



Assessment of the antioxidant activity and antimicrobial activity of *Flemingia vestita* root extract

Bhrianka Bharadwaj¹, Rahnam B Jyrwa³, Rahul Nayak¹, Surjya Loying², Sangita Boro^{1*}

¹ Assistant Professor, Programme of Microbiology, Assam Down Town University, Khankar Gaon, Assam, India

² Assistant Professor, Programme of Biotechnology, Assam Down Town University, Khankar Gaon, Assam, India

³ Post Graduate Student, Programme of Microbiology, Assam Down Town University, Khankar Gaon, Assam, India

Abstract

Many diseases have been treated with herbal remedies throughout the history of mankind. A well-known objective for studying them is the discovery of new bioactive components, in the search for promising drugs. *Flemingia vestita* is traditionally used to kill intestinal helminths. Many studies have proved the anti helminthic effect of the root extract of the plant in-vitro. The present study was carried out to evaluate the antioxidant activity of *Flemingia vestita*'s root extract. Extraction was done using Soxhlet apparatus with methanol, ethanol and acetone as solvents for extraction. The antioxidant activity of the extracts was evaluated using DPPH by spectrophotometry. The tuberous root possesses antioxidant properties, scavenging DPPH with IC₅₀ values 14µg/ml, 71µg/ml and 66µg/ml of methanol, ethanol and acetone extract respectively against a standard reference of 5µg/ml of ascorbic acid. TLC evaluation showed presence of bio-molecules such as flavonoids with Rf values 0.55, 0.67 and 0.38 in the methanol extract, ethanol extract and acetone extract respectively. The antimicrobial activity performed by disc diffusion method using methanol, ethanol and acetone extract showed inhibition zone of 26mm, 21mm and 19mm against *E. coli* and 39mm, 30mm and 16mm against *Klebsiella* species. The positive control discs (antibiotic discs) mostly showed inhibition zones of 30mm, while that of the negative control (2% DMSO) was negligible. Thus, the study reveals that this plant has the potential to be a better drug compared to the prevailing antibiotics and may be effective to treat various diseases.

Keywords: DPPH, IC₅₀, scavenging, antioxidant activity, TLC, *Flemingia vestita*

Introduction

Flemingia is a genus of flowering plants in the legume family, Fabaceae. It belongs to the sub family Faboideae (Papilionaceae). It is the major group of Angiosperms and is a native of the tropical and subtropical regions of the world. *Flemingia vestita* is a nitrogen fixing herb with

characteristic tuberous root which are edible. Found as a wild herb along the mountain slopes of Himalayas, it is distributed in Sichuan and Yunnan provinces of China, Nepal and Khasi & Jaintia Hills of Meghalaya in Northeast India. It is also sparsely found in Laos, Philippines and Vietnam.

Table 1: Scientific classification/Taxonomy ^[1]

| | |
|---------------|---|
| Domain | Eukaryota |
| Kingdom | Plantae |
| Division | Magnoliophyta |
| Class | Magnoliopsida |
| Order | Fabales |
| Family | Fabaceae alt. Leguminosae |
| Sub family | Faboideae |
| Tribe | Phaseoleae |
| Sub tribe | Cajaninae |
| Genus | <i>Flemingia</i> |
| Species | <i>vestita</i> |
| Binomial name | <i>Flemingia vestita</i> (Benth. Ex Baker, 1876) |
| Other names | <i>Flemingia procumbens</i> (Roxb.), <i>Moghania vestita</i> (Benth. ex Baker Kuntze); <i>Moghania procumbens</i> (Roxb., Mukerjee), famously known as Sohphlang. |

The juicy tuber is a highly priced vegetable among the Garo, Khasi and Jaintia tribes of Meghalaya, India. In fact, its demand as foodstuff has increased so much that it has been cultivated as a cash crop and is regularly available in the local markets. In terms of nutritional value, it is particularly rich in phosphorus and proteins. The tuber has been an

indigenous vermifuge among the Khasis, it is called *soh-phlang*. The raw tuber or the root peel is directly consumed for the treatment of soft-bodied intestinal worms. In 1991, an isoflavone- genistein was isolated from the tuber extract which was demonstrated to be the major anthelmintic principle ^[2].

