Total Phenol, Flavonoid content and Antioxidant Activity of aqueous extract of Kachnar (*Bauhinia variegate) leaves*

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ABSTRACT

Bauhinia variegata plant is known as Kachnar in Hindi. Parts of the plant such as leave, stem, flower, roots, bark are used in several native medicinal systems. The parts of plant are rich in phenolic compounds that are secondary plant metabolites and also commonly present in other plants of medicinal value. Phenolic compounds contribute to the antioxidant potential of plant by neutralizing free radicals and further not allowing the decomposition of hydroperoxides into free radicals. In the present study, the total phenolic content (TPC) of the aqueous extract of Kachnar leaf was examined using Folin- ciocalteu's (FC) method, Flavonoid content using aluminium chloride assay, and antioxidant activity (AOA) using DPPH assay for free radical scavenging activity. TPC, flavonoid and DPPH radical was found to be 8.10-9.85mg/g gallic acid equivalent, 0.9365 µg/g of quercetin equivalents and IC50 value was estimated1224.98µg/ml respectively in the 10mg/ml extract of the sample. The result of the current study shows that Kachnar leaves may offer several health benefits by virtue of presence of high phenolics and antioxidant activity.

1. Introduction

With the changing global scenario, there is an increased awareness on nutrition and wellness. Recent transitions in human behavior show that people are looking towards natural food as an alternative of traditional medicines. Lot of evidence has emerged on the therapeutic value of plants and their various parts (D. Hockings, 1993).

Bauhinia variegata plant is known as Kachnar in Hindi, Raktakanchan in Sanskrit, and Mountain Ebony in English. *Bauhinia variegata* is a tropical tree belonging to the family *Leguminosae* (Casesalpiniodeae). The tree grows to an average height of 10 m to12m. It grows in tropical region (K. Sinha & A.K Verma, 2012). In India, it grows widely in Punjab and Delhi (Anonymous, 1995).

Several parts of the plant like leave, stem, flower, roots and barks are used in native medicinal system. There has been mention of the therapeutic value of kachnar in Ayurveda. The plant is a good source of antioxidants and anti-inflammatory compounds (CV. Filho, 2009; A. Acharya & N Kafle, 2009). These compounds not only maintain the health but also help to protect the body from damage by free radicals induced by oxidative stress (Anonymous, 2001 & Arvind et al; 2012).The parts of tree are used for making indigenous medicines to treat ailments like dyspepsia, leprosy, obesity, ulcer. It has been found that Kachnar leaves contain several components like lignins, saponins, tannins, kaempferol-3- glucoside, rutin, apigenin, quercetin, proteins, sugars, vitamin C, calcium and phosphorus (M. Ayyanar & S. Iganacimuthu, 2005; R.Y Koteswara et al., 2008).

Antioxidants derived from plant sources are beneficial to fight against oxidative stress responsible for causing several diseases and disorder like liver disease, neurological disease, cancer, alzheimer and ageing (A. Mishra et al., 2013). Antioxidants inhibit the chain reaction by acting as H+donors and free radical acceptor (C. Bharti et al., 2022). The polyphenolic compounds are considered as wonderful oxygen scavenger due to lower electron reduction potential of phenolic radical as compared to oxygen radical. The Folin-Ciocalteu method for the measurement of antioxidant activity depends on electron transfer from phenolic compounds to phosphomolybdic acid complex under alkaline conditions that further forms a blue colored complex. The solvents and extraction procedures are responsible for the dissolution of these endogenous compounds. They also improve the nutritional quality of foods because of their capability to retard oxidative degradation of lipids. In addition, they also exhibit metal chelating potential (K. Sowndhararajan & S.C Kang, 2013; S. Aryal et al., 2019).

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Received: 27 September, 2024
Available online: 25 October, 2024

The polyphenolic compounds present in plant have strong antioxidant mechanism which protect the cell parts from oxidative injury, thus defending the macromolecules like proteins, nuclei materials and lipids present in the cells (K.N Bopanna et al.,1997 & C.P Khare, 2007). Plants having high phenolic compounds show higher antioxidant activity (G. Balamurugan & P. Muralidharan, 2007). The redox potential of phenolic compounds makes them function as hydrogen donor, reducing agent and singlet oxygen scavengers accounting for the high antioxidant potential of these medicinal plants. The phenolic content of the plant is an important constituent as its hydroxyl group has considerable scavenging ability (N. Sharma et al., 2010). Flavonoids are secondary metabolite present in the plant and are significant as they act as antioxidant due to their high ability to scavenge free radicals. Flavonoids prevent hydroxyl radical induced damage by donating electron (A. K. Gupta et al., 1980).It has been observed that the flavonoid content is significantly affected due to genetic diversity, biological, environmental and yearly variations. Flavonoids are characterized by benzopyrone structure, which is widely distributed in plant phenolic compounds. The total flavonoid content is estimated colorimetrically after extraction by solvent. In the current study the flavonoid concentration was determined with aluminium- chloride assay. This method relies on the development of chelates of aluminium-flavonoid. Flavonoids contain several oxo and hydroxyl group which bind to aluminium ions. Total flavonoid content is estimated using the standard curve. Quercetin, rutin, and catechin are mostly used as standard compounds for flavonoid estimation (E.A. Ainsworth & K.M Gillespie, 2007).

