



QUALITATIVE PHYTOCHEMICAL SCREENING, EVALUATION OF ANTIOXIDANT AND ANTI-INFLAMMATORY POTENTIALS OF *VITEX PINNATA* L. LEAF EXTRACT

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Abstract

The purpose of the study was to screen phytochemicals and assess the antioxidant as well as anti-inflammatory properties of *Vitex pinnata* leaf extracts. Alkaloids, glycosides, tannins, terpenoids, saponins, phlobatannins, steroids, quinine, phenol, coumarins, and flavonoids were found during the phytochemical investigation. *V. pinnata* leaf extract exhibited an IC₅₀ value of 154.33 µg/ml in the DPPH free radical scavenging assay (standard: IC₅₀ = 25.48 µg/ml) IC₅₀ value of 218.8 µg/ml in the albumin denaturation inhibition assay (standard: IC₅₀ = 33.74 µg/ml). According to the findings, the leaves of *V. pinnata* contain a variety of pharmaceutically important secondary metabolites capable of scavenging DPPH and inhibiting albumin denaturation, demonstrating their antioxidant and anti-inflammatory properties.

Key words: Albumin denaturation, anti-inflammatory, antioxidant, phytochemicals, *Vitex pinnata*

Introduction

Phytochemicals are some bioactive compounds produced by plants, commonly known as "secondary metabolites." Secondary metabolites are found in almost every parts of the plant, such as barks, leaves, stems, roots, flowers, fruits, and seeds (Bansode and Salalkar 2015). It is well known that plants produce these chemicals to save themselves from adverse environmental situations as well as protect themselves against pests and diseases (Ajuru et al. 2017).

A substantial quantity of antioxidants is produced by plants such as polyphenols, flavonoids, anthocyanins, and phenolics in the form of secondary metabolites. These antioxidants can avert oxidative stress, a process that can create cell damage. Considering the growing interest in evaluating the antioxidant potential of natural products, it is necessary to study the phytochemistry of plants with antioxidant activity (Sati et al. 2010).

A chemical that can reduce inflammation has an anti-inflammation property. Most of the palliatives are synthetic anti-inflammatory drugs, which can reduce pain by shrinking the inflammation. These anti-inflammatory drugs have been found comparatively effective and safer than that of sedatives. There are various plant species that have antioxidant as well as anti-inflammatory properties that can be used in medicinal fields. Medicines derived from plant sources are generally considered to be risk-free for health, but using untested plant products as medicines can often be harmful (Farzaei et al. 2020).

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Vitex pinnata L. which belongs to the family Lamiaceae (formerly under the family Verbenaceae), is indigenous to south and south-east Asia, particularly forests in tropical regions. In Bangladesh, it has been reported from Chattogram and the Chattogram Hill Tracts. It is a slow-growing, small to moderate-sized evergreen tree with quadrangular branches and a crown that often spreads. In tropic conditions, flowering and fruiting are almost constant from January to December (Thenmozhi and Subasini 2016, Prasetyaningtyas et al. 2021).

In Indian traditional medicine, *V. pinnata* is used to oust intestinal worms, as an antipyretic, analgesic, anti-inflammatory, antioxidant, wound healer, antibacterial, and to treat stomach aches (Thenmozhi and Subasini 2016). Sometimes the young leaf shoots are eaten raw to treat fever and hypertension. A bark decoction is used to treat stomach aches. A root tea is taken for body aches, backaches, and fatigue (Thenmozhi and Subasini 2016). A poultice of the leaves is used to cure fever and wounds, and Barks are occasionally used for treating wounds and to reduce spasms (Thenmozhi and Subasini 2016). No report was found on the phytochemical analysis of *Vitex pinata* obtained from Bangladesh. Moreover, it is believed that this plant is becoming rare in Bangladesh (Mia 2009). Considering all these factors, this research has been carried out.

