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PHYTOCHEMICAL EVALUATION AND DETERMINATION OF ANTIMICROBIAL ACTIVITY OF ROOT EXTRACTS FROM ALOE VERA

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ABSTRACT

Antibiotics are the most successful family of drugs as yet developed for improving animal and human health. Due to the increasing antibiotic resistance to of many bacteria, plant extracts are of new interest as antiseptics and anti-microbial agents in medicine. Aloe vera is a medicinal plant part of the family Liliaceae. This study investigated the phytoconstituents and anti-microbial activity of Aloe vera root extract. Phytochemical screening confirmed that the methanolic section showed the best possible number of phytochemicals compared with solvents like Acetone and chloroform. Extracting of roots in chloroform exhibits the presence of rich phytochemicals compared with Acetone and methanol. The anti-microbial activity of Aloe vera root extract was examined using the disc diffusion method in 3 human pathogens like Escherichia coli, Staphylococcus aureus, Bacillus subtilis, and fungi like Aspergillus Niger and Penicillium chrysogenum. The extract has inhibitory effect against both bacteria and fungus. This research study would be helpful for the development of new medications and formulations.

Keywords: *Anti-Microbial Activity, Microorganism, Aloe Vera Root.*

Introduction

The use of herbal medicine is widespread worldwide and is a significant component of primary healthcare in many countries, including India [1]. Many places around the world, especially in underdeveloped nations where traditional medicine is relied upon to maintain human and animal health, the use of medicinal plants to cure illnesses is an ancient practise [2, 20]. In order to promote and incorporate traditional medicine within their national health care system, they have taken this into consideration [3]. 25 percent of the solid component in aloe plants are sugars. Sugar has the capacity to enhance and slow both the immunological response [4]. Anthraquinone is a phenolic chemical, and these molecules have potent analgesic and antibacterial properties as well as powerful purgative actions [5]. Aloe vera's biological actions include promoting the healing of wounds, antifungal activity, hypoglycaemic or antidiabetic effects, anti-inflammatory, anticancer, immunomodulatory, and gastro protecting properties [6]. Due to its immune-modulating properties, several Aloe species have been utilised as an antiviral, for ulcer treatment, and even to treat melanoma [7]. As a result, there is a pressing need to develop some novel plant-derived antibacterial agents.

Leaves of aloe-vera include phytochemicals like acetylate mannans, poly-mannans, anthraquinone, glycosides, anthrones, emodin, and different lectins that are being investigated for their potential bioactivity [8]. Aloevera gel is a transparent, clear, and soothing gel applied topically for a variety of skin issues, including pimple. It has enzymes that lessen inflammation and ease pain.

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Additionally, it lessens inflammation and redness and has antifungal and antibacterial properties. Due to its capacity to stop blistering and scarring, A. vera has the ability to cure serious burns [9]. It serves as a versatile skin treatment. The presence of saponin, a chemical molecule that functions as an antibacterial agent, may be partially to blame for this [10,20,21].

Materials and Methods

Phytochemical Test

- **Preparation of Extract:** Fresh Leaves and roots of Aloe vera were collected from the outer place of town. The Leaves and roots of Aloe vera were air-dried and grinded into fine powder. Four grams of grinded powder had been percolated with one 150 ml of solvent (Methanol, Acetone, and Chloroform) for extraction and kept at Soxhlet 150^0 C temperature for 24 hours. After extraction, the extract used for phytochemical evaluation.
- **Alkaloid**: Wagner's test: 2 ml of filtrates, few drops of Wagner were mixed by the side wall of the test tube. A brownish-red precipitate confirmed the test as positive [11,18].
- **Saponins**: Foam test: -1ml of extract was shaken with little quantity of water. The foam produced persists for 10 minutes confirms the presence of saponins.
- **Flavonoids**: NaOH test: 1ml extract was treated with aqueous NaOH and HCl, the colour changes to orange yellow in presence of flavonoid.
- **Tannins**: Ferric chloride test: 1ml extract mixed with 1ml 1% ferric chloride solution gives blue, green or brownish green colour.
- **Steroids**: Salkowski tests: 1ml extract when shaken with 1ml concentrated sulphuric acid and on standing yield red colour.
- **Anthraquinones**: Chloroform layer test: 5 millilitre of extract was taken in test tube which was dried and 5 ml of $CHCl_3$ was added and vigorously shaked for 5 minutes. Then complete extract was filtered and the filtrates mixed with equal volume of 10% of ammonium solution (Dissolve 40 ml of 25% NaOH and make the proportion to 100 ml with distilled water). A pink violet or red colour in the ammonical layer indicates the presence of anthraquinones [11,19].
- **Cardiac Glycosides**: Sodium hydroxide test: 1 ml of extract was dissolved in 1 ml of 1 N sodium hydroxide solution. A yellow colour confirms the presence of glycosides in extract.
- **Terpenoid**: Salkowski test: Added 0.5 ml of chloroform with 2ml of extract mix and then added 1ml of concentrated Sulphuric acid from the side to form a ring. Formation of reddish-brown precipitate at the interface indicates the presence of terpenoids [19].

