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Phytochemical and Antimicrobial Efficacy of *Vernonia amygdalina*

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Abstract: ***Popularly known as bitter leaf (due to its bitter taste), Vernonia amygdalina is an edible shrub plant which is grown in major parts of Africa, particularly in the western parts of the continent. V. amygdalina is greatly consumed due to its nutritious and medicinal properties and has come be considered a very essential medicinal plant in herbal medicine and pharmaceuticals). V. amygdalina is known to be naturally found along river lakes and forest margin. It also can be found in woodlands and grasslands. It is fast-growing plant which can grow up to a height of 1 - 3m, with leaves being 6mm in diameter.Studies have shown that V. amygdalina contains bioactive compounds which work against helminthes and other protozoan, including bacteria and fungi. Further studies are being carried out on the plant to fully understand its mechanism of action and its full potency against other bacterial and fungal diseases. Present finding focuses on preliminary phytochemical screening of bioactive compounds and its anti-microbial activities against microbes.***
Keywords: V. amygdalina ; phytochemical ; antimicrobial

I. INTRODUCTION

A lot of plant research is ongoing in various fields of plant development and improvement. A part of the plant (known as an explant) is usually taken and grown under controlled (*in vitro*) conditions. The necessity of crop improvement and development is due to the reasons most plants have highly beneficial properties for humans and animals, such as nutritional and medicinal benefits. Some species of plants are being discovered and studied in order to figure out how beneficial they are in pharmaceuticals and drug development, foods and other industries[5]. The essence of plant research is basically to improve human health and subsequently provide sustainable better living conditions for all in the society. Plants, since time immemorial have been known to be a good source of food and nutrition. They have also been considered to have various bioactive compounds which enhance the immune system in the fight against infections and diseases [3]. Many of these bioactive compounds have been categorized and classified, while research is still in process to discover more of these compounds or sub-compounds which act against pathogenic microbes and protozoans [4].



A) Habit of ***Vernonia amygdalina***

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II. MATERIALS AND METHODS

A. Prelimnary Phytochemical Screening using Ethanolic Extract:

Fresh leaves of *V. amygdalina* were harvested, washed thoroughly, dried in the hot air oven for 3 days and grinded to powder and then stored in air-tight containers for further use. Twenty-five grams of the pulverized plant material was extracted for 2 days in absolute ethanol (Sigma-Aldrich). The separated extracts were then filtered through Whatman's No. 1 filter paper and the filtrates were then separately condensed to dryness using rotary evaporator. The thick extracted mass was then dried at room temperature. Dried extract was collected in an air tight container and stored at 4°C for further analysis.

1) *Preparation of Ethanolic Extract:* 4gm of the dried extracted mass was dissolved in 20ml of absolute ethanol as stock and tests for various bioactive compounds were carried out using the ethanolic extract.
2) *Phytochemical Tests:*

a) *Test for Saponins:*1ml of the stock solution was diluted with 20ml of distilled water. The whole solution was shaken by hand for 15 min. The formation of a foam layer was obtained. This indicated the presence of Saponins.

b) Test or steroids: 0.1gm of dried extract was dissolved in 10ml of chloroform. And equal volume of sulfuric acid was added to the tube. The formation of a red layer at the top of the sample was seen with a yellow with green fluorescence upon addition of sulfuric acid. This observation indicated the presence of steroids.

c) Test for tannins: From the prepared stock solution, 1ml of the sample was taken and diluted with chloroform (1ml). Acetic anhydride (1ml) was then added to the solution. The appearance of green colour indicated the presence of tannins.

d) Test for Flavonoids: To 1ml of the stock extract, few drops of sodium hydroxide (NaOH) were added (showing intense yellow colour) which became colourless after the addition of dilute acid. The appearance of deep yellow upon addition of NaOH and disappearance of colour after adding dilute acid showed the presence of flavonoids.

e) Antibacterial Activity: The anti-microbial activity of the plant was tested by the Disc Diffusion Method, using the ethanolic extract. Test organisms used were *E.coli* and *Bacillus subtilis* [1]. The test organisms were gotten from the microbiology laboratory of the Department of Biotechnology in Loyola College of Arts and Science, Chennai- India.