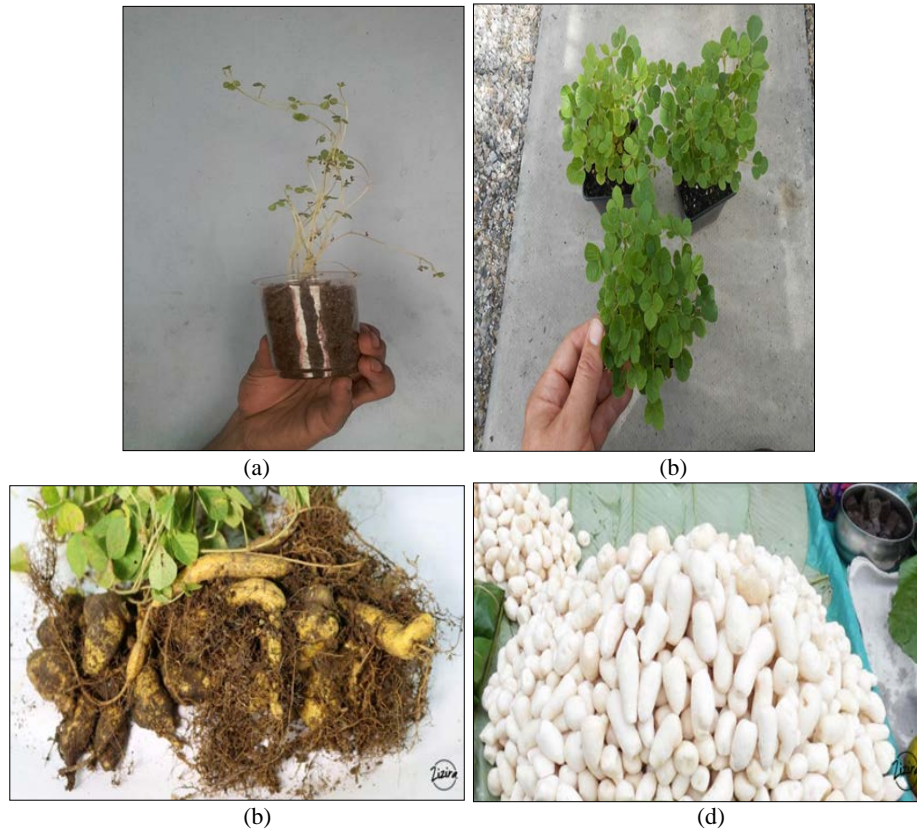


Fig 1: (a, b) *Flemingia vestita* grown in a pot, (c) Uprooted *Flemingia vestita* and (d) Ready for sale edible tuber of the plant

As not much research has been done on this species of *Flemingia* and traditionally the roots have been used to treat various intestinal infection, therefore this study has been planned with the purpose to analyse the antioxidant activity, to estimate the compounds present in the roots and also to find the antibacterial activity against enteric bacteria.

Materials and Methods

Sample collection: The roots (tubers) as well as the seedlings of this plant were collected from Smit, East Khasi Hills, Meghalaya. The plant sample was then authenticated by Department of Botany, Gauhati University, bearing Account No. - 181148, Dated-6.5.2016

Extraction: Initially, the tuberous root of *Flemingia vestita* was dried at room temperature. Then, it was ground to powder form. With the help of Soxhlet apparatus, extraction was carried out [3, 4]. Methanol, ethanol and acetone were used as solvents for extraction [5].

Antioxidant activity: The extract was evaluated for its antioxidant activity. The preparation activity maintained in 1:1 ratio with the concentration of 1mg/ml (1000 μ /ml) and then concentrations were prepared, by diluting with methanol (original solvent). Five test tubes were taken and marked as-10, 20, 40, 80 and 160 and pipetted 10 μ l/ml, 20 μ l/ml, 40 μ l/ml, 80 μ l/ml and 160 μ l/ml respectively. They were then diluted with methanol or the original solvent taken to make the final volume of 1ml. So, in the test tube, the final volume was made with 1ml sample + 2ml of DPPH. After that the tubes were incubated at 37°C in the dark. 2ml of this solution was diluted with methanol and the volume was made up to 3ml. The absorbance was taken at 517nm [6]. 3 ml of DPPH was used as a control.

Calculation

Inhibitory concentration was measured with the formula

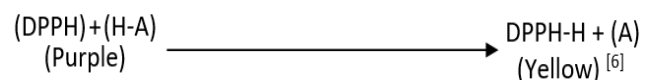
$$I \% = \frac{A_o - A_s \times 100 \%}{A_o}$$

Where, A_o -----> Absorbance for unreduced DPPH.

