

Antioxidant activity and phytochemical analysis of aqua-ethanol extract of *Bauhinia racemosa* Lam. leaves

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Abstract

The objectives of this study were to evaluate antioxidant activity, qualitative phytochemical analysis and LC-MS/MS analyses of aqua-ethanol extract of *Bauhinia racemosa* Lam. plant leaves. The extract afforded the rich content of different phytochemicals reported in qualitative phytochemical analysis, LC-MS/MS spectral analysis the leaves and antioxidant activity pharmacological activity. The extract was subjected to assess their antioxidant potential using DPPH in vitro system. Using DPPH (2, 2-Diphenyl-1-picrylhydrazyl) free radical scavenging assay, the aqua-ethanol extract was found to have significant antioxidant activity with an IC_{50} value $42.17 \pm 0.03 \mu\text{g/mL}$. A qualitative phytochemical analysis shows that the plant leaves contains carbohydrate, protein, amino acids, glycoside, tannins, saponin, flavonoids and phenolic compounds. It is concluded that the aqua-ethanol extract of *Bauhinia racemosa* Lam. leaves have been strong antioxidant potential. Further study is needed for isolate and characterizes the active antioxidants, which may serve as a potential source of natural bioactive antioxidants.

Keywords: *Bauhinia racemosa* Lam; Phytochemical; LC-MS/MS; Pharmacological; DPPH; Antioxidant

1. Introduction

Natural biodiversity and knowledge of rich ancient traditional systems of medicine built a strong base for the utilization of a large number of medicinal plants with bioactive contents in general healthcare and alleviation of common ailments of the humankind suffering from various disorders [1]. Antioxidant activity has been reported to prevent oxidative damage caused by free radicals and it used in curing various human diseases. The medicinal plants exhibited potent antioxidant activities due to the presence of phenolic compounds and flavonoids. This type of medicinal plants can act as scavengers in preventing oxidative damages caused by the free radicals [2]. The presence of bioactive phytochemical compound in the aqueous and methanolic extracts of *Bauhinia racemosa* Lam. plant showed good phenolic content and radical scavenging activity. Widely distributed phenolic compounds in medicinal plants have gained much attention, due to their free radical scavenging ability with potential antioxidant activity [3]. Extensive information on the bioactive phytoconstituents, ethno pharmacology, and scientifically verified medicinal uses of *Bauhinia racemosa* Lam is available in the reviewers' literature. It has been widely used as curative agents for variety of ailments. Several alkaloids, carbohydrates, proteins, tannins, oil and fats, phenolic compounds, flavonoids, saponins and carbohydrates have been reported to be present in different parts of *Bauhinia racemosa* Lam [4]. Hence plant shows various types of pharmacological activities such as analgesic, antipyretic, anti-inflammatory, antispasmodic and antimicrobial activity which may be due to the presence of the investigated active chemical constituents [5,6]. When antioxidants added in foods, it reduces rancidity, retard the formation rate of toxic oxidation products and increases life of patients. These antioxidants may help to relieve from oxidative stress. The antioxidant activity of *Bauhinia racemosa* Lam. medicinal plants extracts has been extensively studied by researchers and reported significantly. This plant might be helpful in preventing and slowing the process of diseases involved as result of oxidative stress [7]. Due the adverse effects of synthetic antioxidants such as toxicity and carcinogenicity [8,9] consumer preferences natural antioxidants

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are in highly demands for application as nutraceuticals, bio-pharmaceuticals and food additives. There is no more information pertaining to the antioxidant potential of *Bauhinia racemosa* Lam. leaves. Therefore, from this traditional knowledge of medicinal plant system, the present study was carried out to evaluate the antioxidant activity of leaves aqua-ethanol extracts of *Bauhinia racemosa* Lam. plant.

2. Material and methods

2.1. Collection of Plant material

Leaves of *Bauhinia racemosa* Lam. plant were collected from local area identified and authenticate with the help of our institute botanists. The collected leaves are cleaned with distilled water to remove dirt and air dried in shade.

