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Aqueous Extract of the Leaves of *Phaseolus vulgaris* Linn. and its Bioactivity against *Propionibacterium acnes*

A.R. Shaikh[1] and M.R. Badole[2]

Department of Chemistry, Ramnarain Ruia Autonomous College, Matunga, Mumbai, University of Mumbai 400 019, M.S, India

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ABSTRACT

In the present work the aqueous extract of the leaves of *Phaseolus vulgaris* Linn. was extracted using a sequential extraction method. The extracts were screened for alkaloids, flavonoids, steroids, glycosides, saponins, and tannins as reported in the literature. The Zone Inhibition Method (Kirby-Bauer method) was used to check the antibacterial activity against *Propionibacterium acnes.* The results showed that a 1000 g dose of crude extract, along with the positive control ciprofloxacin, exhibited a zone of inhibition of approximately 7.5 mm in diameter and 40 mm, respectively. The bioactivity of the crude aqueous extract of a plant is attributed to the synergistic effects of Phyto constituents such as alkaloids and flavonoids. The sequential extraction method will aid in isolating and characterizing compounds with bioactivity, thereby facilitating the discovery of new antibacterial drugs.

Key words: *Fabaceae, Propionibacterium acnes, Disk diffusion method, Phaseolus vulgaris Linn.*

Introduction

Skin is a covering of tissue that covers and shields from outside factors. A beautiful appearance and self-assurance come from having disease-free, healthy skin. Skin acne is caused by an unhealthy lifestyle, stress, and a hormonal imbalance. Acne affects the majority of youngsters, which lowers their self-esteem and life confidence. *Propionibacterium acnes* is the causative agent of acne on the skin (McLaughlin *et al.*, 2019). Many studies and efforts to raise awareness about skin conditions have been made within the past ten years. Although there are many cosmetic goods in the market, demand for plant-based products is rising because they have no detrimental effects on our health (Kumar *et al.*, 2007). By choosing plant-based skin care products, individuals can help reduce the demand for environmentally harmful practices such as deforestation, habitat destruction, and overuse of chemicals. Sustainable development and plant-based skin care both promote human health and well-being. Sustainable development promotes practices that promote healthy communities and lifestyles, such as access to clean air, water, and nutritious food. Herbal skin care products enriched with natural ingredients such as fruits, vegetables, and plant extracts have many benefits for skin health. These products are often free of harsh chemicals and synthetic additives, reducing the risk of allergic reactions and long-term skin damage, and they also provide additional nutrients and antioxidants that pro-

([1]Research Scholar, [2]Associate Professor)

mote skin vitality. Sustainable development emphasizes the importance of minimizing damage to the environment while meeting the needs of the present without compromising the ability of future generations to meet their own needs. Similarly, the production and use of plant-based skin care products often has a lower environmental impact compared to products of animal origin or products made from synthetic chemicals (Tolnay *et al*., 2019). Herbal medicine, particularly leaves, has long been a subject of research. *Phaseolus vulgaris* Linn. seeds has antioxidant and anti-inflammatory properties, researchers are currently interested in learning more about its effects on skin (Ganesan and Xu, 2017).

The current aim of the study is preliminary phytochemical screening of aqueous extract of the leaves of the plant as per standard procedure given in literature and to check the antibacterial activity of *Phaseolus vulgaris* Linn. The disk diffusion method is used.

Materials and Methods

Collection of plant

The plant was collected from Dapoli, Maharashtra. Authentication of plant species was done from Xavier's college, Mumbai, and it matches with herbarium specimen No. D. P 2275. After collection of the plant, the leaves of the plant are washed and dried under the shed. After complete drying, they are ground into a fine powder and stored in an airtight container.

Experiment

Chemicals and Instruments

All the solvents used for extraction are laboratory grade of Fisher scientific. The sonicator used of model ultrasonic bath. Sonicator used for increasing solubility of solvents to crude powder. The rota evaporator used for evaporating each solvent of Buchi RE (Model 120). The lyophilizer used for freeze drying of aqueous extract is Vertis (model-Super modulyo).

Extraction

5 grams of dried powder plant was taken in a dry conical flask and 100 ml of pet ether was added in it. The mixture allowed for sonication for about 10 minutes. After sonication, filtrate is filtered through Whatman no.1 filter paper. The filtrate was collected and dried on a rotary evaporator. The residue was again extracted with ethyl acetate, dichloromethane, methanol, and water by the same process. In a sequential method of extraction, a solvent is chosen from the non-polar to polar range (Agatonovic-Kustrin *et al*., 2018).The solvents chosen for the study are as shown in the flow chart:

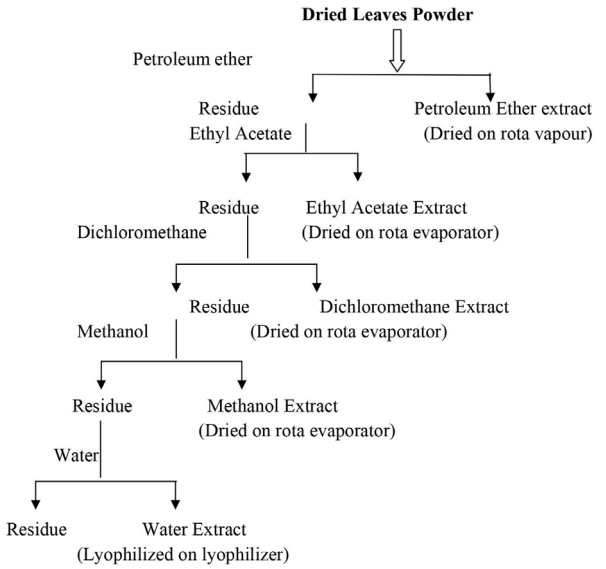

Fig. 1. Schematic Representation of Sequential Method of Extraction.

The aqueous extract is lyophilized. Lyophilization or freeze-drying is a process in which water is frozen and removed from a sample, first by sublimation and then by desorption. Freeze-drying is a drying process that sublimates moisture from a product after it has been frozen and stored for an extended period. The drying process is applied in the production of certain chemicals, pharmaceuticals, and biological products that are heat-labile or unstable in sand-water solutions (Khandagale *et al*., 2016). Each extract was evaporated on a rotary evaporator and the yield of the extract was determined using the following formula: (Abbas *et al*., 2021)

$$\textbf{Percentage yield} = \frac{\textbf{Weight of crude extract obtained}}{\textbf{Weight of dry powder taken initially}} \times 100$$

Preliminary phytochemical Screening

Preliminary phytochemical screening was carried out for all the extracts for the presence of alkaloid, flavonoid, steroid, glycoside, saponin, and tannin as reported in the literature (Waterman, 1993), (Shaikh and Patil, 2020) and (Harborne, 1967).

Detection of alkaloid

Mayer's Test

2 drops of Mayer's reagent were added to 1 cm^3 of each extract. Brown red precipitation confirms the presence of alkaloids.

Detection of Flavonoid

Alkaline reagent Test: To 1 cm^3 of each plant extract alkaline solution of 1 cm^3 10% NaOH was added. The presence of yellow coloration confirms the presence of flavonoids.

Lead acetate Test:

1 cm^3 of extract was taken in a test tube and a few drops of lead acetate solution were added to it. The formation of yellow precipitation is an indication of the presence of flavonoid.

Detection of Steroid

Salkowski's test

5 cm^3 of each extract treated with 2 cm^3 of each chloroform and hydrochloric acid and it gives red colouration which indicates that steroids are present in the extract.

Test for Glycoside

Keller Killiani test

2 cm^3 of each extract was combined with a 0.5 cm^3 solution of glacial acetic acid and two to three drops of ferric chloride. 1 cm^3 of H_2SO_4 added along the walls of the test tubes. The formation of a brown ring confirms the presence of glycoside.

Test for Saponin

Frothing Test

0.5 mg of each extract was added to distilled water and shaken vigorously. The appearance of frothing indicates the presence of saponin.

Test for Tannin

10% NaOH test

1 cm^3 of 10% NaOH was added into 0.5 cm^3 of each extract. The formation of greenish coloration indicates the presence of tannin.

Antibacterial Zone Inhibition test-*Propionibacterium acnes (p. acnes)*

Procedure

The Antibacterial activity was checked by the Zone Inhibition Method (Kirby-Bauer method). The Mueller-Hinton agar (MHA) plates were inoculated by spreading with 100 ìl of bacterial culture, *P. acnes* (adjusted to 0.5 McFarland Unit - Approx. cell density (1.5 X 108 CFU/ml) and followed by placing the discs containing 10 ìl of different concentrations. One disc in each plate was loaded with solvent alone which served as vehicle control and the Ciprofloxacin disc (10 ìg) was taken as positive control. The plates of *P. acnes* were incubated (Basil Scientific Corp. India- Incubator) at 37 °C for 24 hrs. Clear zones created around the disc were measured and recorded.

Statistical data analysis

The results were expressed as mean ± standard error of mean (SEM). One-way analysis of variance is used to test repetition of results and the level of significance was considered at $p<0.05$

Result and Discussion

Preliminary phytochemical screening

The biological activity of the plant is attributed to the phyto constituents that it possesses. Preliminary phytochemical profiling of leaves of *Phaseolus vulgaris* Linn. from different leaf extracts, as shown in Table 1.

Of all extracts, only petroleum ether, methanol, and aqueous extracts show the presence of alkaloids. Flavonoids are present in all the extracts. Only petroleum ether, methanol, and water extracts show the presence of tannins. Steroids were absent in all the extracts tested. The extraction process used is simple and quick. The calculated yields of each extract are shown in Table 2. Compared to other extracts, aqueous extracts show the highest percentage yield.