A_s -----> Absorbance for sample.

With similar concentration the values were compared with standard (L-ascorbic acid). [7]

The scavenging reaction between DPPH and an antioxidant, H-A can be written as



Thin layer chromatography

The mobile phase was prepared by making a solution of ethyl acetate, methanol, glacial acetic acid and distilled water in the ratio 10:1:1:1:2.6. The TLC set-up was then run in one dimension. After the development of the TLC plates, the plates were air dried and then introduced in an iodine chamber for visualization or detection.

Calculation

The Retention factor value (Rf value) was measured with the formula

$$Rf \text{ value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$



Fig 2: TLC plates of the extracts

Antimicrobial evaluation

The antimicrobial activity was performed by Disc Diffusion Method. The bacteria selected to observe the activity were *E. coli* and *Klebsiella*. The bacterial samples were collected from Down Town Hospital Guwahati, Assam.

Result and Discussions

DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) is stable free radical with purple colour, the intensity of which is measured at 510 nm spectrophotometrically. The results of DPPH scavenging of the different extracts of *Flemingia vestita* are in Figure 1 below.

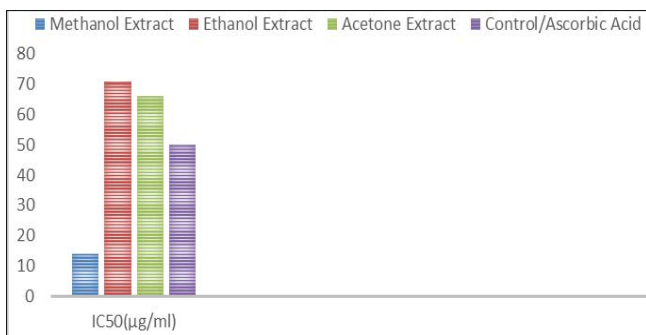


Fig. 3: Graphical representation of the IC₅₀ value of the different extracts

The extracts were evaluated against DPPH using the standard ascorbic acid. An inhibitory concentration of 71% was observed by the ethanolic extract of the roots. Compared to the control Ascorbic Acid, the inhibitory concentration shown both by Ethanolic and Acetone extracts of the roots were very significant. This possibly was because of the phytochemicals like flavonoid present in the extracts. Free radicals are known to be major factor in biological damages and have been used to evaluate the free radical scavenging activity natural antioxidants [7]. DPPH is a radical itself which is purple in colour. It changes into a

stable yellow compound by reacting with an antioxidant, and the extent of the reaction depends on the hydrogen donating ability of the antioxidant [8]. The reduction capability of the DPPH radical is determined by the decrease in its absorbance of 510 nm, induced by antioxidants [9, 10, 11].

In order to evaluate the antioxidant potency through free radical scavenging by the test sample, the change of optical density of DPPH radicals was monitored [11, 12]. The tuberous root extract of *Flemingia vestita* was found to possess antihelminthic properties [2]. And the antioxidant property of the tuber was not evaluated earlier. The antioxidant activity evaluation of the root extracts shows that the root possesses adequate number of antioxidants in it. This is majorly due to the presence of some flavonoids found in the genus *Flemingia* such as isoflavone, genistein, etc [2, 13]. The flavonoid antioxidant properties are linked to their ability to bind metallic ions, which can be involved in free-radical genesis. Radical chain reactions are broken by flavonoid hydrogen donors and also by their capacity to regenerate α -tocopherol. Flavonoids possess a remarkable spectrum of biochemical and pharmacological activities that have been attributed, in part, to their antioxidant activities [14]. In a study conducted by S. Madan *et.al.* using various solvent extracts of *F. strobilifera*. The extracts showed significant antimicrobial activity against many Gram positive and Gram negative bacteria [16]. Kavita Ghalot *et.al.*, collected information in an attempt to cover the more recent developments in the ethnobotany, pharmacology and phytochemistry of the genus *Flemingia*. The tuberous root showed presence of Carbohydrates, Alkaloids, Glycosides, Tannins, Flavonoids, Steroids, Terpenoids, Phenolic compounds and Proteins. Similar results were observed by Rajendra Prasad Gujjeti and Estari Mamidala on their study of *Aerva lanata* root extract [10], except that they found Saponins present which is absent in *Flemingia vestita*'s root peel.