Anti-Microbial Analysis

- **Preparation of Extract:** The powdered plant material was subjected for successive extraction using solvents according to the polarity (ethyl acetate, aqueous extract, ethanol, methanol, chloroform and Acetone). One gram of the powder was taken and added into 10 ml of solvent then kept it for 24 hours at room temperature. Then, extract filtered with Whatman No. 1 filter paper. The filtered extract centrifuges at ten thousand rotations per minute (rpm) for 20 minutes at – 4^o Centigrade [12].
- **Bacterial Cultures:** Bacterial (Gram-positive and Gram-negative) and fungal culture were collected from Department of Biotechnology, Rajasthan University for anti-microbial assay. All the bacterial and fungal strains were cultured in Nutrient broth in an incubator for 48 hours at 37°C. The anti-bacterial and anti-fungal activity of Aloe vera root extract was tested using Agar the disc diffusion technique [7].
- **The Disc Preparation for Microbiological Analysis:** The 6-millimetre (diameter) the discs were prepared from Whatman No. 1 filter Paper and sterilized by autoclave at 121°C. By completion sterilization the moisture the discs had been dried on hot air oven at temperature 50°C. Then various type of discs was prepared for determination by placing a specific quantity in micrometer with the various extracts. Then prepared discs placed in prepared solidified media plates and measured the zone of inhibition [17]. The zone of inhibition should be more than 8 mm then only it can be considered as effective against different microorganisms.

Anti-bacterial and Anti-fungal Activity

Nutrition and suitable environment are always primary requirement for the organisms. The potato dextrose agar (PDA) plates and sterile nutrition agar plates were made for evaluation. The bacterial test organisms like Staphylococcus aureus, Bacillus subtilis, and Escherichia coli were spread over the nutrient agar plates. The fungal organism like Aspergillus Niger and Penicillium chrysogenum were spread over the potato dextrose agar plates. Following the development of the microbial grass, three distinct plants extract the discs were utilized, each with a considerable variance in the amount used, and distance control the discs were also made. All of the bacterial plates were incubated for 24 hours at 27°C and the fungal plates for 72 hours at 24°C. Anti-microbial activity was determined via measuring the diameter of zones of inhibition (millimetre) produced after incubation. For each test, three replicates had been carried out and the final results were calculated accordingly [11].

Result and the Discussion

- **Phytochemical Analysis**

The outcomes of phytochemical screening for the leaves and roots of Aloe vera in different solvent extracts presented in Table No. 1. Alkaloids were present in all the solvent extracts, and Anthraquinones were totally present in all plant extracts. Cardiac glycosides were evident high quantity in chloroform but low amount in Methanol and Acetone extracts of the Leaves. It additionally shows that Flavonoids and terpenoids had been confirmed in methanol and chloroform. Tannins found present in low amount in Chloroform, Methanol and Acetone extracts. Saponins were present in all of the solvent extract whereas Steroids found present in low amount in all the extracts of the leaves. Phytochemical screening of extract of root barks of Aloe vera using methanol, Acetone and chloroform have been compared. Alkaloids and anthraquinones have been present in low amount in all the solvents' extract. Cardiac Glycosides only confirmed in good concentration in the methanol solvents' extracts in comparison Acetone and chloroform solvents' extract in root of the plant. Saponins, Flavonoids Tannins and Terpenoids were present in low amount in all extracts of Aloe vera root. Steroids had been located highly present most effective in Acetone extract compared to other two solvent extract of the plant's root. Cardiac Glycosides were highly present in the methanol solvents' extracts compared Acetone and chloroform solvents' extract in root of the plant. The alkaloids, saponins and tannins in more than a few antibiotics used in treating long-established pathogenic traces [9, 11]. Steroids are reward in all leaf and root plant extract while highly present in Acetone extract of root. Steroids have been reported to have antibacterial properties [12]. It also demonstrates that while flavonoids and terpenoids are found in modest concentrations in the root of the plant, they are highly beneficial in the methanol and chloroform extracts of the plant's leaves. Flavonoids and tannins are phenolic compounds, according to a recent review, and plant phenolics are a significant class of chemicals that function as main antioxidants or scavengers of free radicals [13].