Materials and Methods

Collection and preparation of plant materials

Plant materials were collected from the Chittagong University campus, examined carefully, and identified. A herbarium specimen (CUMED 20191) has been deposited for future reference. The leaf samples of *V. pinnata* were used for the qualitative estimation of secondary metabolites. The disease-free and fresh leaves were selected and thoroughly washed with tap water three times and finally with distilled water, air dried, and later dried in an oven at 60°C for 72 hours. It was then ground into coarse powder using a grinding machine and stored in an airtight bag at 4°C. Five hundred grams of leaf powder were macerated in methanol with constant shaking using a shaker. Then the macerates were filtered through filter paper and concentrated by a vacuum evaporator. For future utilization, the crude extract was kept at 4°C. Alkaloid tests were performed with fresh leaf samples.

Qualitative tests of phytochemicals

Following standard procedures (Soloway and Wilen 1952, Kapoor et al. 1969; Harborne 1973, Trease and Evans 1989, Sofowora 1993, Edeoga et al. 2005, Kolawale et al. 2006, Vijayameena et al. 2013, Auwal et al. 2014, Akter et al. 2018), qualitative tests were performed on the fresh leaf sample and crude extract of *V. pinnata* leaf. The +, ++, +++, and ++++ marks were used to indicate the presence of low, medium, significant, and heavy amount of phytochemicals respectively. The "-" symbol denotes the absence of phytochemicals. Qualitative tests for the following secondary metabolites were done:

Alkaloid test

Rapid spot testing (Webb 1949) was used with a slight modification (Aplin and Cannon 1971) to carry out the alkaloid detection test. In the qualitative test of alkaloids, alkaloid-detecting reagents were used. Five grams of fresh leaf were chopped and pasted with a mortar and pestle to moisten with 10 ml of 2% HCl. Then the mixture was heated in a water bath at 60°C for one hour, and then the extract was filtered through filter paper. To detect alkaloids, we mixed 2 ml of each extract with 1 ml of 1% HCl in five test tubes, then added 6 drops of Meyer's, Wagner's, Dragendroff's, Hager's, and Tannic Acid reagents to each test tube, and observed yellowish, brownish red, orange, yellow, and reddish precipitation, respectively. The reagents were prepared by following standard methods (Cromwell 1955). If the plant extract reacts with different chemical reagents and produces the described colors, it is considered the presence of alkaloids.

Flavonoids: A portion of the powdered plant sample was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered, and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow coloration was observed, indicating a positive test for flavonoids.

Saponins: About 2 g of crude powder was boiled with 20 ml of distilled water in a water bath and filtered. Ten ml of filtrate were mixed with 5 ml of distilled water and shaken vigorously for a stable, persistent froth. The persistent froth indicates the presence of saponins.

Tannins: If dark green or deep blue color develops in a mixture of 5 ml of extract and 1 ml of 5% ferric chloride, it indicates the presence of tannin in the extract.

Phenols: The presence of phenols was revealed by the emergence of a blue or green color after a few drops of 10% aqueous ferric chloride were added to the plant extract.

Terpenoids: The 0.5 g of crude extract was diluted in 5 ml of methanol and combined with 2 mL of chloroform. Seven ml of concentrated H₂SO₄ added to the mixture result in the formation of a reddish-brown interface, which denotes the presence of terpenoids.

Steroids: Five ml of methanol were added to 0.5 g of crude extract. One ml of this solution was taken into a test tube. Ten ml of chloroform was added to it, and then an equal amount of concentrated H₂SO₄ was added from both sides of the test tube. The presence of steroids was determined by the yellow-green fluorescence of the H₂SO₄ layer and the red-colored upper layer.

Glycosides: In 5 ml of methanol, the crude extract (0.5 g) was dissolved. Two ml of methanolic extract were combined in a test tube with ten ml of 50% HCl. It was then heated for 30 minutes in a water bath. Five ml of Fehling's reagent were added to the mixture, and it was then boiled for five minutes. The presence of glycosides was assumed based on the brick-red color of the precipitation.

Cardiac glycosides: Five ml of plant extract were added along with 2 ml of glacial acetic acid, one drop of ferric chloride solution, and 1 ml of concentrated H₂SO₄. A brown or green ring indicates the presence of cardiac glycosides.

