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ASSESSMENT OF ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF ALOE VERA

Nidhi Rathore*[1] and Dr. Swati Goyal[2]

Dr. A. P. J Abdul Kalam University Indore M.P.

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***Corresponding Author**
Nidhi Rathore
Dr. A. P. J Abdul Kalam
University Indore M.P.

ABSTRACT

Aloe vera has been a popular medicinal plant for centuries and is used in our lives in many ways. It produces about six natural antiseptics, which have the power to kill mold, bacteria, fungi, and viruses. It contains major high potential beta-carotene i.e. Vitamins A, B, C, enzymes, minerals, sugar, lignin, saponins, and salicylic acid. On the basis of various phytochemicals, it is major used in the pharma sector and cosmetic industry. The present study was examining the phytochemical screening and antibacterial propriety of Aloe vera. The extracts were prepared using ethanol solvents. The phytochemical analysis and antimicrobial activity test of extracts were performed. The presence of Choline, Tinosporin, Isocolumbin, Palmatine, Tetrahydropalmatine, Magnoflorine, 18-norclerodane glucoside, Flavonoid, diterpene glucoside, Tinocordiside, Cordioside, Cordifolioside A, Cordifolioside, Tinocordifolioside, alkaloids, glycosides, carbohydrates, steroids, polyphenol, saponins, and terpenoids were indicated by testing.This experiment to determine antimicrobial resistance of Aloe vera by Zone of inhibition method has been used to examine the antimicrobial resistance of different microorganisms group i.e. Gram-negative bacteria i.e. Pseudomonas aeruginosa ATCC No.9027 and Gram-positive Bacteria i.e. Staphylococcus aureus ATCC No 6538. The sample extracts of plant has been shown the inhibition of microorganisms in the experiment soyabean casin digest agar media plate. Ethanolic extracts sample results showed that Gram-positive bacteria Staphylococcus aureus ATCC No 6538 of zone of inhibition area is high compared to Gram-negative bacteria i.e and Pseudomonas aeruginosa ATCC No.9027 of zone of inhibition area. Phytochemicals test results of Aloe Vera plant extracts is compliance against test method pre determine accepted criteria. The results of Phytochemicals and antimicrobial activity is confirmed that ethanolic extracts of plant is shown highly antimicrobial resistance.

1. INTRODUCTION

The history of medicine in India is very old. Indian medicinal system has been told in ayurveda. About 5000 plants have been included in Ayurveda. The medicinal plants are useful for healing as well as curing of many diseases because of the Phyto chemical constituents present in them. According to World Health Organization (WHO), medicinal plants are the best source to obtain a variety of drugs and compounds. Therefore, such plants should be investigated to better understand their properties, safety, efficiency and their capability or utility. Aloe vera is the plant which is used very frequently as a local medicine. Its vernacular name is Ghrit kumari, and gwar patha. It belongs to family Asphodelaceae. Africans called it the "the burn plant" and native Americans "the miracle plant that heals itself. Aloe is an ornamental succulent plant, found very commonly in gardens and pots. It is a cactus-like plant that grows in hot, dry climates and currently due to high demand; it is cultivated in large quantities. In the pharmaceutical industry, it has been used in the synthesis of topical products such as ointments and gel preparation and also in the development of tablets and capsules. In food industry it is used as source of functional foods or parts of the ingredients in other food products (Hamman, 2008).

More than 200 chemical components have been identified from the leave pulp and exudates of Aloe vera plant. It includes vitamins, enzymes, minerals like calcium, zinc, chromium, potassium, sugars (monosaccharides and polysaccharides). Leaves of aloe vera contains many polysaccharides such as glucomannan. Anthraquinones like aloin and emodin. Aloe vera gel contains phenolic constituents such as Aloin A and B, Aloenin (B), Aloesin and Chrysophanol. It is observed that aloe vera have other compounds like salicylic acid, lignin and sapoins. Some vitamins like A, B^1, B^2, B^6, B^{12}, C and E cannot be prepared by human body. That are available in Aloe vera. The gel consists of 99.3% water and the remaining 0.7% containing a range of active compounds including polysaccharides, vitamins, amino acids, phenolic compounds, and organic acids.