Table 2. Percentage yield of each extract of leaves of *Phaseolus vulgaris* Linn.

Extract	Percentage Yield
1. Pet ether	03.11%
2. DCM	15.30%
3. Ethyl Acetate	17.54%
4. Methanol	28.08%
5. Aqueous	30.95%

Table 1. Preliminary phytochemical screening of various extracts of the leaves of *Phaseolus vulgaris* Linn.

Extract	Petroleum				
	Ether	Dichloromethane	Ethyl Acetate	Methanol	Aqueous
Alkaloid	+	-	-	+	+
Flavonoid	+	+	+	+	+
Steroid	-	-	-	-	-
Glycoside	+	-	-	+	+
Saponin	-	+	+	-	+
Tannin	+	-	-	+	+

+ indicates Presence and – indicates absence

Antibacterial zone inhibition test

Plants have been utilized as a reliable source of medicine since ancient times. Kirby-Bauer disk diffusion used to checkthe ability of pathogenic microorganisms to respond differently to different antibiotic substances. *Propionibacterium acnes* is gram-positive bacteria that prefers anaerobic growth conditions and is involved in the pathogenesis of acne in humans (Mollerup *et al*., 2016). The zone of inhibition is the region surrounding a disk on an agar plate where an antimicrobial agent is present and prevents the growth of bacteria. It is used to determine if a particular test organism is sensitive to an antibiotic's activity or not(Arullappan, Zakaria, and Basri, 2009).

Figure 2. Shows that a 1000 µg dose of crude aqueous extract with positive control gives a zone of inhibition of about 7.5 mm diameter and 40 mm diameter respectively. The zone of inhibition is measured by Vernier Calliper (Bhargava *et al*., 2016). The information obtained from the zone of inhibition helps to decide whether the crude extract is susceptible, intermediate, or resistant to antibiotics (Jorgensen and Ferraro 2009).Figure: 2 indicates that only 1000 µg of crude extract is active for the *Propionibacterium acnes*. If the zone of inhibition of the test compound is close to the zone of inhibition of the antibiotic, the compound is considered susceptible. If there is a difference between the zone of inhibition of the test compound and the zone of inhibition of the antibiotic, then it is said to be an intermediate. If the zone of inhibition for the test compound is absent, then itis called resistance to antibiotic (Rodloff *et al*., 2008)(Bhattacharjee, 2015). At this concentration, crude aqueous extract acts as an intermediate to present antibiotic.

The study of green solvents and their pharmacological activity has gained more importance as they aid in the study of drug development and formulation. The antibacterial activity of the crude aqueous extract of the plant is due to the synergetic effects of phytoconstituents such as alkaloids, flavonoids, glycosides, saponins, and tannins. The extractive value of extracts depends on the method of extraction. The sequential method of extraction will help to isolate the phyto constituents responsible for antibacterial activity in *P. acnes*. A study of the antibacterial activity of isolated compounds will give a clear picture of

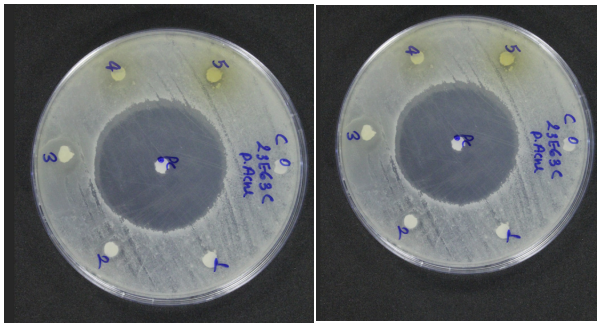

Fig. 1. Plate A and plate B. Zone of Inhibition of aqueous extract of the leaves of *Phaseolus vulgaris* Linn. 1=50 µg/disk, 2=125 µg/disk, 3=250 µg/disk, 4=500 µg/disk, 5=1000 µg/disk, Pc = Ciprofloxacin 10 µg

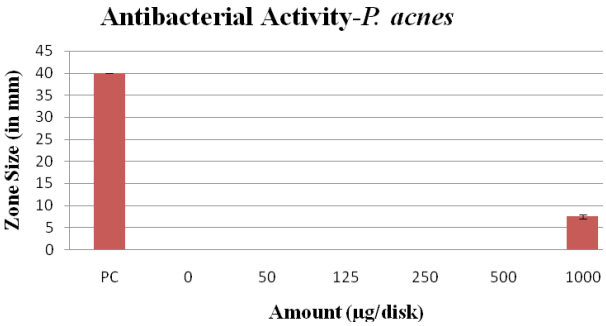

Fig. 2. Graphical representation of antibacterial activity of crude aqueous extract of leaves of *Phaseolus vulgaris* Linn.

their bioactivity. Hence, the isolation and characterization of compounds with bioactivity should be carried out to find new antibacterial drugs.

Conflict of Interest

Authors declare no conflict of interest.

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