2.2. Preparation of Extract

The *Bauhinia racemosa* Lam. dried leaves were rushed and powdered with the help of grinder. 30 g of powdered plant material was macerated in 150 mL of aqua-ethanol and kept on a magnetic stirrer for stirring and extracted using a soxhlet apparatus sequentially in aqua-ethanol solvent. The fraction of extract was collected and solvent was evaporated out to dryness. The extracted material was stored in airtight bottles for further investigation studies.

2.3. *In vitro* antioxidant activity

The *in vitro* antioxidant activity was evaluated by using stable free radical DPPH (2, 2-diphenyl-1-picrylhydrazyl) with the help of UV-spectrophotometer [10-12]. 0.1 mM DPPH stock solution was prepared in ethanol solvent. 1.0 mL of this stock solution was added to 1.0 mL of extract solution in water at different concentrations (5-50 $\mu\text{g}/\text{mL}$) and final volumes were made to 3 mL by adding distilled water. After 20 minutes, the absorbance of each concentrations of test solution was measured at 517 nm. Ascorbic acid was used as standard. The absorbance of the test solutions were decreases with increase in concentration of leaf extract, which confirms presence of free radical scavengers in extracts. Percentage of DPPH free radical scavenges by test solution were measured as

$$\% \text{ Free Radical Scavenged} = (A_{\text{Control}} - A_{\text{Test}} / A_{\text{Control}}) \times 100$$

IC₅₀ values were determined by using graphical method.

2.4. Phytochemical analysis

The leaves aqua-ethanol extract of plant was qualitatively evaluated for the bioactive phytochemical contents reporting such as alkaloids, carbohydrate, protein, amino acids, glycoside, tannins, saponin, flavonoids, steroids, terpenoids and phenolic compounds etc. by the help of standard protocol [13, 14].

2.5. LC-MS/MS analysis

LC-MS/MS analysis technique was used for identification of phytochemical ingredients separated by liquid chromatography. It provides separation of ingredients and detection by MS provides molecular weight of compounds. LC-MS/MS analysis of aqueous solvent extracted material was carried out on Waters UPLC-TQD Mass spectrometer. The ingredients were identified by comparison of mass spectra with the inbuilt Metlin, Lipid and Mass Bank databases.

2.6. Statistical analysis

The tests were carried out in triplicate and its results expressed in mean \pm SD. Values of $P < 0.05$ were considered as statistically significant.

3. Results and discussion

In the present study, aqua-ethanol extract of *Bauhinia racemosa* Lam. plant leaves were evaluated for their antioxidant and preliminary phytochemical analysis using DPPH assay method, standard protocols and LC-MS/MS spectral analysis technique.

3.1. Evaluation of antioxidant activity

In vitro antioxidant activity of *Bauhinia racemosa* Lam. leaves aqua-ethanol extract was tested by using DPPH assay method. The dose response curve of DPPH for ethanol extract of *Bauhinia racemosa* Lam. leaves was correlated with reference standard ascorbic acid (Figure 1).