Aloe vera plant has also been reported to have anti-cancer, anti-diabetic, anti-inflammatory, anti-oxidant, Antimicrobial, skin hydration, wound healing and hepatoprotective effects. It is used to treat stomach ailments, gastro-intestinal problems, skin diseases, constipations, radiations injury, inflammatory effect, healing wounds and burns, ulcer and diabetes. It is found that as a cosmetic, Aloe vera is excellent in maintaining moisture, tightening and smoothing the skin. Aloe gel can help to stimulate the body's immune system. Aloe vera is a

very versatile plant that has many different uses. Numerous scientific studies on Aloe vera are demonstrating its analgesic, anti-inflammatory, wound healing, immune modulating and anti-tumor activities as well as antiviral, anti-bacterial, and antifungal properties. Johnson et al., 2012 found that Aloe barbadenssis miller (Aloe vera) possess a number of therapeutic uses viz: anti-inflammatory, immunostimulatory, antibacterial, antifungal and cell growth stimulatory activity. Aloe vera gel inhibited the growth of both Gram positive and Gram-negative organism and little inhibition on fungi. The efficacy of the Aloe gel as antimicrobial agent is shown to have wide range of activity against Gram positive and Gram-negative bacteria.

2. MATERIALS AND METHODS

2.1 Plant Material

Aloe Vera plant was collected from Barwani M.P. India in January 2020. The collected whole plant material was identified by Dr. A. P. J Abdul Kalam University Indore M.P. The whole plant was cleaned and washed with distilled water. After completion of the cleaning and washing activity, Plant material was collected in a beaker. Plant materials were dried in Laboratory Room. Then plant is converted into powder form with the help of a homogenized instrument and stored in the air-glass bottle till future use.

2.2 Preparation of plant extracts

2.2.1 Soxhlet Extractor Method was used and ethanol extract preparation. The extract was filtered with Whatman paper. The liquid was collected and stored in a glass bottle.

2.3 Phytochemical Test

Phytochemicals experiments were done.

2.3.1 Examination for Alkaloids

a) Dragendorffs test

Take 01 mL of sample and 01 mL of dragendorftts reagent into 05 mL test tube.

Result: The solution shows a red-orange color precipitate.

(b) Mayer's test

Take 01 mL of sample and 01 mL of Mayer's reagent into a 05 ml test tube.

Result: The solution shows a whitish-yellow/cream-color precipitate.

(c) Hager's test

Take 01 mL of sample and 01 mL of Hager's reagent into a 05 ml test tube.

Results: The solution shows a yellow color precipitate.

(d) Wagner's test

Take 01 mL of sample and 01 mL of Wagner's reagent into 05 ml test tube.

Results: The solution shows a reddish-brown precipitate.

2.3.2 Examination for saponins

Take 01 mL of sample, 01 mL alcoholic solution and mix 20 mL water with shaking. Keep the solution on stand by for 15 minutes.

Results: Approximately 01-02 cm foam-layer appeared in solution.

2.3.3 Examination for Glycosides

(a) Legal test

Take 01 mL of sample, pyridine and sodium nitroprusside solution (For used alkaline)

Results: The solution shows a pink-red to red color.

(b) Baliet test

Take 01 mL of sample and 01 mL of sodium picrate.

Results: Liquid shows a yellow to orange color.

(c) Keller-killiani test

Take 01 gm of sample and 10 mL of 70% IPA for 02 minutes. The solution is filtered.

Take filtered 0.5ml of lead acetate solution and 05 mL of chloroform. The chloroform layer is parted by the evaporation dish and evaporation. After the cooled residue is collected and add 03 mL of glacial acid and 01-04 drops with the help of a dropper of 5% ferric chloride solution. Pour Carefully and slowly add 02 mL of concentrated H_2SO_4.

Results: At the intersection of both liquids, a reddish-brown layer forms, and the upper layer gradually turns bluish-green, darkening with time.

(d) Borntrager's test

Take a sample and mix 0.1 to 0.4 mL dilute H_2SO_4. Test tubes are boiled and filtered in the solution with the use of chloroform. The solution is treated with 01 ml of ammonia.

Results: The ammonia layer is show red color.

2.3.4 Examination for Carbohydrates and sugar

(a) Molisch's test

01 ml of α-naphthol solution and mix the same quantity of sample. After that few drops of concentrated H_2SO_4 by the pipette.

Results: The junction of the two liquids has a purple or reddish-violet coloration.

(b) Fehling's test

Take Fehling solution A and B and mix sample.

Results: The tested liquid show a brick-red precipitate after heating. Sugar is present.