3) Disc Diffusion Method: The **Disc Diffusion assay**, also known as **agar diffusion test**, or (disc-diffusion antibiotic susceptibility test, is a test of the antibiotic sensitivity of bacteria. It makes use of **antibiotic discs** to test the extent to which bacteria are affected by those antibiotics. In this test, wafers containing antibiotics are placed on an agar plate where bacteria have been placed, and the plate is left to incubate. If an antibiotic stops the bacteria from growing or kills the bacteria, there will be an area around the wafer where the bacteria have not grown enough to be visible. This is called a zone of inhibition. The antibiotic used in this test was Ampicillin.

4) Preparation of Media:

a) Luria-Bertani (LB) Broth:

10ml of LB broth was prepared in two separate tubes for the test organisms *E.coli* and *B. subtilis*. The media was sterilized by autoclaving and allowed to cool down. Under sterile conditions, the test organisms were inoculated in the broth culture and incubated for five hours in the incubator shaker.

B. Procedure:

50ml of nutrient agar was prepared and autoclaved with petri-plates for 20 min. After autoclaving, the media was allowed to cool down and in sterile conditions; the media was transferred to the plates and allowed to solidify. Three wells were made in the plates and labeled respectively as control (antibiotic inhibition), negative (without antibiotic inhibition) and positive (test sample showing inhibition) and the test organisms each was swabbed on a medium. The antibiotic wafer was placed in the control well, non-antibiotic wafer in the negative well and test sample (V.amygdalina leaf extract) in the positive well. The plates were labeled according to the test organisms, i.e. *E .coli* and *B. subtilis* respectively and incubated for 24h. After 24h, the plates were observed for zones of inhibition. For both test organisms, a zone of inhibition was observed on each plate showing a positive result, thus, indicating that *V. amygdalina* possesses potency against bacteria (Abosi and Reseroka, 2003).

III. RESULTS AND DICUSSION

V. amygdalina is also known to contain various bioactive compounds that work indepently, or in combinations. These compounds belong to various class including: alkaloids, flavonoids, saponins, tannins, steroids and many more classes of the compounds. The ethanolic extract of the *V. amygdalina* from leaves was screend for the above stated compounds with positive results, indicating the presence of these of compounds.

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Further studies on the antimicrobial activities of the *V. amygdalina using* using the disc diffusion method against *E. coli* and *B. subtilis*, showed that the plant shows efficacy against bacteria species. Thus, it can be used in treatments of bacterial diseases.

Table Showing Phytochemical Screening Of Bioactive Compounds

Bioactive compounds	Present/Absent
Saponins	+
Tannins	+
Steroids	+
Flavonoids	+

Test for Antibacterial Activity

Zone of inhibition **Zone of inhibition**

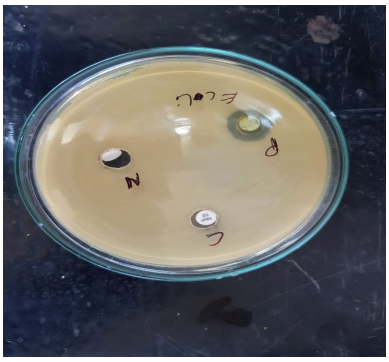




Figure 1: Antimicrobial potency of *V.amygdalina* against *E. coli* **Figure 2: Antimicrobial potency of *V. amygdalina* against *B. Subtilis***