Quinine: One ml of plant extract was mixed with 1 ml of H₂SO₄, and the mixture was left to stand for a while to acquire color. The appearance of a red color indicates the presence of quinine.

Coumarin: One ml of extract was added to one ml of 10% NaOH, and the mixture was let to stand for a while. The emergence of a yellow color indicates the presence of coumarin.

Phlobatannin: The presence of phlobatannins was detected when two ml of the aqueous solution of the crude sample were added to dilute HCl.

Methods for antioxidant activity test

The antioxidant activities of a methanolic crude extract of *V. pinnata* leaf and the standard antioxidant ascorbic acid were assessed by comparing the free radical scavenging effect of the 2, 2-diphenyl-1-picrylhydrazyl (DPPH, MWt. 394.32) (Brand-Williams et al. 1995). Crude extracts of *V. pinnata* leaf were used to prepare a range of concentrations (50, 100, 150, 200, and 250 µg/ml) in methanol. Ascorbic acid in different concentrations (50, 100, 150, 200, and 250 µg/ml) was prepared in methanol and a 0.004% DPPH solution was prepared by dissolving the DPPH in methanol.

Three ml of the DPPH solution were mixed with 3 ml of the extract solution and the standard solution separately. These solution mixtures were kept in the dark for 30 minutes. The degree of DPPH purple decolorization to DPPH yellow indicated the scavenging efficiency of the extracts. The absorbance of the

DPPH solution was measured at 517 nm using a UV-visible spectrophotometer. Ascorbic acid served as a positive control. The lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The scavenging activity against the DPPH was calculated using the following equation:

$$\text{Scavenging activity (\%)} = \frac{A-B}{A} \times 100$$

Where,

A = Absorbance of control (DPPH solution without the sample).

B = Absorbance of the DPPH solution in the presence of the sample (extract / ascorbic acid).

Standard and test solution preparation

A stock solution comprising 10 mg/ml of ascorbic acid and plant extract was made up. The estimation of antioxidant activity was carried out in triplicate. For methanolic crude extract, 15 test tubes were taken. Besides, 15 test tubes were taken for standard and 3 for control. Each test tube was labeled as 50, 100, 150, 200, and 250 µg/ml. Three ml of methanol were added to each test tube. Then 50, 100, 150, 200, and 250 µg/ml of the concentrated stock solution were added using a micropipette, and finally three ml of the 0.004% DPPH solution were added to the test tubes.

Methods for anti-inflammatory activity test

The inhibition of albumin denaturation technique was used to investigate the anti-inflammatory properties of *V. pinnata* (Mizushima and Kobayashi 1968, Sakat et al. 2010). The reaction mixture consisted of test extracts (50 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml and 250 µg/ml) and a 5% aqueous solution of egg albumin. The pH (5.6 ± 0.2) of all the reaction mixtures was adjusted by 1N HCl. The sample extracts underwent a 20 minute incubation period at 37°C and a 20 minute heating period at 60°C. A spectrophotometer was used to measure the turbidity at 660 nm after the samples had been cooled. Three replications of the experiment were run (Shinde et al. 1999). The following equation was used to calculate the anti-inflammatory activity:

$$\% \text{ inhibition} = \left(\frac{A-B}{A} \right) \times 100$$

Where,

A = Absorbance of 5% egg albumin solution and methanol (Control)

B = Absorbance of 5% egg albumin solution and plant extract (test groups) or,

B = Absorbance of 5% egg albumin solution and acetyl salicylic acid (standard solution).

Results

Preliminary qualitative phytochemical tests were done using the leaf extract of *V. pinnata*. The intensity of presence of alkaloid contents and eleven other phytochemicals in the extract of the test sample was expressed by plus (+) signs. One plus (+) indicated the lowest, two pluses (++) indicated moderate, three pluses (+++) indicated substantial, and four pluses (++++) indicated the highest quantity of phytochemicals in the samples. The absence of the secondary metabolites was indicated by the minus (-) sign. Tables 1 and 2 show the results of the qualitative phytochemical analysis. Results from Table 1 indicate the presence of a substantial to moderate amount of alkaloids in the leaf extract of *V. pinnata*.