(c) Benedicts test

Take a 02 mL sample and mix Benedicts reagent. Heating for 02 minutes and cool.

Results: Red precipitate formation is shown, and sugar is present.

2.3.5 Examination for tannins and phenolic compounds

Take a 01 mL sample and add lead acetate solution.

Results: White precipitate is shown, and tannins are present.

2.3.6 Examination for Flavonoids

Take approximately 01 ml of ethanol extract sample and mixed with ammonia solution.

Results: The appearance of fluorescence in ultraviolet and visible light indicates the presence of flavonoids.

2.3.7 Examination for Steroids

Libermann-Burchard test

Take a 01-gm sample and 0.3 ml of chloroform,03ml of acetic anhydride, and 03 mL of glacial acetic acid. Used tap water for cooling and put down some drops of concentrated sulphuric acid.

Results: The presence of sterols is indicated by the appearance of a bluish-green color.

2.4 Test of Specific spiked microorganism

The presence antimicrobial activity test of ethanol extract sample was performed against two Specific spiked microorganisms i.e ***Staphylococcus aureus*** ATCC No,6538 and ***Pseudomonas aeruginosa*** ATCC No.9027

2.4.1 Media Preparation

Always use a clean and dry flask for the preparation of media. Measure the required amount of water or equivalent to purified water with the help of a Clean and dry measuring cylinder. Weight accurately the 30.00 g of media. Slowly add media to the flask with purified water taking care that media does not spill. Take pH of media before sterilization in an autoclave. After completion of sterilization, media was unloaded from the autoclave. Cool to 45 degrees centigrade and pour media in under LAF. After sonification of the media plate, after pH was done and a Growth promotion test was performed. The media plate is ready for testing.

2.4.2 Culture preparation of *Staphylococcus aureus* ATCC No.6538 and *Pseudomonas aeruginosa* ATCC No.9027

Handle microbial cultures carefully to avoid contamination of the area. Clean the Biosafety cabinet and under Biosafety cabinet culture ampoule open. Proceed further as per in-house. Incubate SCDM tubes at 30°C to 35°C for 24 hours and SDM tubes at 20- 25°C for 72 hours. After completion of the incubation period, observation was done and noted. Then cultures of Staphylococcus *aureus* ATCC No.6538 and *Pseudomonas aeruginosa* ATCC No.9027 are ready for testing.

2.4.3 Zone of the inhibition Test method

Take media plate of SCDA agar and transfer to biosafety cabinet. Take culture tube of *Staphylococcus aureus* ATCC No 6538 and spared plate method proceed. Use 02 SCDA plate (for testing and make 01 cups of 8.0 mm diameter with cork borer on each plate. In each petri-dish, pour 100 µL, of each of the ethanol extract sample solutions. Keep the plates as such for 1 h for the diffusion of solution. Transfer the plates carefully into an incubator set at 30-35 °C so that there is no spill of dilution filled into each cup. Incubate the Petri-dishes at 30-35 °C for 24 h. Measure the diameter of the white zone produced by ethanol extract sample solution after incubation on a suitable antibiotic zone reader or Vernier caliper. Same procedure are apply for *Pseudomonas aeruginosa* ATCC No.9027.

3. RESULTS AND DISCUSSION

In the present study, the phytochemical test of ethanol extract sample extracts of Aloe Vera demonstrated the presence of alkaloids, glycosides, carbohydrates, steroids, polyphenol, saponins, and terpenoids. Refer to table 01.

Table 1: Phytochemical analysis of ethanol extract sample extracts of Aloe Vera.

S.No	Name of test	Results
1.	Test for alkaloids	Present
2.	Test for saponins	Present
3.	Test for Glycosides	Present
4.	Test for carbohydrates and sugars	Present
5.	Test for tannins and phenolic compounds	Present
6.	Test for flavonoids	Present
7.	Test for steroids	Present