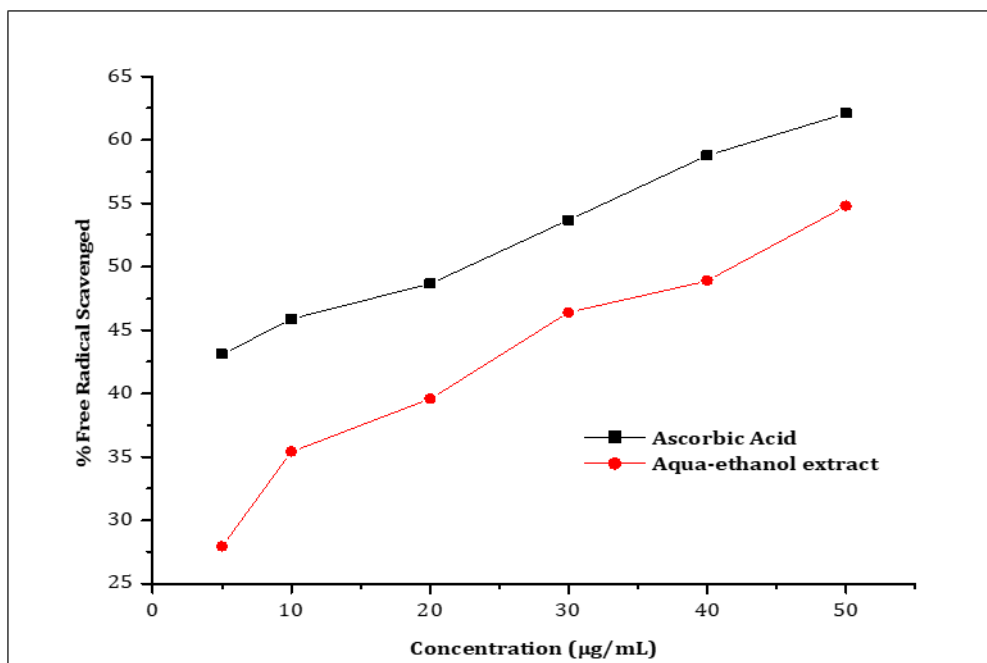


Figure 1 Antioxidant activity of *Bauhinia racemosa* Lam. Leaves aqua-ethanol extract

The antioxidant activity of the aqua-ethanol extract increased in a concentration dependent manner [15]. In DPPH radical scavenging assay, the IC_{50} value of the extract was $42.17 \pm 0.03 \mu\text{g/mL}$. The ascorbic acid was used as a standard reference compound; it shows IC_{50} value at $23.08 \pm 0.02 \mu\text{g/mL}$ in DPPH assay (Table.-1).

Table 1 % DPPH Free Radical Scavenging Activity of *Bauhinia racemosa* Lam. leaves extract

Sr. No.	Concentration (µg/mL)	% Scavenged Ascorbic Acid	% scavenged Extract
1	5	43.08 ± 0.02	27.92 ± 0.03
2	10	45.89 ± 0.01	35.41 ± 0.04
3	20	48.67 ± 0.03	39.58 ± 0.02
4	30	53.68 ± 0.02	46.37 ± 0.03
5	40	58.77 ± 0.03	48.91 ± 0.04
6	50	62.09 ± 0.01	54.78 ± 0.02
IC ₅₀ Value (µg/mL)		23.08 ± 0.02	42.17 ± 0.03

This indicates that aqua-ethanol extract of *Bauhinia racemosa* Lam. leaves has good potential as a source for natural antioxidants.

3.1 Phytochemical analysis

The extract was preliminary analyzed for the active phytochemicals using standard methods. The preliminary phytochemical tests are helpful in finding chemical constituents in the plant material that may lead to their quantitative estimation and also in locating the source of pharmacologically active chemical compounds. The analysis study reported

that leaves extract showed the presence of carbohydrates, protein, amino acids, glycosides, tannins, saponin, flavonoids and phenolic compounds [16,17] (Table-2).

