powdered leaves of *B. magnifica* (20 g) were subjected to extraction using solvents of increasing polarity: chloroform, ethyl acetate, ethanol, and methanol (500 mL each). This extraction process was conducted in a Soxhlet apparatus over a duration of 8 h to optimize the retrieval of bioactive compounds. Subsequent to the extraction, the solvents were efficiently removed under reduced pressure utilizing a rotary evaporator (Heidolph, Germany), resulting in concentrated extracts rich in valuable phytochemicals. The dried extracts were accurately weighed to calculate the extractive yield and were stored in airtight vials at 4 °C to ensure their stability for subsequent analysis.

Qualitative analysis of phytochemical constituents of *B. magnifica*

Preliminary phytochemical analysis was carried out for the extract as per standard procedures described by Harborne [9].

Detection of Carbohydrates

Benedict's test: An equal volume of Benedict's reagent was added to the extracts and heated in a boiling water bath for 5 min. The appearance of green, yellow, or red color indicates the presence of reducing sugars. Glucose was used as a positive control.

Detection of Proteins and Aminoacids

Biuret test: Sodium hydroxide (4%) and copper sulfate (1%) were added to extract and observed for the formation of violet or pink color indicating the presence of proteins. Bovine serum albumin (BSA) was used as a positive control.

Detection of Tannins and Phenolic compounds

Ferric Chloride test: A few drops of 5% ferric chloride ($FeCl_3$) were added to 2-3 mL of the extract, and the mixture was observed for the development of a deep blue-black color. Tannic acid served as the positive control.

Gelatin test: A 1% gelatin solution containing 10% sodium chloride was added to 1 mL of the extract, and the mixture was observed for the formation of a precipitate. Tannic acid was used as the positive control.

Detection of Saponins

The extract (2 mL) was mixed with 2 mL of distilled water and shaken vigorously, resulting in the observation of persistent foam. Pea powder served as the positive control.

Detection of Flavonoids

1 mL of extract was combined with 1 mL of 10% lead acetate solution and then observed for the formation of a yellow precipitate. Lemon peel juice served as the positive control.

Detection of Terpenoids

Salkowski's test: Chloroform (1 mL) was added to 1 ml of the extract. A few drops of concentrated sulfuric acid were added carefully along the sides of the test tubes. The mixture was then observed for the formation of a yellow-colored upper layer, which indicates the presence of terpenoids. Tocopherol was used as a positive control.

Detection of Alkaloids

Dragondroff's test: 3 mL of each extract was stirred with 3 mL of 1% HCl on a steam bath. Dragendorff's reagent was then added to the mixture. The turbidity of the resulting precipitate was taken as evidence of the presence of alkaloids.

Detection of Glycosides

Liebermann's test: 1 mL of extract is dissolved in 1 mL of chloroform and 1 ml of acetic acid. The solution is cooled thoroughly on ice, and a few drops of concentrated sulfuric acid are added carefully. A color change from violet to blue to green indicates the presence of a steroidal nucleus (the glycone portion of a glycoside). Calotropis latex was used as a positive control.

Detection of Cardiac glycosides

Keeler Kiliani's test: 1 mL of extract was treated with 1 mL of glacial acetic acid and a few drops of ferric chloride. After that, 2 ml of concentrated sulfuric acid was added carefully along the sides of the test tube. The formation of a reddish-yellow brown color at the junction of the two layers, along with a bluish-green upper layer, indicated the presence of glycosides. Calotropis latex was used as a positive control.

Detection of Steroids

Liebermann-Burchard's test: 1 mL of extract was mixed with 1 mL of chloroform. Then, 1 mL of acetic anhydride was added to the mixture, followed by the careful addition of 2 ml of concentrated sulfuric acid along the sides of the test tube. The mixture was observed for the formation of a red-violet colored layer at the junction, which indicates the presence of steroids. Cholesterol was used as a positive control.

Quantitative analysis of phytochemical constituents of *B. magnifica*

Quantitative phytochemical analysis was studied for Total Alkaloids, Flavonoids, Phenols, Tannins, and Terpenoids content, which were responsible for the major biological activities of plants.

