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Preliminary Phytochemical Analysis of *Brugmansia suaveolens* and Study of its Antimicrobial Activities

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Abstract: ***Brugmansia suaveolens (family:solanaceae) is a medicinal semi-woody shrub which is known as angel's tears and snowy angel's trumpet. Every part of Brugmansia suaveolens is poisonous as well as exhibit curative activity against several ailments like headache, reduce inflammatory swellings of joints in rheumatic attacks etc. In the present study, the bioactive compounds present in the flower of Brugmansia suaveolens were investigated for antimicrobial activity against some pathogenic bacteria (Staphylococcus aureus, Escherichia coli) and fungus(Penicillium atramentosum).dried flower samples were used to obtain various extracts viz.Chloroform, Acetone,Ethanol, and water. The phytochemical analyses showed the presence of phenol, steroids, saponins, Tannins flavonoids glycosides terpenoids alkaloids phlabatannins extracts used in this study.under the well diffusion method had a considerable antimicrobial activities increased with higher concentration. The Ethanol extract from dry flower Brugmansia suaveolens shows more effective against Escherichia coli, The Chloroform extract from dry flower Brugmansia suaveolens shows more effective against Staphylococcus aureus. In case of fungi Chloroform extract from dry flower Brugmansia suaveolens showed the highest inhibition zone against Penicillium atramentosum.***
Keywords: Brugmansia suaveolens, Phytochemical, Antimicrobial, Well diffusion method, Secondary metabolites

I. INTRODUCTION

Medicinal plants, also called medicinal herbs, have been discovered and used in traditional medicine practices since prehistoric times. Plants synthesize hundreds of chemical compounds for various functions, including defense and protection against insects, fungi, diseases, and herbivorous mammals[1].

The earliest historical records of herbs are found from the Sumerian civilization, where hundreds of medicinal plants includes. The Greek physician Dioscorides, who worked in the Roman army, documented over 1000 recipes for medicines using over 600 medicinal plants in De material-medica.

Drug research sometimes makes use of ethno-botany to search for pharmacologically active substances, and this approach has yielded hundreds of useful compounds. These include the common drugs aspirin, digoxin, quinine, and opium. The compounds found in plants are diverse, with most in four biochemical classes: alkaloids, glycosides, polyphenols, and terpenes. Few of these are scientifically confirmed as medicines or used in conventional medicine. Medicinal plants are widely used as folk medicine in non-industrialized societies, mainly because they are readily available and cheaper than modern medicines. In many countries, there is little regulation of traditional medicine, but the World Health Organization coordinates a network to encourage safe and rational use. The botanical herbal market has been criticized for being poorly regulated and containing placebo and pseudoscience products with no scientific research to support their medical claims [2].

Ayurveda is a traditional medicine system from India. Ayurveda is becoming increasingly popular in Europe, with many chronic conditions responding to it well. Charak is known as the father of Ayurveda or the father of Ayurvedic medicine. He wrote a book named Charak Samhita, on medicine which contained the description of a large number of diseases and discusses their treatment. Apart from medicinal use, Ayurvedic herbs can also be used for purposes like pest control, natural dyes, and formulation of food items, teas and perfumes among others. If we look at various researches from across the world, a sudden spurt in cases of people turning to natural herbs for treatments and usage in everyday life has gone up significantly. Going back to the basics, people have realized the threat chemically treated products pose to their life and are rightly so adopting healthier ways of life by including Ayurveda and its principals as the mainstay of their life.

Traditional Indian medicine (ayurveda) is becoming increasingly popular, with many chronic conditions responding to it well. Traditional Indian medicine, or ayurveda, is based on a traditional medical system, in the same way as traditional Chinese-medicine, with both being developed in their respective geographic regions. Ayurvedic practice is around 3000 years oldest with a long history of managing disease.