Table 1. Qualitative tests for alkaloids of *V. pinnata* leaves.

Qualitative estimation of alkaloids in leaf extract by different reagents				
Dragendroff's (D)	Hager's (H)	Mayer's (M)	Wagner's (W)	Tannic acid (T)
++	+++	++	++	++++

Table 2. Qualitative test for eleven phytochemicals of *V. pinnata* leaves.

Phytochemicals	Gly.	Tann.	Ter.	Sap.	Phlo.	Ste.	Car. Gly.	Qui.	Phe.	Cau.	Flav.
Intensity of presence in the extract	++++	+++	++++	++	++++	++++	+++	+++	++	+++	+++

Gly. = Glycosides, Tann. = Tannins, Ter. = Terpenoids, Sap. = Saponins, Phlo. = Phlobatannins, Ste. = Steroids, Car. Gly. = Cardiac glycosides, Qui. = Quinines, Phe. = Phenols, Cau. = Coumarins, Flav. = Flavonoids.

The results of Table 2 showed that the leaf extract possess different amounts of phytochemicals. And the highest amounts of phytochemicals present in the leaf extract are glycosides, terpenoids, phlobatannins and steroids. Coumarins, tannins, quinine, flavonoids, and cardiac glycosides are present in moderately high amounts. Saponins and phenols are present in moderate quantities.

Antioxidant activity

Antioxidant activity of *V. pinnata* leaves was carried out by the DPPH scavenging assay (Brand-Williams 1995). Five various concentrations (50, 100, 150, 200 and 250 µg/ml), were utilized in this investigation. The results of the DPPH scavenging assay indicated that the leaves were potentially active as antioxidants. Later, the scavenging activity was compared with that of ascorbic acid, a common antioxidant. The study measured the percentage of scavenging action at five different concentrations (50, 100, 150, 200 and 250 µg/ml) is presented in Table 3.

Table 3. DPPH scavenging activity of methanolic leaf extract of *V. pinnata* and standard solution.

Con. (µg/ml)	50	100	150	200	250
<i>V. pinnata</i> (%)	13.46±0.04	26.92±0.07	51.92±0.14	69.23±0.18	80.77±0.21
Ascorbic acid (%)	51.92±0.72	67.30±0.47	78.85±0.56	84.62±0.49	94.23±0.23

Results showed that the highest mean scavenging activity of ascorbic acid was 94.23±0.23% at the concentration 250 µg/ml and the lowest mean scavenging activity was 51.92±0.72% at concentration 50 µg/ml. In the case of methanolic crude extracts of *V. pinnata* leaves, the highest mean scavenging activity was 80.77±0.21% at a concentration of 250 µg/ml and the lowest mean scavenging activity was 13.46±0.04% at a concentration of 50 µg/ml.

For comparison, different extract concentrations (50, 100, 150, 200, and 250 µg/ml) were placed on the X-axis, and the relative scavenging activity (RSA) of ascorbic acid and methanolic crude extract of *V. pinnata* leaf was placed on the Y-axis (Fig. 1). A sequential change occurred as concentration increased, and the

highest concentrations showed the highest RSA. The leaf sample's IC₅₀ value was 154.33 µg/ml, while that of ascorbic acid was 25.48 µg/ml.

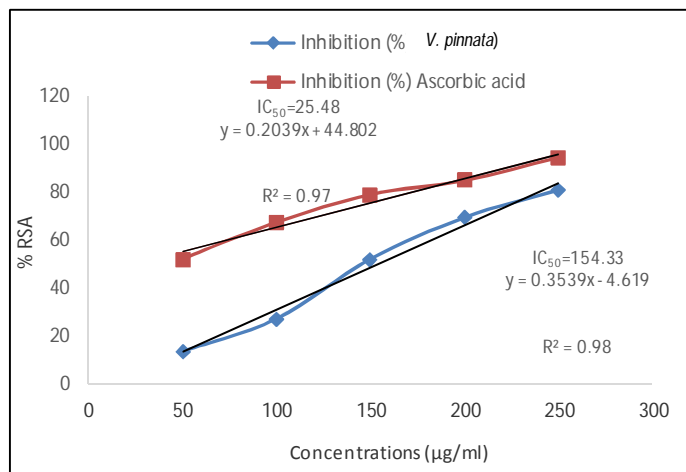


Fig. 1. Relative % DPPH scavenging activity (SCV) of ascorbic acid and methanolic crude extract of *V. pinnata* leaf.