Estimation of Total Phenols

A 20 µL aliquot of the extract was taken, and the volume was adjusted to 200 µl using distilled water. The sample was then mixed with 1 mL of 10% Folin-Ciocalteau (FC) reagent and incubated at 37 °C for 5 min. After this, 1 ml of 6% sodium carbonate (Na_2CO_3) was added, and the mixture was incubated again at 37 °C for 30 min. Following incubation, the absorbance was measured at 750 nm using a UV-Vis spectrophotometer, with a reagent blank used as a reference. The total phenolic content was calculated using a standard graph based on various concentrations of gallic acid (10-100 µg). The phenolic content was expressed as an equivalent amount of gallic acid [10].

Estimation of Total Flavonoid

Leaf extract (20 µL) was mixed with 4 mL of distilled water. Next, 0.3 mL of 5% $NaNO_2$ and 0.3 mL of 10% $AlCl_3$ were added to the mixture. This mixture was then incubated at room temperature for 5 min. After incubation, 2 mL of 1 mM NaOH was added, and the total volume was adjusted to 10 mL using distilled water. The absorbance was measured at 510 nm with a UV-Vis spectrophotometer, using a reagent blank as the reference. The total flavonoid content was calculated using a correlation equation generated from various concentrations of quercetin. The flavonoid content is expressed as the amount equivalent to quercetin [11].

Estimation of Total Tannins

The total tannin content in the sample was estimated using the method described by Makkar [12]. First, 0.05 mL of the sample was placed in a test tube, and the volume was adjusted to 0.5 ml with distilled water. Next, 0.25 mL of the Folin-Ciocalteu reagent (1N) was added, followed by 1.25 mL of a 20% sodium carbonate solution. The test tubes were then vortexed, and the absorbance was recorded at 725 nm after 40 min. The total tannin content, expressed as tannic acid equivalents, was calculated based on a calibration curve prepared from different concentrations of tannic acid (1 mg/mL), and the results were reported on a dry matter basis.

Estimation of Total Terpenoids

The total terpenoid content in each extract was determined using the method outlined by Suica-Bunghez [13]. A volume of 200 µL of the extract was mixed thoroughly with 1.5 mL of chloroform and allowed to sit at room temperature for 3 min. After this period, 100 µL of concentrated sulfuric acid was carefully added while keeping the test tubes on ice. The test tubes were then placed in the dark for 1.5 to 2 h without disturbing them. Following the incubation period, the supernatant was decanted, and the reddish-brown precipitate was retained. This precipitate was then dissolved in 1.5 mL of methanol and vortexed thoroughly. The absorbance of the solution was measured at 538 nm. A mixture containing distilled water instead of the extract was used as a blank for calibration. The total terpenoid content was calculated as tocopherol equivalents using a calibration equation generated from a tocopherol standard.

Estimation of Total Alkaloid

The total alkaloid content in each extract was estimated according to Ezeonu and Ejikene [14] with slight modifications. The reaction began by mixing 40 mL of 10% acetic acid in ethanol with 1 g of the extract, which was then allowed to stand for 4 h. After incubation, the filtrate was collected and concentrated in a water bath to 1/4th of its original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitate was complete. The solution was allowed to settle, and the collected precipitate was washed with 0.1 M ammonium hydroxide and then filtered. Finally, the residue was dried and weighed.

Alkaloids (%) = [(weight of Alkaloids) / (Volume of sample)] × 100

Phytochemical profiling Using HR-LCMS analysis

Phytochemical compounds present in the ethyl acetate fraction was assessed using High Resolution- Liquid Chromatography Mass Spectroscopy (HR-LCMS)-Orbitrap (facility: SAIF, IIT Bombay, India) to identified the phytochemical compounds based on the comparison of mass and MS/MS spectra data with database library.