Antioxidant activity is measured using the quenching ability of stable purple colored 2,2- diphenyl-1-picylhydrazyl (DPPH). It is a dark crystalline powder with stable free radicals. DPPH accepts electrons from free radicals getting reduced to form a yellow colored complex. The DPPH assay is simple, widely acceptable methodology to determine the radical scavenging potency of plant extracts. It is sensitive, replicable, requires small amount of sample and allows testing of both hydrophobic and lipophilic substances. Individual parts of *B. variegata* like leaf, stem, bark, bud, flower and root show significant in-vitro and in-vivo activity against dysentery, diahorrea, hemorrhoids, oedema and skin diseases. The alcoholic extract of leaves, stem and flowers posses antibacterial and antimicrobial properties. The leaves of the tree are shallowly cordate and subcoriaceous. They are studied as a potential drug for research in treatment of disease including free radical induced disorders like cancer, diabetes atherosclerosis etc. (K Adawia, 2007; Joaquim M Duarte-Almeida, et al., 2004). Leaves also contain crude protein, phosphorus and volatile oils. Leave contain flavonoids such as quercetin, rutin, kaemferol. It has also been identified that blood glucose level is significantly affected by flavonoid content. Studies reveal the reduction of elevated plasma glucose level by the aqueous extract of leaves (C.R. Azevedo et al., 2006).

In the current study aqueous extract of the leaves of *B. variegata* was analysed for total phenolic activity, total flavonoid content and DPPH.

2. Material and methods

Bauhinia variegata leaves were collected from trees in Vasundhara Enclave, Delhi, India. DPPH was obtained form HiMedia Labs. Pvt. Ltd. Mumbai. Gallic acid, aluminum chloride ,quercetin dihydrate, sodium hydroxide, sodium carbonate, sodium nitrite were procured from SRL, Mumbai, India. Folin's reagent was obtained from Molychem, Mumbai, India.

2.1 Preparation of leave extract

The leaves of the plant were washed with distilled water. 20 gm of leaf tissue was weighed and crushed in mortar and pestle with deionized water. The crushed leaves were boiled in 80ml deionized water for 20 mins. The extract was filtered using Whatman No.4 after 1 hour, centrifuged at 2000g and stored in refrigerator till further usage.

2.2 Total Phenolic content

The extract was tested for total phenolic content (TPC) using Folin-Ciocalteu method. Supernatant (10mg/ml) was used for estimation. 1 ml of the sample and standard Gallic acid (0, 20, 40, 60, 80, 100, 120 µg/ml) were introduced in the test tubes and the volume was made up to 5 ml using distilled water. 0.5ml of Folin-Ciocalteu reagent was added and the test tubes were shaken.

1.5ml of 20% sodium carbonate was added in the test tubes after 5 minutes and final volume was made up to 10 ml with distilled water. The tubes were kept for incubation at room temperature for 2 hrs such that the development of intense blue colour was observed. Absorbance was measured at 750nm. All the readings were performed in duplicates. The calibration curve was plotted using standard Gallic acid and total phenolic content was calculated from the standard curve expressed as mg of Gallic acid equivalent (GAE).

2.3 Total Flavonoid content

Briefly, the aqueous extract (1ml) was mixed with distilled water (4ml). 300µl of 10% AICI3 was added after 5 minutes followed by 2 ml of 1M NaOH after 1 min and distilled water was added to make up the volume to 10ml. Absorbance was measured after1 hour at 510nm. Flavonoid content was calculated from standard quercetin curve and experessed as milligrams of quercetin equivalents (QE) per gram extract.

2.4 Determination of Free Radical Scavenging Activity

Free radical scavenging activity was measured by taking 2ml aliquots of ethanol with different concentration of

sample in test tubes. 4ml DPPH solution was added to each tube. The solutions were vortexed and incubated for 30 min. 2ml ethanol was taken as control. The absorbance was measured at 517 nm and the activity was calculated using the following formula :

$$\%\text{scavenging activity} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100$$

3. Results and Discussion

Polyphenols or phenolics are secondary plant metabolites with very high antioxidant potential. They neutralize free radicals and do not allow decomposition of hydro peroxides into free radicals. Hence, it is very essential to quantify phenolic compounds and to assess their antioxidant activity. The total phenolics from plant leaf extracts were examined using Folin-Ciocaltea reagent. The standard curve was plotted, regression equation (y = 0.008x + 0.101, R^2= 0.989) was obtained and the total phenolic content was calculated. The total phenols were depicted as µg/g gallic acid equivalent. In the current study, the total phenolic content was found to be 8.975 ±0.87 mg/g gallic acid equivalent as shown in Fig.1. A study has reported phenolic content as 6.386±0.058 mg/g gallic acid equivalent (A. Thakur et al., 2020). Our results are closer to the literature cited and often the difference may be attributed to various interfering factors. However, a study has also depicted total phenols as 45µg/mg in leaf extract (Y. Kamal et al., 2022).