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A. Phytochemicals

Phytochemicals are chemical compounds produced by plants, generally to help them resist fungi, bacteria and plant virus infections, and also consumption by insects and other animals. Some phytochemicals have been used as poisons and others as traditional medicine. As a term, phytochemicals is generally used to describe plant compounds that are under research with unestablished effects on health, and are not scientifically defined as essential nutrients. Regulatory agencies governing food labeling in Europe and the United States have provided guidance for industry to limit or prevent health claims about phytochemicals on food product or nutrition labels. From ancient time, the belief has been that the plants contain some biologically active compounds with therapeutic properties useful for treatment of various ailments, including asthma, gastro-intestinal problems, skin disorders, respiratory and urinary complications, hepatic and cardiovascular disease etc. The medicinal value of these plants signifies a great potential for the discovery and development of new pharmaceuticals due to its chemical substances that produce a positive physiological action on the human body[3][4].Different parts of the plants, such as in the roots, stems, leaves, flowers, fruits or seeds are the deposited areas of phytochemical and are often seen as pigmented molecules in the outer layer of plant tissue[5].

Medicinally important plants having its pharmacological benefits due to accumulation of bioactive phytochemicals in the plant tissue considered as primary and secondary metabolites. Primary metabolites as organic compounds that comprises of glucose, starch, polysaccharide, protein, lipids and nucleic acid which are helpful for growth and development of the human body. Plants produce secondary metabolites which include alkaloids, flavonoids, saponins, terpenoids, steroids, glycosides, tannins, volatile oils etc [6.7].Phytochemicals are secondary plant metabolites can be classified based on the chemical composition (containing nitrogen or not), chemical structure (for example, having rings, containing a sugar), the biosynthetic pathway (e.g., phenylpropanoid, which produces tannins) or their solubility in various solvents. many of plants contain secondary metabolites can be divided into three chemically distinct namely alkaloids, terpenes and phenolics that could be potential sources for several effective drugs.

B. Alkaloids

Alkaloids are generally present in higher plants, particularly in dicots, whereas only a few have been noted in lower plants. The alkaloids can occur in the whole plant or in the specific plant organ. Alkaloids are derived from amino acid mostly contain one or more carbon rings which usually contain nitrogen. The type of Alkaloids and plant families depend upon the position of nitrogen atom in the carbon ring. Alkaloids play important roles in plants as it checks the feeding of herbivores, protects from pathogenic hit, and inhibitions of competitors . Alkaloids have several pharmacological importance like antihypertensive (many indole alkaloids) and antiarrhythmic (quinidine, spareien) effects, antimalarial activity (quinine) and anticancer actions (dimericindoles, vincristine, vinblastine). A few alkaloids contains caffeine, nicotine, and morphine etc possessing the stimulant property used as the analgesic and quinine as the anti-malarial drug [5].[6].

C. Flavonoids

Flavonoids are secondary metabolites that are very abundant in plants, fruits, and seeds, responsible for the color, fragrance, and flavor characteristics. In plants, flavonoids perform many functions like regulating cell growth, attracting pollinators insects, and protecting against biotic and abiotic stresses[8].Flavonoids are included in the large family of phenolic compounds or polyphenols and comprise more than 6000 different structures[9].Flavonoids are phytochemical compounds present in many plants, fruits, vegetables, and leaves, with potential applications in medicinal chemistry.

D. Phenols

Plant phenolics are generally involved in defense against ultraviolet radiation or aggression by pathogens, parasites and predators, as well as contributing to plants' colors. They are ubiquitous in all plant organs and are therefore an integral part of the human diet. Phenolics are widespread constituents of plant foods. Phenolics are compounds possessing one or more aromatic rings with one or more hydroxyl groups. They are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants, with more than 8,000 phenolic structures currently known, ranging from simple molecules such as phenolic acids to highly polymerized substances such as tannins [10].