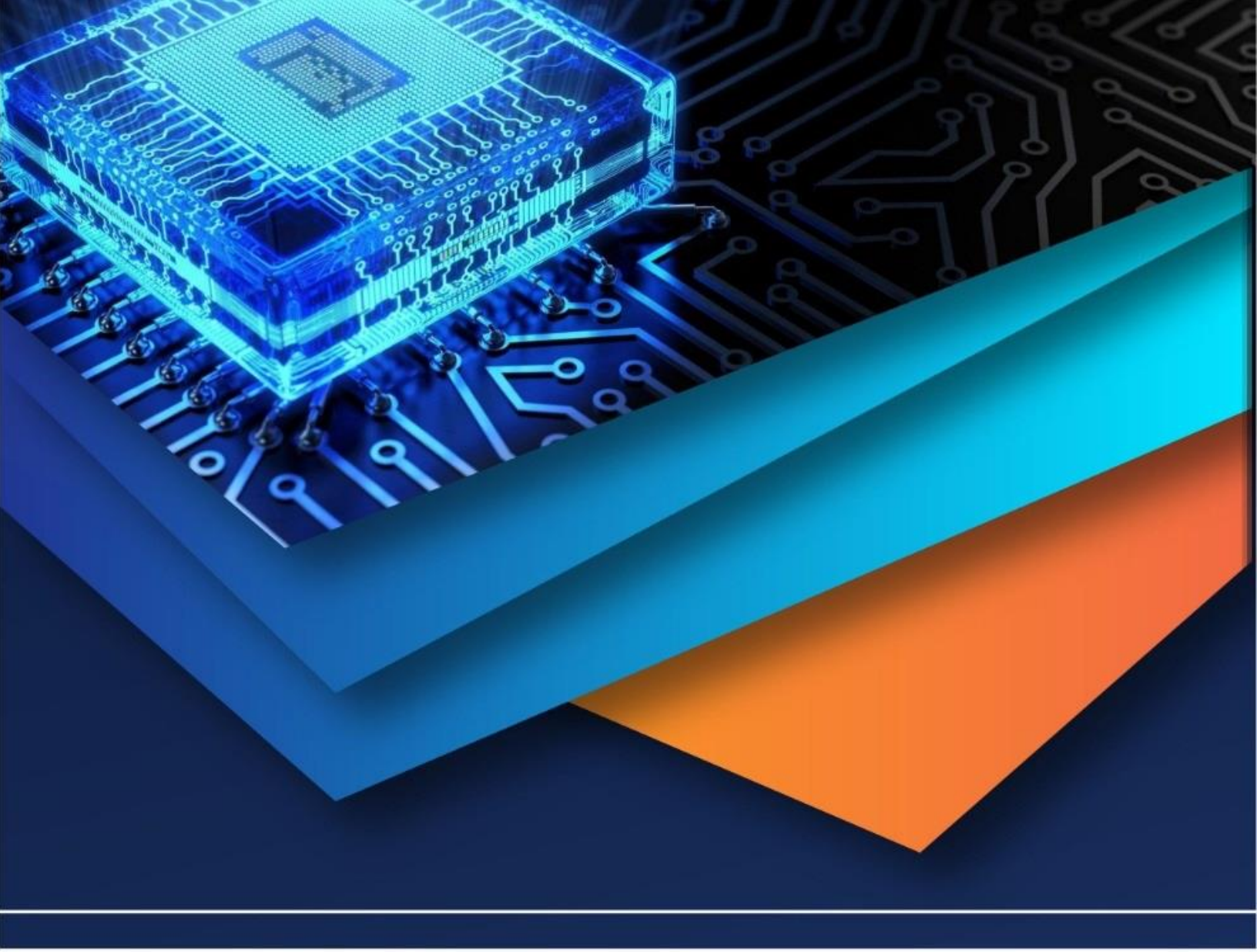
IV. SUMMARY AND CONCLUSION

Popularly known as bitter leaf (due to its bitter taste), *Vernonia amygdalina* is an edible shrub plant which is grown in major parts of Africa, particularly in the western parts of the continent. *V. amygdalina* is greatly consumed due to its nutritious and medicinal properties and has come be considered a very essential medicinal plant in herbal medicine and pharmaceuticals. *V. amygdalina* is known to be naturally found along river lakes and forest margin. It also can be found in woodlands and grasslands. It is fast-growing plant which can grow up to a height of 1 - 3m, with leaves being 6mm in diameter.Studies have shown that *V. amygdalina* contains bioactive compounds which work against helminthes and other protozoan, including bacteria and fungi. Further studies are being carried out on the plant to fully understand its mechanism of action and its full potency against other bacterial and fungal diseases. Present finding focuses on the preliminary phytochemical screening of bioactive compounds and its anti-bacterial activities against selected microbes such as *Escherichia coli* and *Bacillus subtilis*, which is due to the presence of flavonoids and tannins in *V. amygdalina*.

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