Anti-inflammatory activity test

Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well-documented cause of inflammation. The ability of methanolic plant extracts to denature proteins was studied as part of the investigation into the mechanism of anti-inflammation activity. It had a modest impact on preventing heat-induced albumin denaturation when present in high doses. Acetyl salicylic acid had the maximum inhibitory activity (90.89±0.009%) at a concentration of 250 µg/ml. The lowest inhibitory activity for methanolic crude extracts of leaves was 14.29±0.009% at a concentration of 50 µg/ml, and the most inhibition activity was 57.14±0.002% at a concentration of 250 µg/ml.

Table 4. Albumin protein denaturation assay for anti-inflammatory activity against the methanolic crude extract of *V. pinnata* leaf.

Con. (µg/ml)	% Inhibition of albumin denaturation	
	Leaf	Acetyl salicylic acid
50	14.29±0.009	48.55±0.006
100	21.43±0.013	67.88±0.004
150	28.57±0.001	75.56±0.005
200	50.00±0.001	82.18±0.006
250	57.14±0.002	90.89±0.009

For comparison, different extract concentrations (50, 100, 150, 200, and 250 µg/ml) were plotted on the X-axis, and the percentage inhibition of albumin protein denaturation by methanolic crude extractives of *V.*

pinnata leaf and acetyl salicylic acid was plotted on the Y-axis (Fig. 2). The highest concentrations showed the highest inhibition, and the graph showed a sequential change with the increase of concentrations. The leaf sample and acetyl salicylic acid had IC₅₀ values of 218.8 µg/ml and 33.74 µg/ml, respectively.

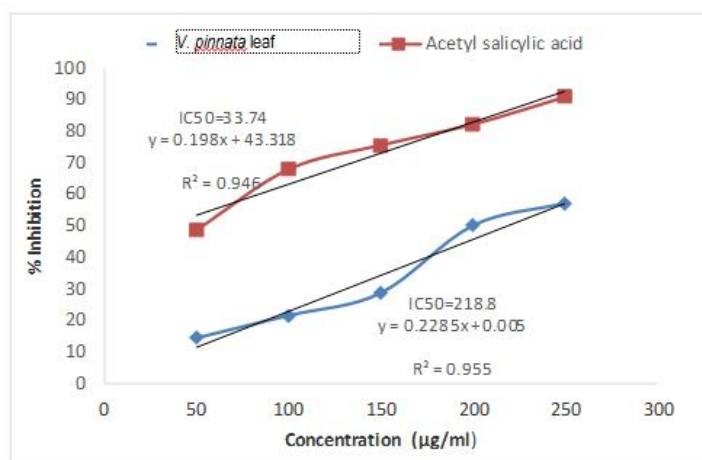


Fig. 2. Percent (%) inhibition of albumin protein denaturation by the methanolic crude extract of *V. pinnata* leaf.