E. Saponins

They are commonly occurring compounds that are widely distributed in all cells of legume plants. Saponins, which derive their name from their ability to form stable, soaplike foams in aqueous solutions, constitute a complex and chemically diverse group of compounds. In chemical terms, saponins contain a carbohydrate moiety attached to a triterpenoid or steroids. Saponins are attracting considerable interest as a result of their diverse properties, both deleterious and beneficial [11].

II. OBJECTIVES

The present study is undertaken with the following objectives

1) Collection and extraction of plant material.
2) Analysis of the plant extract for phytochemicals.
3) To study the antimicrobial activity against selected gram negative bacteria and gram positive bacteria.

III. MATERIALS AND METHODS

A. *Collection of plants*

Brugmansiasuaveolens plant flowers were collected from Madikeri, Karnataka, India.

B. *Preparation of fresh flower extract*

Fresh flowers were plucked from the plant, washed cleanly under the tap water to remove contaminants then left for shade dry for a week. Then flowers were powdered and stored in an air tight container until further use.

C. *Stock preparation (Flowers extraction)*

40 gm of flowers powder was weighed using electronic balance, and it is placed inside a thimble made from thick filter paper, which was loaded into the main chamber of soxhlet extractor. The soxhlet extractor was placed onto a flask containing the extraction solvent i.e., 280 ml of ethyl alcohol. Then the soxhlet was equipped with a condenser with continuous water flow.The solvent is heated up to its boiling point i.e., 78°C. Then the soxhlet apparatus was run for 48 hrs, and waited for the completion of the extraction process. Then the liquid extract was collected in a clean conical flask, covered and stored for further use for conducting phytochemical tests and to perform antibacterial activities.

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D. Antimicrobial assay

It is the method of measuring growth inhibition of selected bacteria and fungi. The principle is that the organism is inoculated into a medium containing all growth factors needed and antimicrobial substance. The zone of inhibition is proportional to the concentration of extract added.

E. Antibacterial assay

Escherichia coli species were collected from the Microbiology laboratory, Yuvraja's college, Mysuru. Obtained the sub-culture on nutrients agar slants for further use.

1) *Preparations of Nutrient Agar Medium:* The nutrient agar medium was dissolved in required amount of distilled water with little amount of agar agar and then made up to 250ml and then agar was added pH was adjusted to 7. Media was sterilized by autoclaving at 121°c for 15min.
2) *Antibacterial Activity:* The antibacterial activity was tested by well diffusion method.
3) *Well Diffusion Method:* The effect of the flower extract of *Brugmansia suaveolens* on bacterial growth was determined by using agar well diffusion technique. Sterilized nutrient media was poured in sterile Petri plates. Once the media get solidify, the plates were incorporated with 5 wells with the help of sterile borer [5 mm diameter], then a loopful suspension of tested bacteria were spread uniformly with sterile cotton swab. Following this, the wells were poured with different concentrations of various extracts i.e 25µl,50µl,75µl. Other hand Finally, well containing distilled water and ampicillin were served as negative and positive control respectively, following the plates were incubated at 25°C for 2-3 days , then the inhibition zone was observed. All these procedure is done inside the laminar air flow chamber to maintain the aseptic condition.

F. Antifungal assay

1) *Collection: Penicillium atramentosum* species were collected from the Microbiology laboratory, Yuvaraja's college, Mysuru. Obtained the sub-culture on nutrients agar slants for further use.
2) *Preparations of Nutrient agar Medium:* 39g of Potato nutrient agar medium was dissolved in required amount of distilled water with little amount of agar agar and then made up to 250ml and then agar was added pH was adjusted to 7. Media was sterilized by autoclaving at 121°c for 15min. After sterilization the PDA media was poured into sterile Petri plates under aseptic condition and allowed to solidify.
3) *Antifungal Activity:* The antifungal activity was tested by well diffusion method.
4) *Well Diffusion Method:* The fungal inoculums prepared were used to test the antifungal potential. The PDA media was poured into sterile Petri plates in aseptic condition then plates were allowed to solidify in laminar air flow chamber. Once the media get solidify, the plates were incorporated with 5 wells with the help of sterile borer [5 mm diameter], then a loopful suspension of tested fungi were spread uniformly with sterile cotton swab. Following this, the wells were poured with different concentrations of various extracts i.e 25µl,50µl,75µl. On the other hand Finally, well containing distilled water and Bavistin were served as negative and positive control respectively, following the plates were incubated at room temperature for 4-5 days , then the inhibition zone was observed. All these procedure was done inside the laminar air flow chamber to maintain the aseptic condition. And finally antifungal activity was calculated by measuring the diameter of inhibitory zones in mm.