Table 2: Rf values of the different extracts

| Extracts | Methanol extract | | | Ethanol extract | | | Acetone extract | | |
|----------|--------------------------|---------------------------|----------|--------------------------|---------------------------|----------|--------------------------|---------------------------|----------|
| | Distance moved by solute | Distance moved by solvent | Rf value | Distance moved by solute | Distance moved by solvent | Rf value | Distance moved by solute | Distance moved by solvent | Rf value |
| Values | 8cm | 14.5cm | 0.55 | 11.5cm | 17cm | 0.67 | 1.9cm | 5cm | 0.38 |

TLC analysis of methanol, ethanol and acetone extract separated the bio-molecule flavonoids. The Rf values are 0.55, 0.67 and 0.38 respectively. *Bülent Kaya et.al* also found flavonoid compounds identified in *Alchemilla l.*

species. Three more flavonoids with Rf values of Rf1= 0.36, Rf2=0.54 and Rf3=0.68 were also identified for the first time in their study^[12]

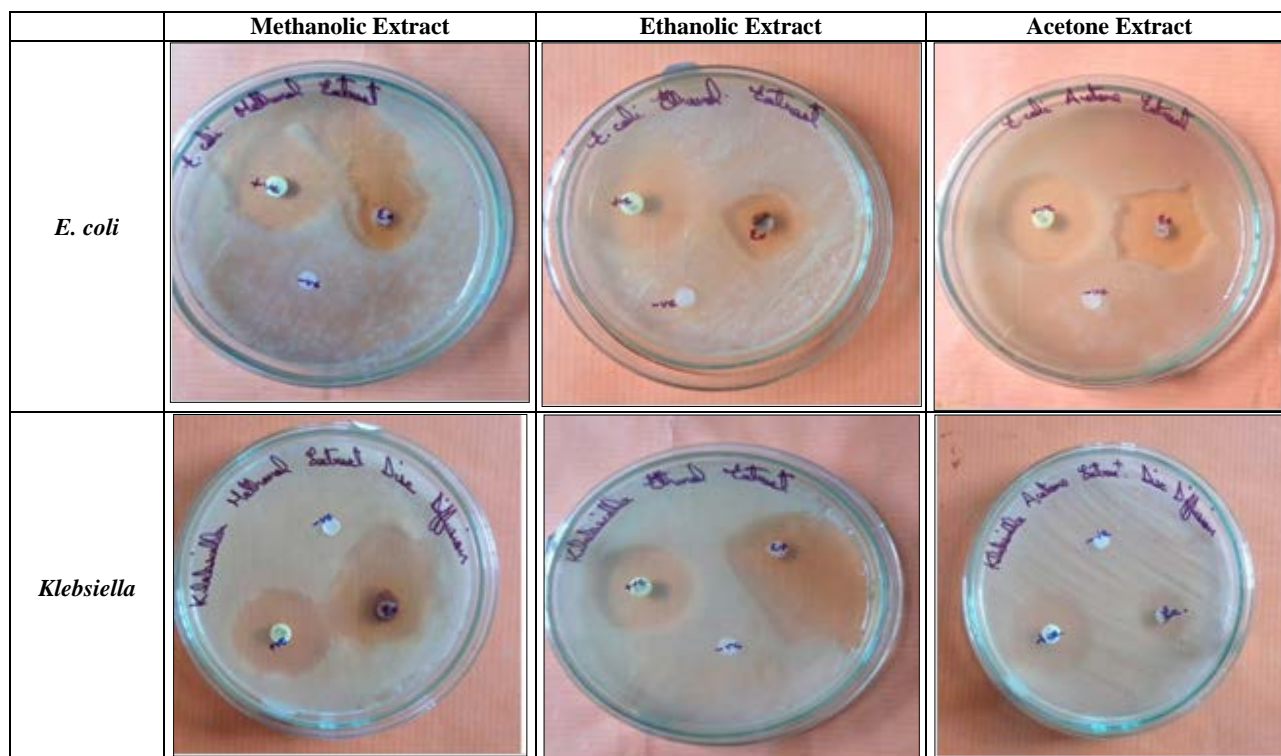


Fig 4: Plates showing the antimicrobial zone formed by the various extracts along with the controls.