Ferric reducing antioxidant power activity (FRAP) assay

The ferric reducing antioxidant power assay is a reliable analytical technique that evaluates the capacity of antioxidants to reduce Fe^{3+} ions to Fe^{2+} ions in the presence of TPTZ. This process results in the formation of a distinctive blue Fe^{2+} –TPTZ complex, which exhibits an absorption maximum at 593 nm, thereby providing a quantitative measure of antioxidant activity. The FRAP assay was conducted following the method described by Nair [15] with slight modifications. The FRAP solution was prepared by combining 10 volumes of 300 mM acetate buffer with 1 volume of 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM HCl and 1 volume of 20 mM $FeCl_3 \cdot 6H_2O$. To conduct the assay, 20 µL of the leaf extract were mixed with 3 mL of the FRAP solution, and the reaction mixture was incubated at 37 °C for 30 min. After incubation, the increase in absorbance was measured at 593 nm. FRAP activity was calculated using the correlation equation y = 0.0028x generated using various concentration of ascorbic acid (10-100 µg) and the activity was expressed as the amount equivalent to ascorbic acid.

RESULTS

Qualitative analysis of phytochemical constituents of *B. magnifica*

The phytochemical analysis revealed the presence of various secondary metabolites in the polar and nonpolar solvents of *B. magnifica* leaf extracts. According to the qualitative findings presented in the table 1, the Chloroform and Ethyl acetate extracts of *B. magnifica* showed abundant quantities of Carbohydrates, Tannins, Phenols, Flavonoids, and Terpenoids. The positive results from Benedict's test, Ferric Chloride test, Gelatin test, Lead Acetate test, and Salkowski's test confirmed the presence of these metabolites. Proteins, Amino acids, Alkaloids, and Saponins were found in moderate amounts, although Saponins were absent in the Ethyl acetate extract. These metabolites were identified through positive results in the Biuret test, Dragendorff's test, and Foam test. Glycosides and Cardiac glycosides were present in low amounts in the Chloroform extract but not in the Ethyl acetate extract; their presence was indicated by positive results in Liebermann's test and Keller-Kiliani's test, respectively. Tannins were found in high concentrations in the Methanol extract, while only a low amount was detected in the Ethanol extract. Carbohydrates, Flavonoids, and Terpenoids were moderately present in the Methanol extract, whereas only Terpenoids were found in moderate amounts in the Ethanol extract. Additionally, the Methanol extract showed low levels of Phenols. In the Ethanol extract, Carbohydrates, Tannins, Phenols, and Flavonoids were observed in low amounts, while Steroids were absent in all extracts. Overall, the study indicated that Chloroform and Ethyl acetate extracts contained a high variety of chemical constituents compared to the

Ethanol and Methanol extracts, which indicates significant pharmacological properties of this *B. magnifica* plant.

Quantitative analysis of phytochemical constituents of *B. magnifica*

Estimation of total Phenolic content of extracts

Quantitative estimation of the pharmacologically significant phytochemicals in varying amounts in *B. magnifica* leaf extracts as shown in table 2. In comparison to the chloroform extract (20.85 ± 0.01 mg/g), the ethyl acetate extract had a larger total phenolic content (49.50 ± 0.07 mg/g), suggesting that ethyl acetate is more effective in removing phenolic components from *B. magnifica* leaves. This implies that because of its higher phenolic content, ethyl acetate extract might have more antioxidant activity.

Estimation of total Flavonoid content of extracts

The total flavonoid concentration (4.00 ±0.001 mg/g QE) of the chloroform extract was higher than that of the ethyl acetate extract (2.75±0.001 mg/g QE), and this indicates that the chloroform extract is more effective in extracting flavonoids in the *B. magnifica* leaves. This means that the chloroform extract would be more effective on the antioxidant and bioactive properties of the plant.

Estimation of total Tannins content of extracts

In comparison to the chloroform extract (258.25 ± 0.018 mg/g TAE), the ethyl acetate extract had a larger total tannin concentration (506.00 ± 0.041 mg/g TAE), suggesting that ethyl acetate is more efficient in removing tannins from *B. magnifica* leaves. The ethyl acetate extract's antioxidant capacity may be improved by its increased tannin content.

Estimation of total Terpenoids content of extracts

It is clear that chloroform is more effective in extracting terpenoids from *B. magnifica* leaves because its total terpenoids content was greater (1.42 ± 0.002 mg-TPE/g) than that of the ethyl acetate extract (0.79 ± 0.010 mg-TPE/g). This shows that terpenoid molecules may be more bioactive compounds in the chloroform extract.