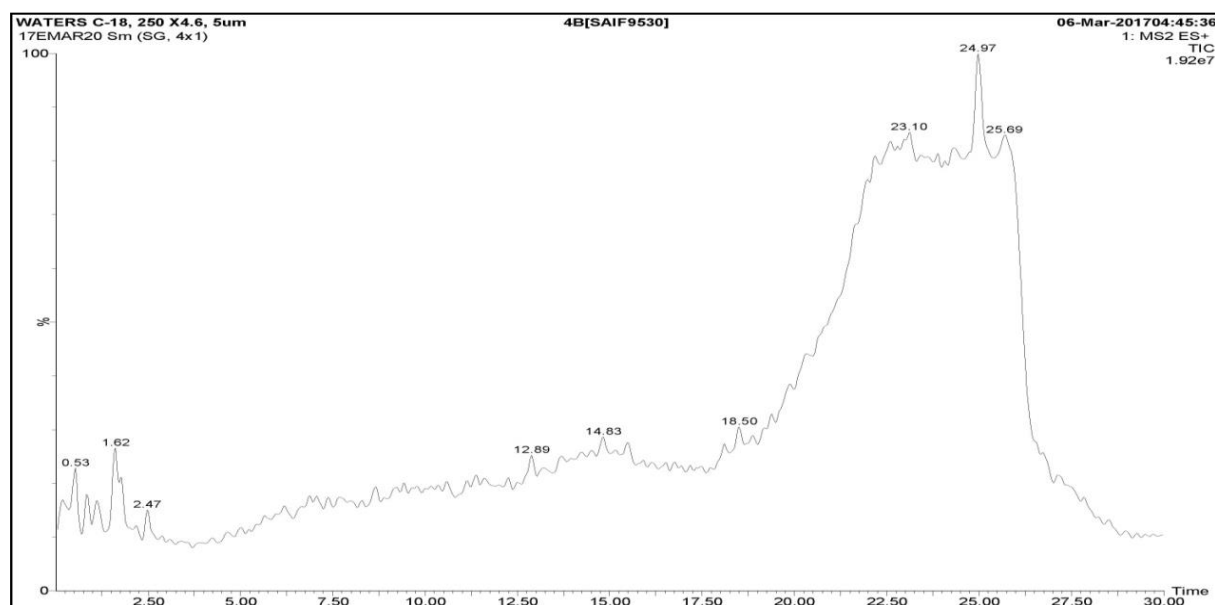
Table 2 Phytochemical analysis of *Bauhinia racemosa* Lam. leaves extract

Phytochemicals	Result
Alkaloid	-
Carbohydrate	+
Protein and amino acids	+
Glycoside	+
Tannin	+
Saponin	+
Flavonoids	+
Steroids	-
Triterpenoids	-
Phenolic compound	+

(+ for present and - for absent)

3.2 LC-MS/MS analysis

The LC-MS/MS analysis of *Bauhinia racemosa* Lam. leaves aqua-ethanol extract were reported the presence of different phytochemical constituents and its intensity peaks chromatogram (TIC and EIC) as shown in (Figure 2).