Table 1: Phytochemical Result

Phytochemicals	Methanol extract		Acetone extract		Chloroform extract	
	Leaf	Root	Leaf	Root	Leaf	Root
Alkaloids	+	+	+	+	+	+++
Anthraquinones	+++	+	+++	+	+++	+
Cardiac glycosides	+	+++	+	+	++	+
Flavonoids	++	+	++	+	++	+
Saponins	+++	+	+++	+	++	+
Steroids	+	+	+	+	+	+
Tannins	+	+	+	+	+	++
Terpenoids	++	+	+	+	++	+

+ mild, ++ moderate, +++ Higher

- **Anti-microbial Analysis**

There are many infectious diseases are still a major challenge for health issues in the world. The emergence of resistance to antibiotics has further compounded the problem [14]. The results of antibacterial activity of root extracts of Aloe vera is presented in figure1.The different solvents extracts i.e., Ethyl Acetate, methanol & aqueous were tested against the human pathogenic bacteria, namely, Escherichia coli, Bacillus subtilis, and Staphylococcus aureus. It was observed that Ethyl Acetate extract showed maximum zone of inhibition 5.2 mm, 4.3 mm & 2.1 mm against the bacterium E. coli Bacillus subtilis, Staphylococcus aureus respectively. Based on the above observation Ethyl acetate extract was again evaluated for anti-microbial activity against Escherichia coli and Bacillus subtilis and Staphylococcus aureus mentioned in Table 2.

Table 2: Anti-bacterial Activity of Aloe Vera Root Extract in Different Solvent

Zone of Inhibition (in Millimetre)			
Microbial Strain	**Acetone**	**Methanol**	**Aqueous**
E. coli	5.2	4.3	3.5
B. Subtilis	4.3	3.9	1.9
S. Aureus	2.1	1.9	0

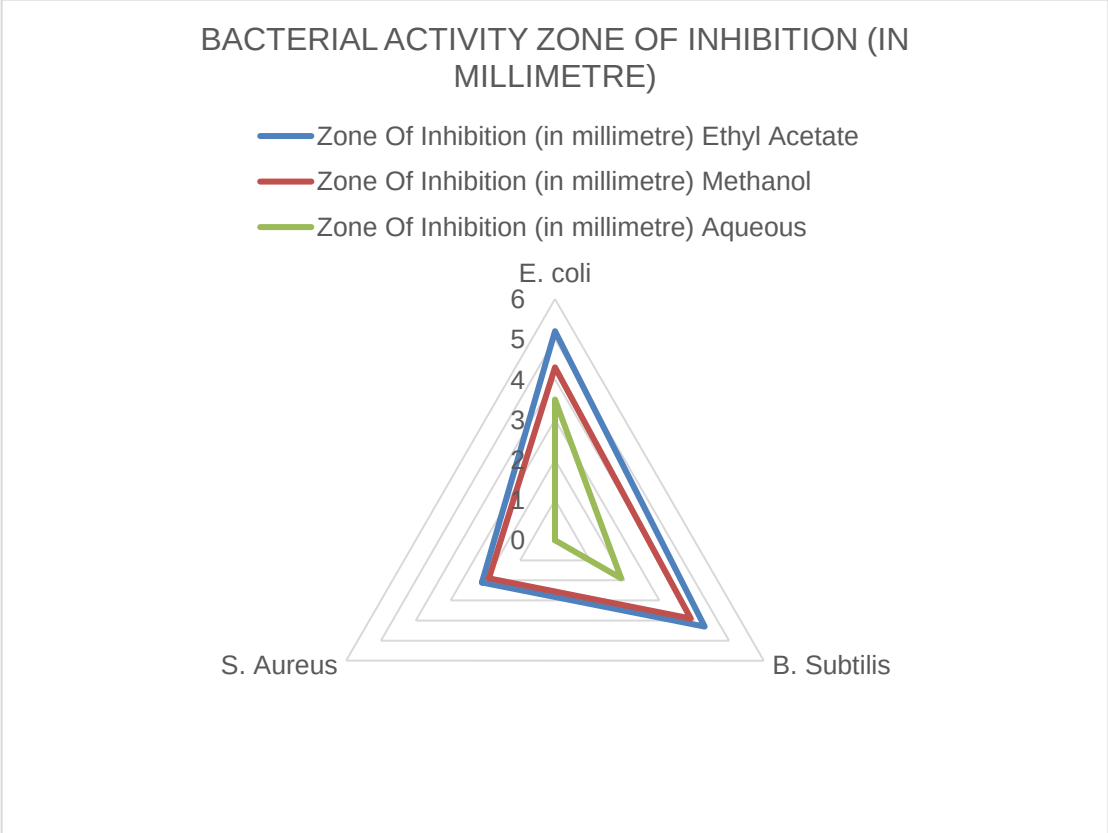

Chart 1: Bacterial Activity Zone of Inhibition (in Millimetre)