Table no. 3: Zone of inhibition of the extracts against bacteria by disc diffusion method

| Extract test organisms | Methanol extract (mm) | Ethanol extract (mm) | Acetone extract (mm) | Positive control (mm) |
|------------------------|-----------------------|----------------------|----------------------|-----------------------|
| <i>E. coli</i> | 26 | 21 | 19 | 30 |
| <i>Klebsiella</i> | 39 | 30 | 16 | 30 |

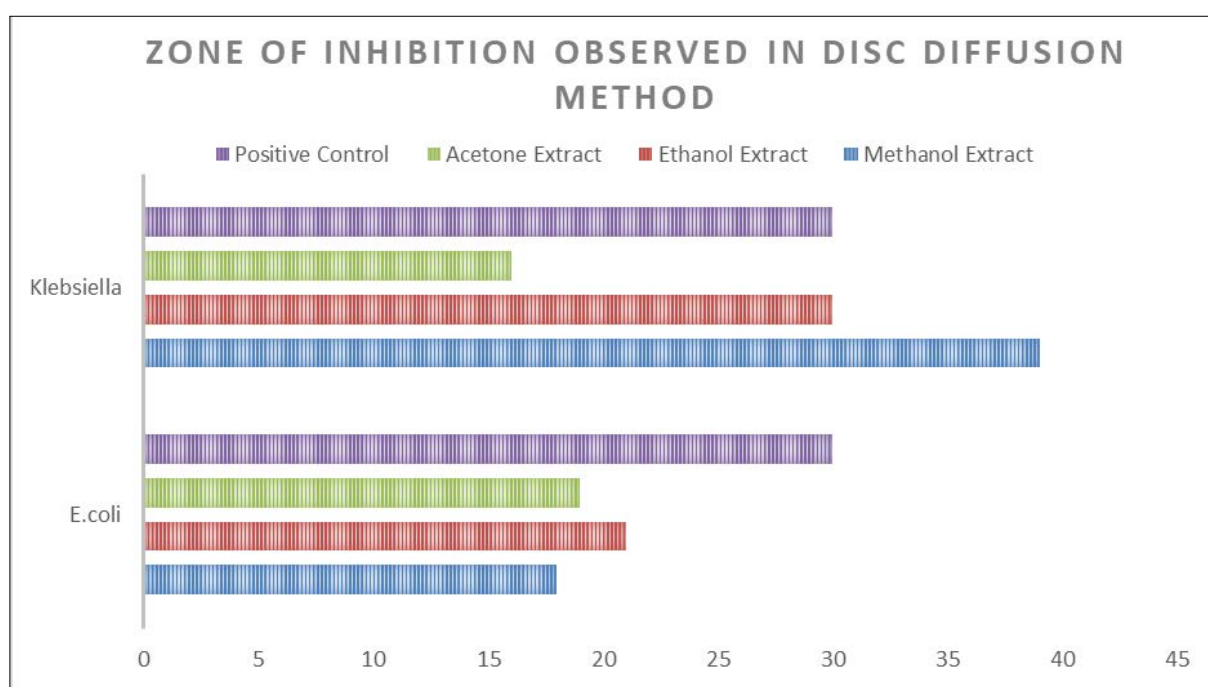


Fig 5: Graph representing the zone of inhibition observed in disc diffusion method against the test micro-organisms

An inhibition zone of 26mm, 21mm and 19mm against *E. coli*; and 39mm, 30mm and 16mm against *Klebsiella*

species was observed by the methanolic, ethanolic and acetone extracts of the root. The positive control discs

showed inhibition zones of 30mm. The methanolic and ethanolic extract happened to show significant zone of inhibition which was very much similar to the positive control. *Parastoo Karimi Alavijeh et al* showed similar results by disc diffusion method.

Conclusion

Flemingia vestita's tuberous root was found to have the potential to inhibit or reduce the rate of oxidation reactions. Anti-oxidant activity tests of the root extracts was performed using DPPH and it was found that *F.vestita's* root extracts possess anti-oxidant properties. Comparatively ethanol extract showed to possess the most scavenging property followed by acetone and methanol extract. The IC₅₀ values are 14µg/ml, 71µg/ml and 66µg/ml of methanol, ethanol and acetone extract respectively. Thus, it could be a potential source of natural antioxidant having great importance as therapeutic agent and preventing oxidative stress related degenerative diseases. Moreover the antimicrobial test too showed considerable activity by the methanolic and ethanolic extract. As the TLC suggests presence of flavonoids, further in depth study is very necessary to explore the other beneficial compounds of this plant. The root of *Flemingia vestita* can provide lead molecules which could be useful substrate for the synthesis of new broad-spectrum antibiotics for the treatment of infections caused by the organisms.

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