Discussion

In the present research, *V. pinnata* leaves were screened for different secondary metabolites. For the detection of alkaloids, five alkaloid-detecting reagents were used, namely Dragendroff's reagent, Tannic acid reagent, Wagner's reagent, Hager's reagent, and Mayer's reagent. Leaf extract gave different results with different reagents. Dragendroff's reagent showed the presence of alkaloids in the extract of *V. pinnata* leaf in a moderate quantity, which is supported by another study (Nuraskin et al. 2020). But another study found negative results for alkaloids in the extract of *V. pinnata* leaf while treating with this reagent (Thenmozhi and Subasini 2016). The tannic acid reagent showed the best result in alkaloid detection. *V. pinnata* leaf extract showed a moderate result with Wagner's reagent. A study found a negative result, whereas another study reported a positive result with Wagner's reagent (Thenmozhi and Subasini 2016). Treatment with Hager's reagent showed a substantial quantity of alkaloids in the extract of *V. pinnata* leaf. This is supported by another experiment conducted by Thenmozhi and Subasini (2016). Mayer's reagent also showed the presence of alkaloids in moderate quantity in *V. pinnata* leaf extract. Meyer's experiment yielded variable results (Thenmozhi and Subasini 2016, Nuraskin et al. 2020).

In addition to alkaloids, the qualitative assessment of eleven other secondary metabolites (glycosides, tannins, terpinoid, saponin, phlobatannin, steroids, cardiac glycosides, quinine, phenol, coumarin, and flavonoids) was done in *V. pinnata* leaf extract. The present study confirms the presence of all those secondary metabolites. Other studies (Thenmozhi and Subasini 2016, Nuraskin et al. 2020) supported the presence of phenol, tannin, steroids, flavonoids, terpenoids, and saponins. Though the presence of glycoside was recorded in the present study, it was absent in the previous report (Thenmozhi and Subasini 2016).

By analyzing previous studies and present research, it can be postulated that the leaf extract of *V. pinnata* contains different types of secondary metabolites, including alkaloids, glycosides, tannins, terpinoid, saponin, phlobatannin, steroids, cardiac glycosides, quinine, phenol, coumarin, and flavonoids. Differences have been

observed in some other experiments compared to the current investigation (Thenmozhi and Subasini 2016). These variations may be due to differences in extraction methods and/or plant environmental conditions.

Plants have been known to be a potent source of medicinal compounds and antioxidants. The oxidative stress that is created by free radicals can have an impact on the pathophysiology of many diseases, including cardiovascular failure, atherosclerosis, inflammation, carcinogenesis, reperfusion injury, and neurological conditions (Aruoma 1998). Alternative sources of antioxidants from natural origins, particularly plants, are currently in demand due to the growing safety concerns with the consumption of synthetic antioxidants (Stankovic et al. 2016). Different endogenous or external activities in the human body can produce free radicals (Lobo et al. 2010). DPPH, hydrogen peroxide, hydroxyl, superoxide anions, and nitric oxide radicals are a few examples of these radicals. On the other hand, excessive free radicals can destroy the immune system and cause a variety of illnesses, such as cataracts, thrombus formation, cancer, diabetes mellitus, renal failure, neurodegenerative diseases, cardiovascular diseases, liver diseases, rheumatoid arthritis, atherosclerosis, angina pectoris, aging, metabolic disorders, DNA damage, and many more (Nigri et al. 2004, Schetter et al. 2009). When DPPH radicals interact with an antioxidant or other substances those gives protons, the absorbance of DPPH is decreased. In order to examine the radical scavenging capacities of various natural compounds, DPPH radicals are widely used (Satyanarayana and Eswaraiyah 2010). This study was aimed at determining whether the leaf extract of *V. pinnata* could be a good source of antioxidants or not. Results showed that the leaf extract of *V. pinnata* contained antioxidants, which was supported by other studies (Shafie et al. 2020).

Protein denaturation is characterized as a process in which the secondary and tertiary structures of the protein become misaligned, and it happens as a result of heat, a strong acid or base, an organic solvent, or a concentrated inorganic salt (Leelaprakash and Dass 2011). The inflammation process is dependent on the activities of enzymes, specifically proteins. Any substance capable of protein denaturation is considered to have anti-inflammatory agents. The egg albumin method, which uses denaturation to test the anti-inflammatory activity of herbal medicine, is a low-cost alternative (Dharmadeva et al. 2018). The present experiment showed the presence of anti-inflammatory competency in the leaf extract of *V. pinnata*. Previous studies also supported the presence of anti-inflammatory competency in the leaf extract of *V. pinnata* (Nam 2006, Thenmozhi and Subasini 2016). By analyzing the previous and present studies, it can be concluded that the leaf extract of *V. pinnata* contains bioactive compounds with antioxidant and anti-inflammatory properties.