Antifungal Activity

The Acetone portion of Aloe root extract showed antifungal activity against all tested fungi between 4 and 42 mm, named Aspergillus niger and Penicillium chrysogenum at the tested concentration. Aloe vera extract in Acetone revealed qualified antifungal activity. Extracts of Aloe vera roots were screened for antifungal activity against Aspergillus niger and Penicillium chrysogenum. The maximum zone of inhibition 44 mm for Aspergillus niger and 26 mm for Penicillium chrysogenum were observed. It is better explained in plotted graph. All the five different concentrations (100, 200,400,600,800 µg/ml) of extracts of Aloe vera showed the inhibitory effect on the both fungal species mentioned in table no.3. However, specific plant compounds of Aloe vera such as anthraquinones and di-hydroxy anthraquinones as well as saponins [3,12] have been proposed to have direct anti-microbial activity.

Table 3: Anti-fungal Activity shown by Aloe Vera Root Extract on Fungi

Name of the Fungus	**Conc. of Aloe vera root extract in µg/ml & Zone of Inhibition (in mm)**				
	Acetone Extract				
	100	**200**	**400**	**600**	**800**
Aspergillus Niger	15	27	32	39	44
Penicillium chrysogenum	6	11	17	18	26

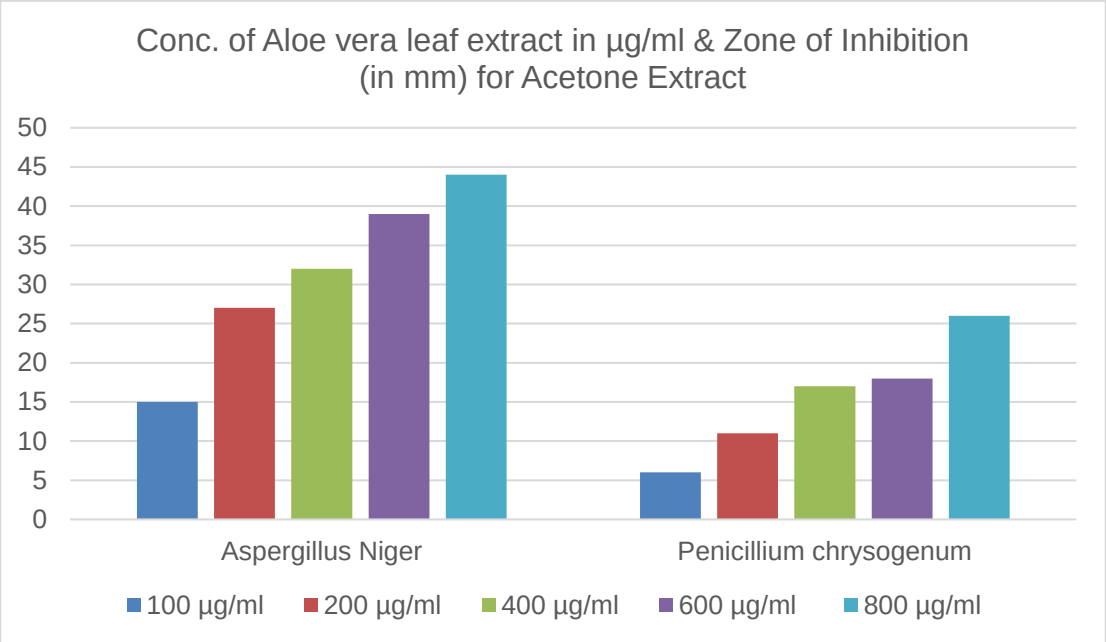

Chart 2: Conc. of Aloe vera root extract in µg/ml / Zone of Inhibition (in mm) for Acetone Extract

Discussion

The main factors that determine the anti-microbial activity are the type and composition of the plant extract, amount used, type of microorganism, pH value and temperature of the environment [13, 14]. The solvent that used to extract the components of the medicinal plant also affect anti-microbial activity of the extract [15]. The results clearly demonstrated that Aloe vera possesses anti-microbial activity. possesses anti-microbial activity amount of the anthraquinones and phenolic antioxidants in the Aloe vera extract could be responsible for the observed anti-microbial activity [16].

Conclusion

It is concluded that plant extracts having great impact on phytochemical and anti-microbial activity against bacterial and fungi pathogens. This can be used in the treatment of infectious diseases. This research study would lead to get valuable information about some compounds that could be used to prepare new and more potent and effective anti-microbial medicines of natural origin.

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