Estimation of total Alkaloids content of extracts

The chloroform extract had a larger total alkaloid concentration (16.50 ± 0.58%) than the ethyl acetate extract (11.50 ± 0.58%), suggesting that chloroform is a more efficient method of removing alkaloids from *B. magnifica* leaves. This shows that the chloroform extract could have more potent alkaloid-related bioactive properties.

Phytochemical profiling Using HR-LCMS analysis

HR-LCMS analysis of ethyl acetate fraction of *B. magnifica* showed major bioactive phytochemicals were detected at various retention times. The HR-LCMS chromatogram obtained (Figure 5) and it depicts multiple prominent peaks from fraction, which identifies many prominent bioactive compounds presents in the sample. The reported bioactive phytoconstituents are alkaloids, flavonoids, glycosides, terpenoids, lipids, steroids, and phenolic compounds. The identified bioactive compounds and their retention time (RT), mass-to-charge ratio (m/z), mass and molecular formula and chemical structure are shown in the table 3.

Ferric reducing antioxidant power activity (FRAP) assay

According to the FRAP experiment, free radical chain breaking occurs through the donation of a hydrogen atom. During this process, the Fe3+ -TPTZ complex is reduced to the blue-colored Fe2+ -TPTZ, which indicates that the ethyl acetate extract (2241.07 ± 0.01 mg/g AAE) has greater antioxidant activity compared to the chloroform extract (1062.50 ± 0.01 mg/g AAE), with Ascorbic acid used as the standard, as shown in Figure 6. This suggests that ethyl acetate is an excellent solvent for extracting chemicals with strong reducing power. The increased presence of phenols and tannins in the ethyl acetate extract is correlated with its higher activity, indicating enhanced antioxidant capability.

Table 1: Qualitative Phytochemical Screening of *B. magnifica*

Parameters	Tests	Chloroform	Methanol	Ethanol	Ethyl acetate
Carbohydrate	Benedict's test	+++	++	+	+++
Proteins and Amino acids	Biuret test	++	-	-	++
Tannins and phenol	$FeCl_3$ test	+++	+++	+	+++
	Gelatin test	+++	+	+	+++
Saponin	Foam test	++	-	-	-
Flavonoid	Lead acetate test	+++	++	+	+++
Terpenoids	Salkowski's test	+++	++	++	+++
Alkaloids	Dragondroff's test	++	-	-	++
Glycosides	Liebermann's test	+	-	-	-
Cardiac glycosides	Keller kiliani's test	+	-	-	-
Steroids	Liebermann-burchard's test	-	-	-	-

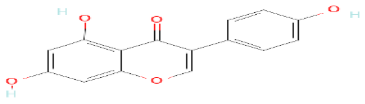
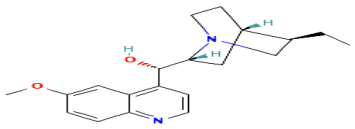
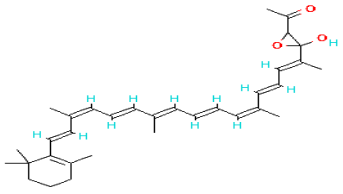
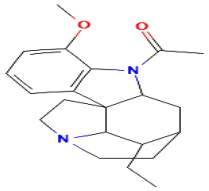
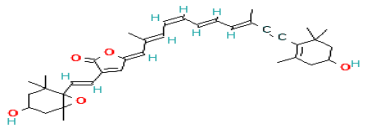
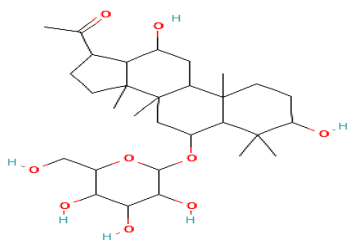
Note: Markings: +++ = Abundantly present; ++ = moderate; + = less; - = not present

Table 2: Quantitative analysis of total phytochemicals in *B. magnifica* leaf extracts