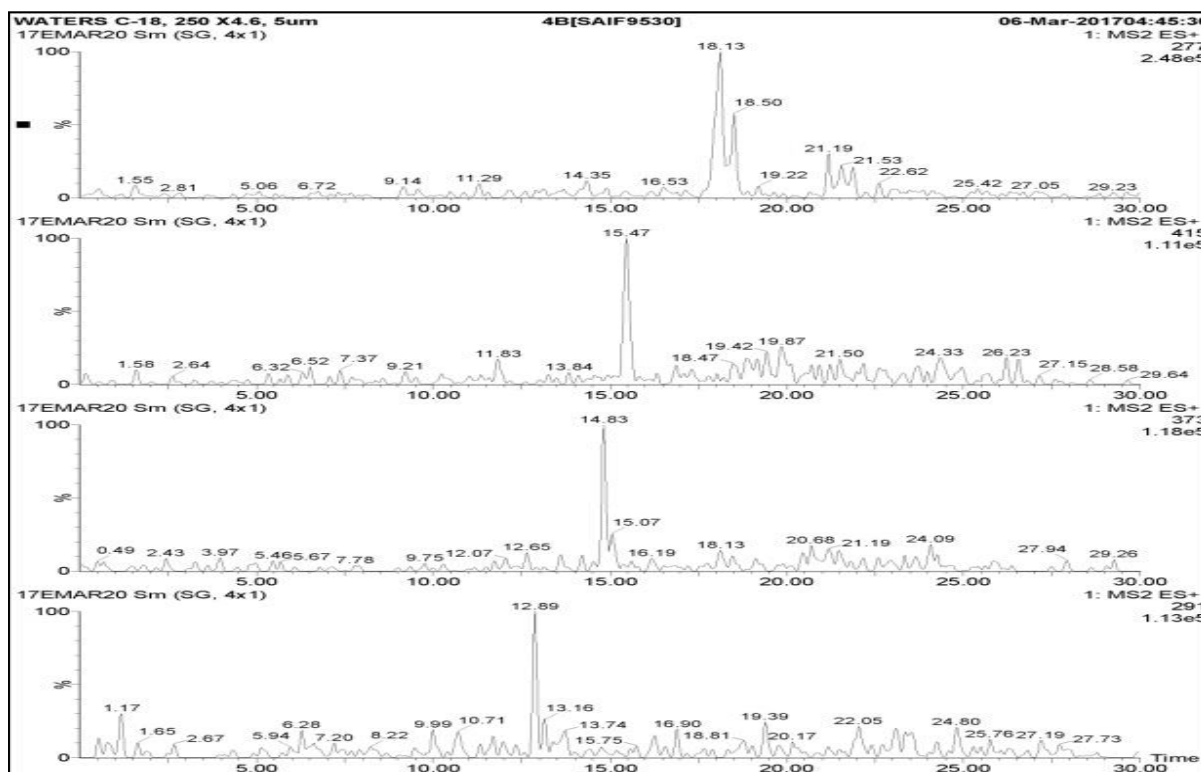


Figure 2 LC-MS chromatogram (TIC and EIC) of *Bauhinia racemosa* Lam. leaves aqua-ethanol extract.

Table 3 Phytochemical constituents detected in leaves aqua-ethanol extract

Peak	R. Time	Name	Base m/z
1	12.89	clavirin I / 9-oxo-12S-acetoxy-2,3,4,5-tetranor-7Z,10Z,14Z-prostatetrienaldehyde-cyclo[8,12]	291
2	14.83	Finasteride	373
3	15.50	Flecainide / N-(2-piperidinylmethyl)-2,5-bis(2,2,2-trifluoroethoxy)benzamide	415
4	18.50	4-([2,2'-bithiophen]-5-yl)but-3-yn-1-yl acetate	277

The chemical constituents tentatively detected in the aqua-ethanol extract of *Bauhinia racemosa* Lam. leaves contribute to antioxidant activity as shown in Table 3 above. Phytochemical analysis and LC-MS/MS analysis of *Bauhinia racemosa* Lam. leaves extract showed the presence of different chemical constituents. That has an important source of medicinally important constituents and reported various pharmacological and therapeutic characteristics like antibacterial activity, astringent property, antiulcer activity, analgesic activity, antitumor activity, antihistaminic effect & antioxidant effect, antihelmintic property & antifilarial property, anti-inflammatory analgesic & antipyretic effect; anti HIV-1 agent, hematology and hepatorenal protective function [18,19]. Phenolic compounds are responsible for antioxidant activity, because they are effective hydrogen donors, which make them antioxidant [20, 21]. *Bauhinia racemosa* Lam. methanol extract has significant antioxidant activity. These activity is due to the high polyphenol content and the interesting major bioactive compounds includes are methyl gallate, gallic acid, kaempferol, quercetin, quercetin 3-O- α - rhamnoside, kaempferol 3-O- β -glucoside, myricetin 3-O- β -glucoside and quercetin 3-O-rutinoside [22]. DPPH free radical scavenging activity was carried out with the leaves aqua-ethanol extracts of *Bauhinia racemosa* Lam. The influence of antioxidants on scavenging of DPPH free radical was supposed to be owed to their hydrogen donating capacity. In the presence of oxygen radicals formed are extremely reactive species vary in their lifespan and organic properties. The free radical scavenging ability may be one of the mechanisms by which herbal medicinal plants exhibit greater antioxidant activity. The phenolic compounds from *Bauhinia racemosa* Lam. leaves possessed the highest

superoxide activity [23, 24]. The observed antioxidant activity of this plant leaves extract could be as a result of the presence of these bioactive phytochemical constituents present.

4 Conclusion

The present studies were indicated that aqua-ethanol extract of *Bauhinia racemosa* Lam. leaves scavenges free radicals significantly. The overall antioxidant activity depends on phytochemical constituents present in the leaves. That could be a source of natural antioxidant and have greater importance as therapeutic agent in preventing or slowing oxidative stress related degenerative diseases. Therefore, it was concluded that aqua-ethanol extract of *Bauhinia racemosa* Lam. leaves showed potent *in vitro* antioxidant activity.

Compliance with ethical standards

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Disclosure of Conflict of interest

Author declares that there is no conflict of interest.

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