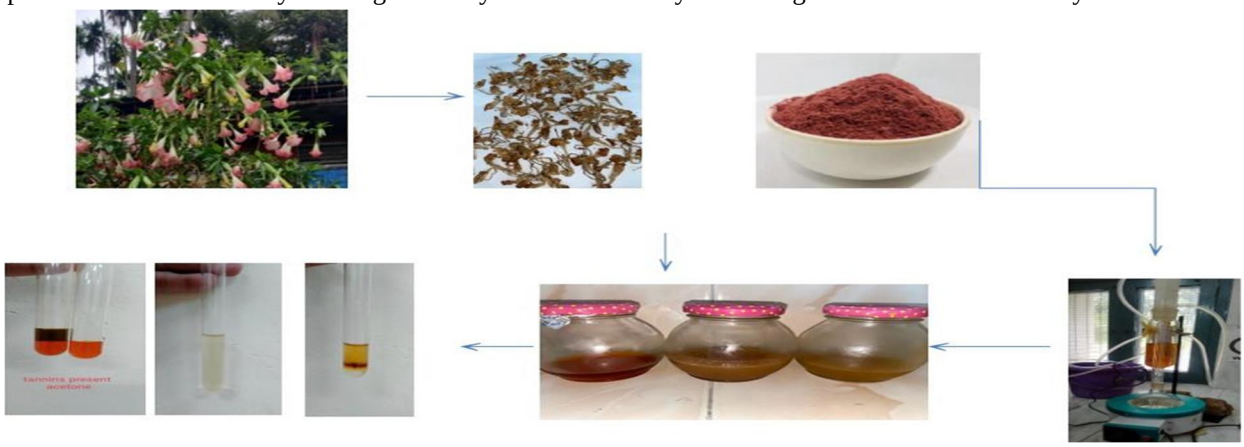

Fig 2: Diagrammatic representation of phytochemical analysis

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IV. RESULT AND DISCUSSION

A. *Results*

In the present study the results were recorded for the phytochemical and Antimicrobial activity of *Brugmansia suaveolens*

Table 1: Phytochemical analysis of *Brugmansia suaveolens*

Sl.No.	Test	Solvents			
		Chloroform	Acetone	Ethanol	Water
01	Steroids				
	Salkowski's test	+	-	+	-
02	Terpenoids				
	Salkowski's test	-	-	+	-
03	Saponins				
	Foam test	+	+	+	-
04	Alkaloids				
	Mayer's test	-	-	+	+
05	Tannins				
	Ferric chloride test	-	+	-	-
06	Flavonoids				
	Ferric chloride test	-	+	-	-
07	Proteins				
	Biuret test	-	-	-	-
08	Carbohydrates				
	Benedict's test	-	-	-	-
09	Phlobatannins				
	Hcl test	-	-	+	-
10	Glycosides				
	Keller-kiliani test	-	+	-	+

Note:-

+ ve :- Present

- ve :- Absent

Zones of inhibition area [in mm] against gram negative and gram positive bacteria in response to the impregnated sample.