Phytochemicals	Chloroform extract	Ethyl acetate extract
Total Phenols	20.85 ± 0.01 mg/g	49.50 ± 0.07 mg/g
Total Flavonoids	4.00 ± 0.001 mg/g QE	2.75 ± 0.001 mg/g QE
Total Tannins	258.25 ± 0.018 TAE	506.00 ± 0.041 TAE
Total Terpenoids	1.42 ± 0.002 mg-TPE/g	0.79 ± 0.010 mg-TPE/g
Total Alkaloids	16.50 ± 0.58**%**	11.50 ± 0.58**%**

Values are mean ± SD (n=2)

Table 3: Bioactive compounds identified by HR-LCMS analysis from ethyl acetate fraction of *B. magnifica*

RT (min)	Compound name	Molecular formula	Mass	MS (m/z)	Structure
13.07	Genistein	$C_{15}H_{10}O_5$	270.05	329.067	
18.19	Hydroquinidine	$C_{20}H_{26}N_2O_2$	326.1967	325.1897	
18.71	7',8'-Dihydro-8' hydroxycitraniaxanthin	$C_{33}H_{44}O_3$	488.3231	547.3364	
19.28	14,19-Dihydroaspidospermatine	$C_{21}H_{28}N_2O_2$	340.2126	339.2057	
20.27	Pyrrhoxanthinol	$C_{37}H_{46}O_5$	570.3389	569.334	
21.54	Notoginsenoside R10	$C_{30}H_{50}O_9$	554.3434	553.3383	

RT: Retention time, MS (m/z): Mass spectrometry is an analytical technique that is used to measure the mass-to-charge ratio of ions

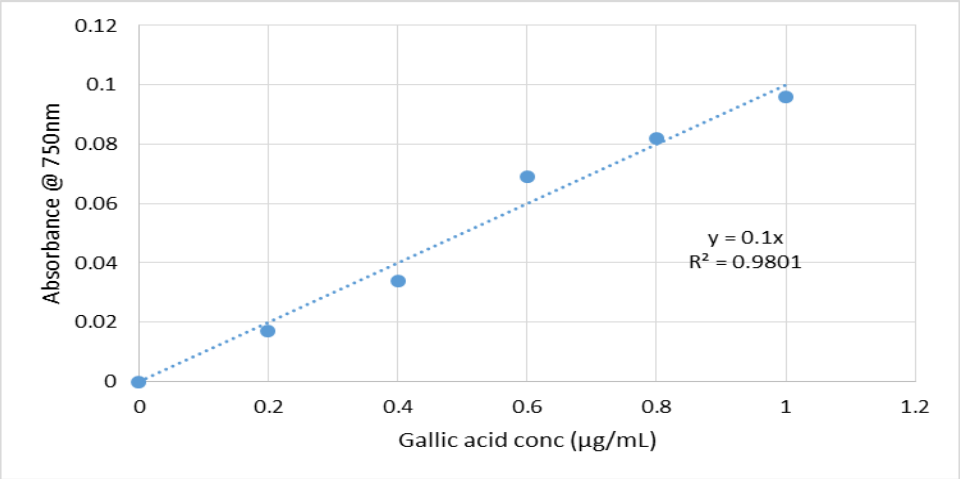


Figure 1: Standard graph for total phenols using different concentration of gallic acid