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References

- Ajuru MG, Williams MF and Ajuru G (2017). Qualitative and quantitative phytochemical screening of some plants used in ethnomedicine in the Niger Delta region of Nigeria. *Journal of Food and Nutrition Sciences* 5(5):198-205. DOI: 5.198.10.11648/j.fns.20170505.16.
- Akter R, Islam MK, Islam R, Alam I, Wahab M and Rahman M (2018). Evaluation of phytochemical screening and cytotoxic activity of different extracts of *Monochoria hastata* leaves. *World Journal of Pharmacy and Pharmaceutical Sciences* 8: 71-82.
- Aplin TAH and Cannon JR (1971). Distribution of alkaloids in some Western Australian plants. *Economic Botany* 25(4): 366-380.
- Aruoma OI (1998). Free radicals, oxidative stress, and antioxidants in human health and disease. *Journal of the American Oil Chemists' Society* 75(2): 199-212.
- Auwal MS, Saka S, Mairiga IA, Sanda KA, Shuaibu A and Ibrahim A (2014). Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of *Acacia nilotica* (Thorn mimosa). *Veterinary Research Forum: An International Quarterly Journal* 5(2): 95-100.
- Bansode T and Salalkar BK (2015). Phytochemical analysis of some selected Indian medicinal plants. *International Journal of Pharmacy and Biosciences* 6(1): 550- 556.
- Brand-Williams W, Cuvelier ME and Berset C (1995). Use of a free radical method to evaluate antioxidant activity. *Food Science and Technology* 28(1): 25-30. [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5).
- Cromwell BT (1955). *Modern Method of Plant Analysis*. Springer-Verlag, Berlin, pp. 373-374.
- Dharmadeva S, Galgamuwa LS, Prasadinie C and Kumarasinghe N (2018). *In vitro* anti-inflammatory activity of *Ficus racemosa* L. bark using albumin denaturation method. *Ayu* 39(4): 239-242. doi: 10.4103/ayu.AYU_27_18.
- Edeoga H, Okwu DE and Oyedemi B (2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology* 4(7): 685-688. doi:10.5897/AJB2005.000-3127.
- Farzaei MH, Bayrami Z, Farzaei F, Aneva I, Das SK, Patra JK, Das G and Abdollahi M (2020). Poisoning by medical plants. *Archives of Iranian Medicine* 23(2): 117-127.
- Harborne JB (1973). *Phytochemical methods: A guide to modern techniques of plant analysis*. Chapman and Hall Ltd., London, pp.1-288.
- Kapoor LD, Singh A, Kapoor SL and Srivastava SN (1969). Antibacterial activity of plant extracts used externally in traditional medicine. *Journal of Ethnopharmacology* 44: 35-40.