Table.01: This table shows antibacterial activity of chloroform extract from dry flower of *Brugmansia suaveolens*

Inhibition zones (mm)					
BACTERIAL CULTURE	+ve control	-ve_control	25µl	50µl	75µl
Escherichia coli	21	0	0	0	0
Staphylococcus aureus	24	0	0	18	22

Graph 1.Graphical representation of antibacterial activity of chloroform extract from dry flower of *Brugmansia suaveolens*

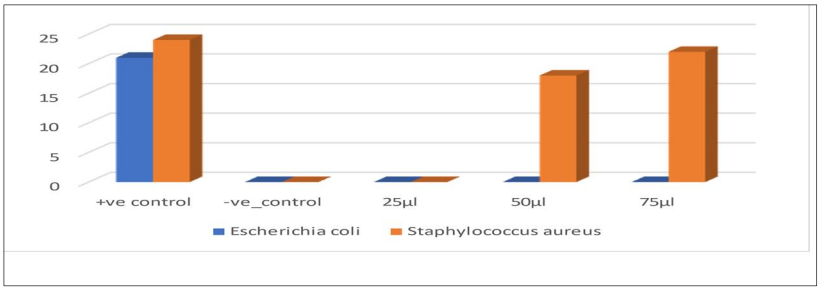

Table.02: This table shows antibacterial activity of Acetone extract from dry flower of *Brugmansia suaveolens*

Inhibition zones (mm)					
BACTERIAL CULTURE	+ve control	-ve_control	25µl	50µl	75µl
Escherichia coli	24	0	0	0	0
Staphylococcus aureus	23	0	11	0	10

Graph 2.Graphical representation of antibacterial activity of Acetone extract from dry flower of *Brugmansia suaveolens*

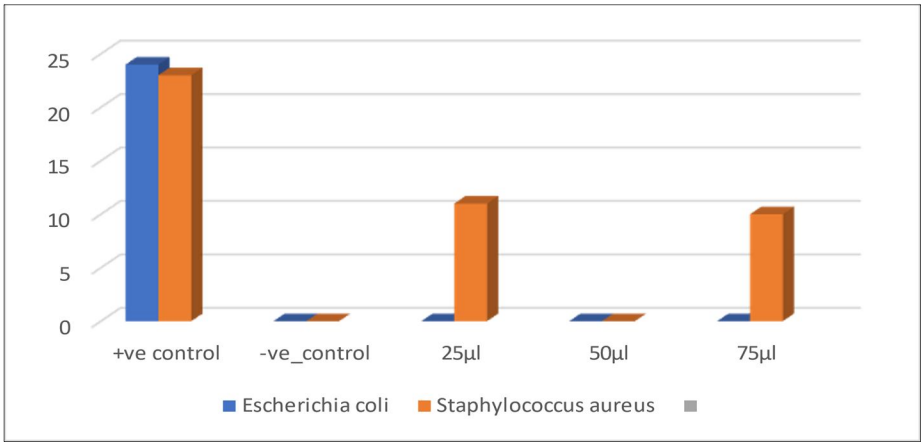

Table.3: This table shows antibacterial activity of Ethanol extract from dry flower of *Brugmansia suaveolens*

Inhibition zones (mm)					
BACTERIAL CULTURE	+ve control	-ve_control	25µl	50µl	75µl
Escherichia coli	17	0	12	15	24
Staphylococcus aureus	24	0	0	0	0

Graph 3.Graphical representation of antibacterial activity of Ethanol extract from dry flower of *Brugmansia suaveolens*