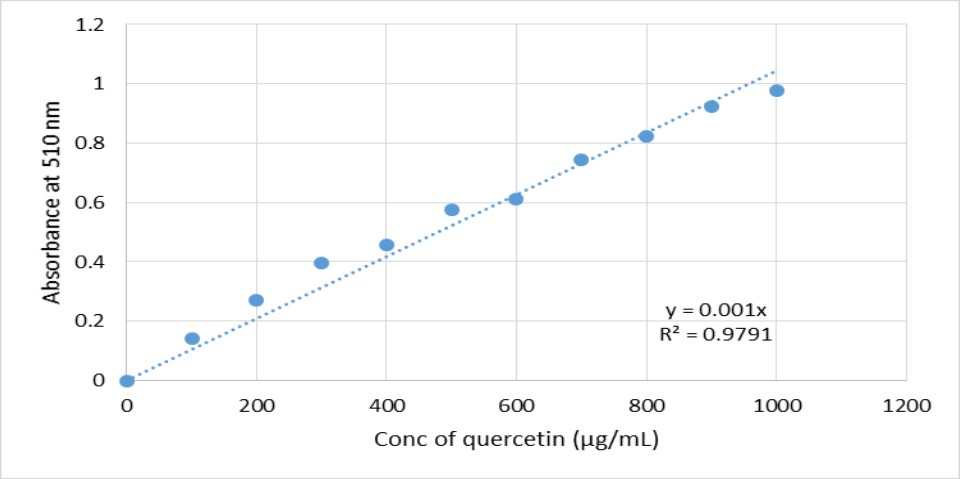


Figure 2: Flavonoid standard graph with different concentration of quercetin

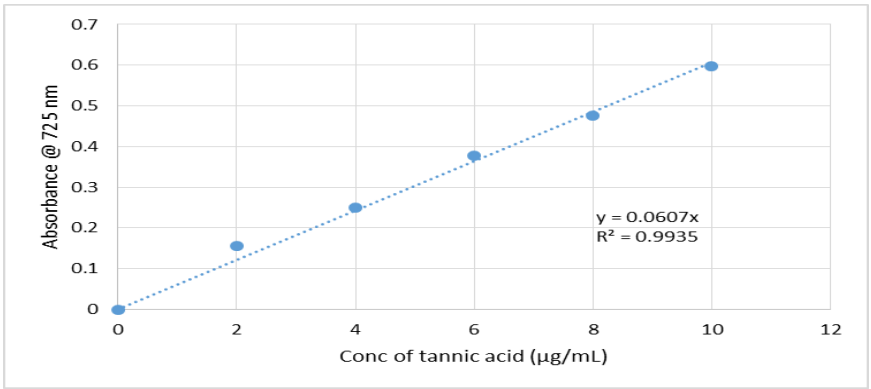


Figure 3: Tannin standard graph with different concentration of tannic acid

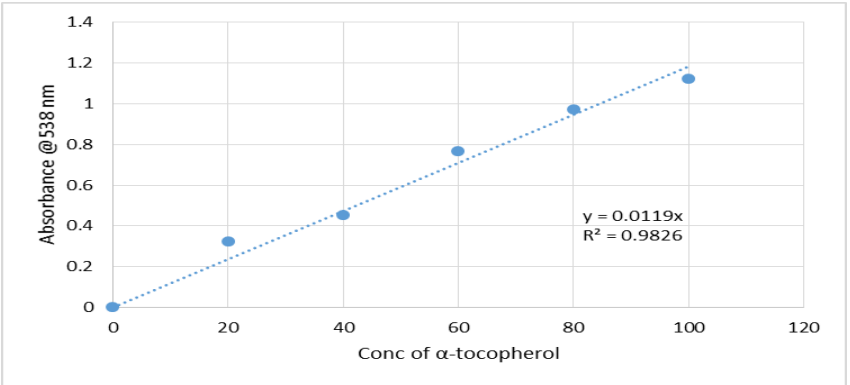


Figure 4: Standard graph for total terpenoids using different concentration of α-tocopherol