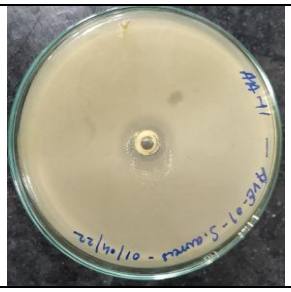
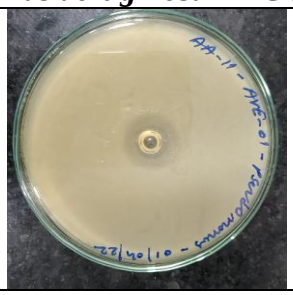
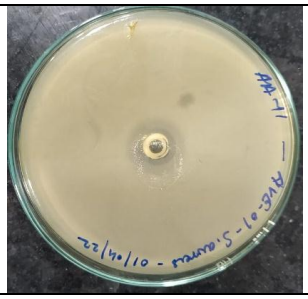
In the present study, the zone of inhibition method was used for antimicrobial activity. The present study has shown that extract possesses significant antimicrobial activity. The antimicrobial effect was found to be significant against Gram-negative bacteria and gram-positive bacteria**.**

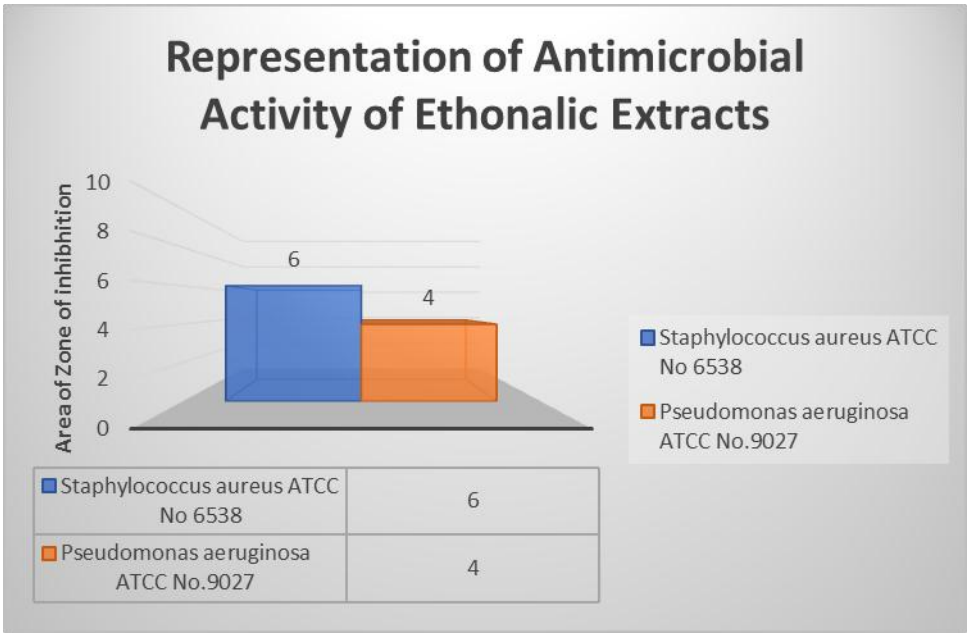
The zone of inhibition area of the sample is recorded low in *Pseudomonas aeruginosa* ATCC No 9027 and high in *Staphylococcus aureus* ATCC No.6538

Table 2: Antibacterial activity of ethanol extract sample extracts of Aloe Vera.

Plate	Name of test	Zone of Inhibition
1	*Staphylococcus aureus* ATCC No 6538	6 Cm
2	*Staphylococcus aureus* ATCC No6538	6 Cm
3	*Pseudomonas aeruginosa* ATCC No.9027	4 Cm
4	*Pseudomonas aeruginosa* ATCC No.9027	4 Cm

Image of *Staphylococcus aureus* ATCC No.6538 and *Pseudomonas aeruginosa* ATCC No.9027.

***Staphylococcus aureus* ATCC No.6538**	***Pseudomonas aeruginosa* ATCC No.9027**
	
***Staphylococcus aureus* ATCC No.6538**	***Pseudomonas aeruginosa* ATCC No.9027**
	



It is concluded that plant extracts are given natural antimicrobial resistance can be applied for different microorganisms group i.e gram positive and gram negative. This study suggests that the antimicrobial properties of Aloe vera may be due to the presence of chemical compounds

Further phytochemical research is needed to identify the isolated bioactive chemicals responsible for these species' antimicrobial properties, which should be used to produce new antimicrobial agents. The reported activity confirms this plant's long-standing use in the treatment of infectious illnesses.

4. CONCLUSION

The present studies high lights on the varying phytochemical contents in the herb, Aloe Vera which makes it a popular choice for folk medicine and also must be considered as a source for alternative medicine.

This plant extract has a variety of antimicrobial properties, and it can be exploited to treat a variety of ailments. Although, further research on characterization and standardization is recommended for this valuable plant.

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