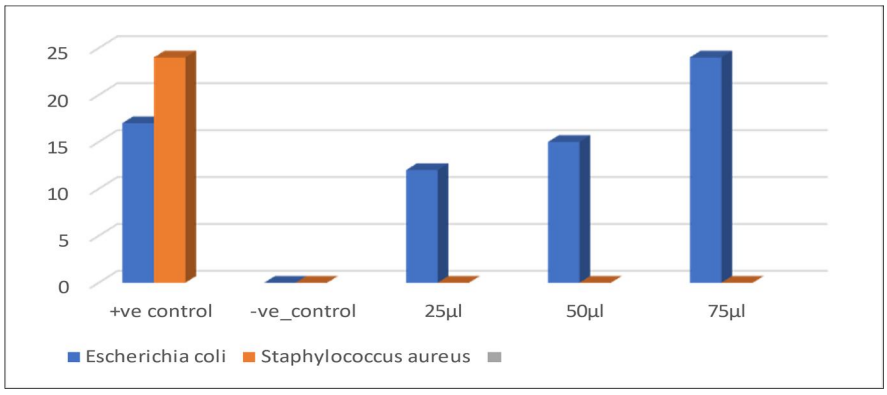

Table.4: This table shows antibacterial activity of water extract from dry flower of *Brugmansia suaveolens*

Inhibition zones (mm)					
BACTERIAL CULTURE	+ve control	-ve_control	25µl	50µl	75µl
Escherichia coli	14	0	15	0	0
Staphylococcus aureus	15	0	0	0	0

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Graph 4.Graphical representation of antibacterial activity of water extract from dry flower of *Brugmansia suaveolens*

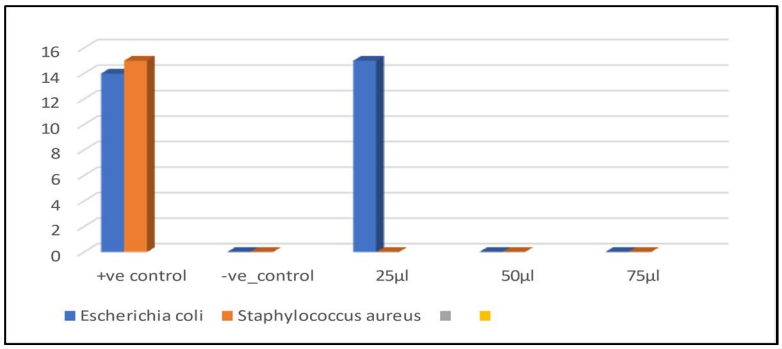


Fig 9:Inhibition zone of *Brugmansia suaveolens* flower extract against *Escherichia coli*

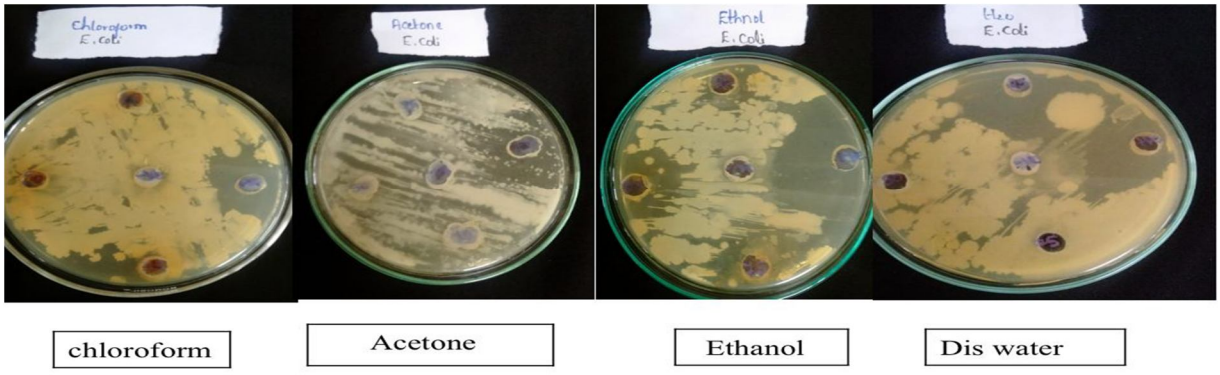
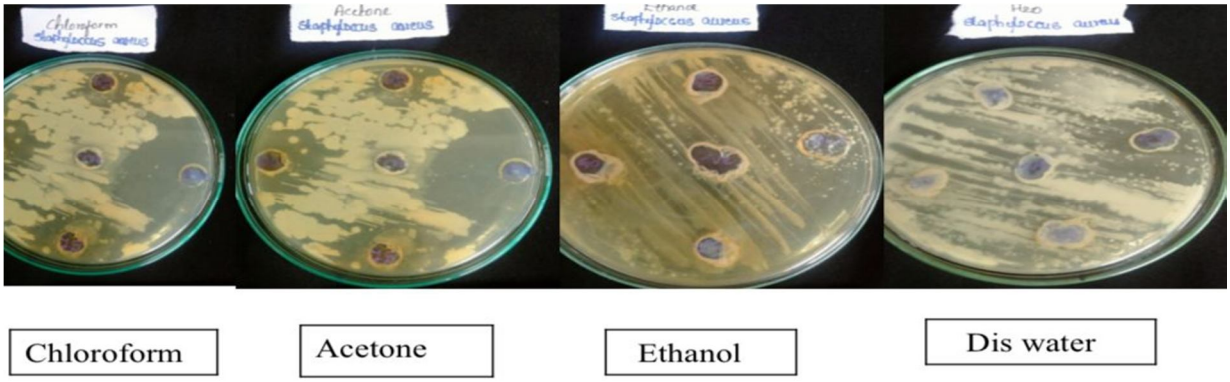