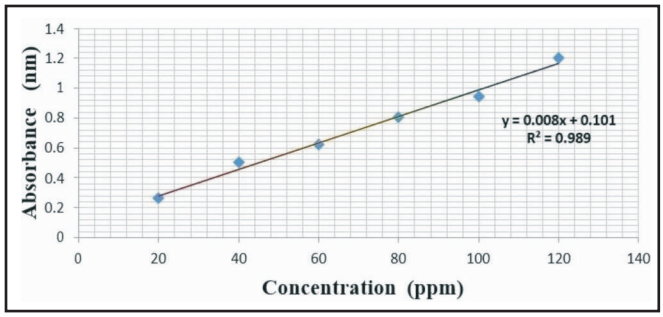

Figure1 : *Standard curve of gallic acid concentration and absorbance*

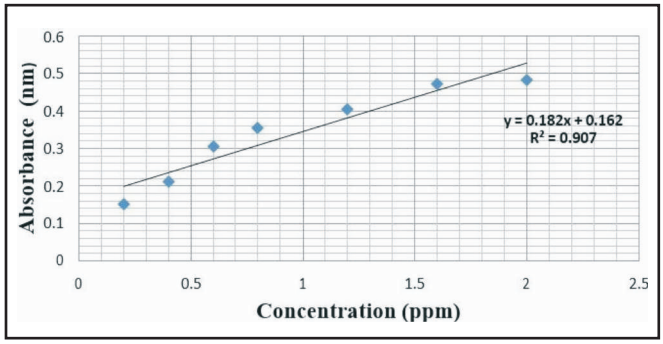

Figure 2 : *Standard curve of quercetin concentration and absorbance*

Flavonoids are present in fruits and vegetables varying in concentration and are responsible for the radical scavenging activity. In the current work, the Kachnar leaf extract was evaluated for the total flavonoid content by using regression equation determined spectrophotometrically. The value was calculated using the equation obtained from the standard curve of quercetin [Y=0.182x+0.162], the regression coefficient (R2) was 0.97, and the flavonoid content in the extract came as 0.9365 µg/mg as shown in Fig.2. Total flavonoid content of 1.64µg/g was reported in a study using aqueous extraction of *Bauhinia* leaf (M. Patil et al., 2017).

The free radical scavenging activities of *Bauhinia varigata* leaf extract was examined by DPPH method. The ability of extract to neutralize the DPPH free radical by donating hydrogen indicates scavenging activity of phytochemicals present in the plant. DPPH is a sensitive test requiring small sample amount and allows assessment of both lipophilic and lipophobic moieties. The IC50 value was calculated using regression equation [Y=0.55x-17.374, R2=0.9989] obtained by plotting curve between % inhibition and concentration. In the current study, an IC50 value of1224.98 µg/ mL was obtained as shown in Fig.3. A study has identified that DPPH assay is a good indicator of the antioxidant activity of different foods. However, studies find that the methanolic extract gives higher percentage of inhibition in comparison to aqueous extract (E.M, Hussen & S.A, Endalew, 2023).

4. Conclusion

Our study investigates the antioxidant potential of Bauhinia varigata leave extract. The plant exhibits noticeable phenolic, flavonoid content and antioxidant activity. The knowledge of phytochemical profile can be used in the preparation of food and nutraceuticals industries from Kachnar leaves. Since Kachnar leaves cannot be consumed raw but they have high antioxidant activity and hence can be incorporated in food preparation. Formulation and recipe can be devised incorporating Kachnar leave like in the soup powders/ premixes, bakery pre-mixes, ready-to-eat preparations like upma, vermicelli, poha. The leaves can be dehydrated and the powder can be innovatively used at home-scale as

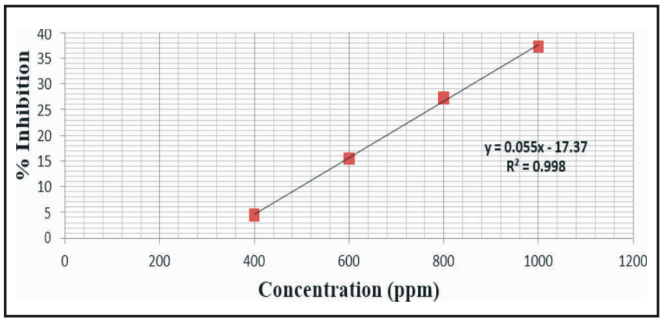

Figure3 : *Graphical representation showing positive correlation of concentration and % inhibition*

well. Additionally similar studies can be conducted on other parts of *Bauhinia varigata* (Kachnar) tree such as flower, seeds, bark, stem etc.

5. References

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