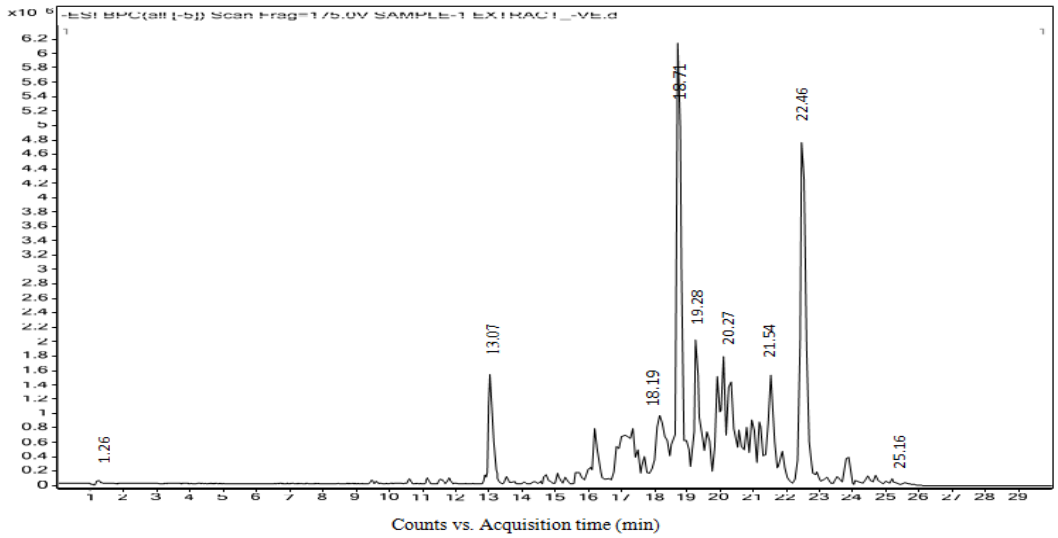


Figure 5: Chromatogram from HR-LCMS analysis of *B. magnifica* leaf ethyl acetate fraction

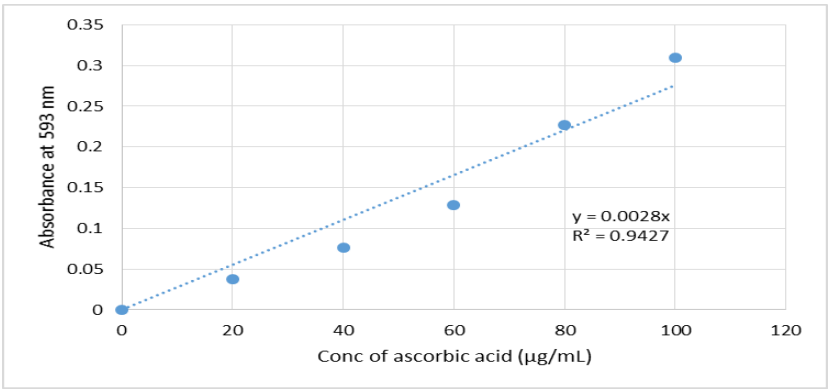


Figure 6: Standard graph for FRAP activity using different concentrations of ascorbic acid

DISCUSSION

The secondary metabolites in the leaves of the *B. magnifica* climbing species are widely distributed. Satyavathi [16] reported that extracts from the leaves and stems of *B. magnifica* contain aucubin compounds, iridoids, triterpenoids or steroids, flavonols, ellagic acid, proanthocyanidins, and polyphenolase activity. Additionally, free amino acids and various phenolic components, such as p-hydroxybenzoic acid, caffeic acid, p-coumaric acid, gentisic acid, and vanillic acid, are present. The ethanol and aqueous extracts of *Bignonia africana* showed the presence of alkaloids, cardiac glycosides, phenolic compounds, polyphenols, carbohydrates, proteins, amino acids, fatty acids, tannins, flavonoids, xanthoprotein, leuco-anthocyanin, coumarin, and terpenoids [17].

In this study, chloroform and ethyl acetate extracts of *B. magnifica* demonstrated a variety of potent phytochemicals, including carbohydrates, tannins, phenols, flavonoids, terpenoids, proteins, amino acids, alkaloids, saponins, glycosides, and cardiac glycosides were found to be more as compared to the ethanol and methanol extracts. Quantitative estimations of total phenols, flavonoids, tannins, terpenoids, and alkaloids were selected due to their high abundance and significant biological activities. These phytochemicals have been linked to various health benefits, including antimicrobial, antioxidant, anti-inflammatory, antidiuretic, anti-arthritic, anti-diabetic, anticancer, analgesic, antimalarial, antiasthmatic, antiviral, antiallergic, and anticarcinogenic properties, as well as effects on cholinomimetic activity, vasodilation, antiarrhythmia, antihyperglycemia, cardiovascular health, and neurodegenerative diseases [18].