- Kolawale OM, Oguntoye SO, Agbede O and Olayemi AB (2006). Studies on the efficacy of *Bridelia ferruginea* Benth, bark extract in reducing the coliform load and BOD of domestic waste water. *Ethnobotanical Leaflets* 10: 228-238.
- Leelaprakash G and Dass SM (2011). *In vitro* anti-inflammatory activity of methanol extract of *Enicostemma axillare*. *Journal of Drug Research and Development* 3:189-96.
- Lobo V, Patil A, Phatak A and Chandra N (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Reviews* 4(8): 118-26. doi: 10.4103/0973-7847.70902.
- Mia MK (2009). *Vitex pinnata* L. In: Ahmed ZU, Begum ZNT, Hasan MA, Khonder M, Kabir SMH, Ahmed M, Ahmed ATA, Rahman AKA & Haque EU (eds.). *Encyclopedia of Flora and Fauna of Bangladesh*, Vol. 10, Angiosperms: Dicotyledons (Ranunculaceae - Zygophyllaceae). Asiatic Society of Bangladesh, Dhaka, pp. 487-888.
- Mizushima Y and Kobayashi M (1968). Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. *Journal of Pharmacy and Pharmacology* 20(3): 169-173.
- Nam NH (2006). Naturally occurring NF-KB inhibitors. *Mini Reviews in Medicinal Chemistry* 6: 945-951.
- Nigri GR, Kossodo S, Waterman P, Fungaloi P and La Muraglia GM (2004). Free radical attenuation prevents thrombosis and enables photochemical inhibition of vein graft intimal hyperplasia. *Journal of Vascular Surgery* 39(4): 843-849.
- Nuraskin C, Marlina M, Idroes R, Soraya C and Djufri (2020). Identification of secondary metabolite of laban leaf extract (*Vitex pinnata* L.) from geothermal areas and non-geothermal of Agam mountains in Aceh province, Indonesia. *Rasayan Journal of Chemistry* 13: 18-23.
- Prasetyaningtyas W, Wati S and Buang R (2021). The use of laban (*Vitex pinnata* L.) leaf as a silk pigment. In *IOP Conference Series: Earth and Environmental Science* 700(1): 012045.
- Sakat S, Tupe P, Hule A and Juvekar A (2010). Anti-inflammatory potential of flavonoid fraction of *Tamarindus indica* Linn (seeds). *Planta Medica* 76(12): 20.
- Sati SC, Sati N, Rawat U and Sati OP (2010). Medicinal Plants as a Source of antioxidants. *Research Journal of Photochemistry* 4: 213-224.
- Satyanarayana T and Eswaraiah MC (2010). *In vitro* antioxidant and free radical scavenging potential of stem of *Calycopteris floribunda* Lam. *Research Journal of Pharmaceutical Biological and Chemical Sciences* 1(2): 117-123.
- Schetter AJ, Nguyen GH, Bowman ED, Mathe EA, Yuen ST, Hawkes JE, Croce CM, Leung SY and Harris CC (2009). Association of inflammation-related and microRNA gene expression with cancer-specific mortality of colon adenocarcinoma. *Clinical Cancer Research* 15: 5878-5887.

- Shafie NA, Suhaili NA, Taha H and Ahmad N (2020). Evaluation of antioxidant, antibacterial and wound healing activities of *Vitex pinnata*. F1000 Research 9(187): 1-16. doi:10.12688/f1000research.21310.2.
- Shinde UA, Phadke AS, Nair AM, Mungantiwar AA, Dikshit VJ and Saraf MN (1999). Membrane stabilizing activity- A possible mechanism of action for the anti-inflammatory activity of *Cedrus deodara* wood oil. Fitoterapia 70(3): 251-257.
- Sofowora A (1993). Phytochemical screening of medicinal plants and traditional medicine in Africa Edition. Spectrum Books Ltd., Nigeria, pp. 150-156.
- Soloway S and Wilen SH (1952). Improved ferric chloride test for phenols. Analytical Chemistry 24(6): 979-983.
- Stankovic N, Mihajilov-Krstev T and Zlatkovic B (2016). Antibacterial and antioxidant activity of traditional medicinal plants from the Balkan Peninsula. NJAS-Wageningen Journal of Life Sciences 78:21-28.
- Thenmozhi S and Subasini U (2016). Morpho-anatomical and physicochemical evaluation of *Vitex pinnata* Linn. leaves. European Journal of Biomedical and Pharmaceutical Sciences 3(4):483-492.
- Trease GE and Evans MD (1989). A text book of Pharmacognosy. 13th Ed. BullerTrindall and Canssel London, pp.176-180.
- Vijayameena C, Subhashini G, Loganayagi M and Ramesh B (2013). Original research article phytochemical screening and assessment of antibacterial activity for the bioactive compounds in *Annona muricata*. International Journal of Current Microbiology and Applied Sciences 2: 1-8.
- Webb LJ (1949). An Australian Phytochemical survey. I. Alkaloids and cyanogenic compound in the Queensland plants. CSIRO, Bull. No. 241. Melborne, Australia, pp. 99. <https://doi.org/10.25919/d3w2-ej09>.

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