Genistein is a plant-derived aglycone isoflavone compound that is most abundantly found in soybeans and other leguminous plants. As a potent phytoestrogen, genistein is utilized in cancer treatments for various types of cancer, including breast, prostate, lung, and gastrointestinal cancers. It exhibits chemopreventive properties by interfering with tyrosine kinases, cell-cycle regulation, and signaling pathways. Additionally, genistein has been investigated for its potential benefits in osteoporosis, metabolic disorders, and cardiovascular protection. It may help inhibit cancer progression by inducing apoptosis or inhibiting cell proliferation. Genistein also demonstrates valuable pharmacological activities such as antimicrobial, antioxidant, and anti-inflammatory effects [19]. Hydroquinidine, on the other hand, is a cinchona alkaloid extracted from the bark of Cinchona ledgeriana, a plant known for its medicinal properties. This compound possesses significant pharmacological effects, including antiarrhythmic, antiviral, and cytotoxic activities against human leukemia cell lines NALM-6 and MOLT-3. Furthermore, hydroquinidine has shown the ability to block potassium and sodium ion channels [20]. 7',8'-Dihydro-8'-hydroxycitraniaxanthin is a xanthophyll-type carotenoid known for its strong antioxidant and anticancer properties [21]. Another compound, 14,19-Dihydroaspidospermatine, is a bioactive strychnos-type alkaloid isolated from the Hedyotis aspera plant, which shows potential nephroprotective effects in the treatment of kidney disorders. Molecular docking studies have demonstrated its ability to inhibit TNF-α, with binding affinities of -6.9, -6.3, -6.3, -6.3, and -6.3 kcal/mol, respectively [22]. Pyrrhoxanthinol, a compound isolated from the muscle of Mytilus edulis found in marine ecosystems, is a carotenoid-derived bioactive compound. It contains an acetylenic unit derived from three carotenoids: diadinochrome A, diadinochrome B, and diatoxanthin, all of which were isolated from dinoflagellate species. This compound also shows significant potential for antitumor (anticancer) activities [23]. Lastly, Notoginsenoside R10 is a bioactive triterpenoid saponin found in the roots and rhizomes of the Panax notoginseng plant. In traditional Chinese medicine, this compound is

utilized for its cardiovascular benefits, neuroprotective effects, and anti-inflammatory properties [24].

The vital role of antioxidants is their ability to interact with reactive oxygen species and neutralize stable oxidative free radicals. In the chloroform and ethyl acetate extracts of *B. magnifica,* the presence of reductants such as antioxidant compounds facilitates the reduction of Fe^{3+} to Fe^{2+}. Consequently, a compound's capacity to donate electrons serves as a key measure of its antioxidant potential. These antioxidants act as electron donors, neutralizing oxidized intermediates in lipid peroxidation pathways; thus, they function as both primary and secondary antioxidants [25]. Anwuchaepe [26] reported that leaf extracts of *Crescentia cujete* (Bignoniaceae) obtained using n-hexane, butanol, ethyl acetate, and methanol demonstrated varying antioxidant activities, with the ethyl acetate extract showing the strongest activity according to the FRAP method. In the current study, the chloroform and ethyl acetate leaf extracts of *B. magnifica* demonstrated strong antioxidant activity as assessed by the FRAP assay. This activity is attributed to the presence of phenolic and flavonoid compounds, which effectively neutralize stable oxidative free radicals by donating hydrogen atoms [27].

CONCLUSION

Several bioactive phytochemicals, such as carbohydrates, proteins, tannins, saponins, flavonoids, alkaloids, and glycosides, were found in *B. magnifica* leaf extracts, according to the current research. The tracts of chloroform and ethyl acetate showed the greatest diversity and abundance. According to quantitative research, the chloroform extract was richer in flavonoids, terpenoids, and alkaloids, while the ethyl acetate extract had the greatest overall phenolic and tannin contents. According to the FRAP test, which measures antioxidants, ethyl acetate extract had a higher reducing capacity, which was consistent with its higher quantities of tannin and phenolic compounds. By employing HR-LCMS technique to reveal unique bioactive compounds in *B. magnifica* provide strong scientific support for many conventional medicinal assertions. These results bolster the potential therapeutic use of *B. magnifica* leaves in the treatment of illnesses linked to oxidative stress by indicating that they are a good source of bioactive chemicals and natural antioxidants.

Conflict of interest

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