Fig10:Inhibition zone of *Brugmansia suaveolens* flower extract against *Staphylococcus aureus*



Zones of inhibition area [in mm] against fungi in response to the impregnated sample.

Table.6: This table shows antifungal activity of chloroform Acetone Ethanol and water extract from dry flower of *Brugmansia suaveolens*

Selected fungi : *Penicillium atramentosum*					
Inhibition zones (mm)					
Flower Extract	+ve control	-ve_control	25µl	50µl	75µl
Chloroform	7	0	4	12	9
Acetone	12	0	13	11	11
Ethanol	18	0	0	13	14
Water	17	0	0	12	8

Graph 5.Graphical representation of antifungal activity of chloroform Acetone Ethanol and water extract from dry flower of *Brugmansia suaveolens*

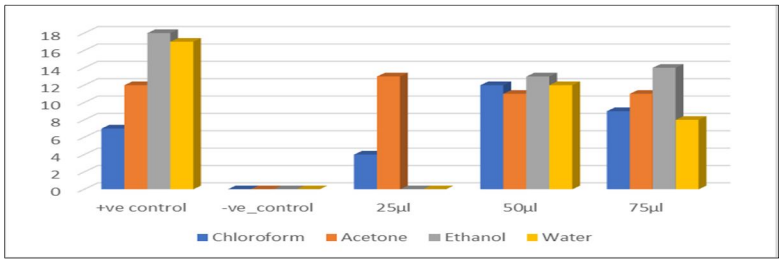
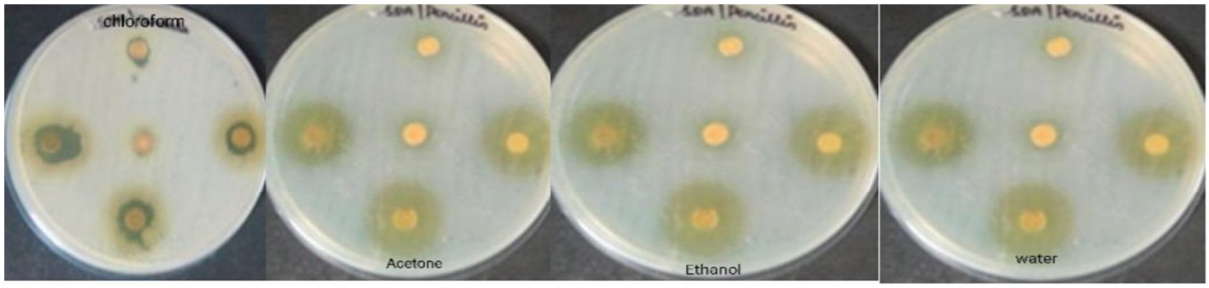


Fig11:Inhibition zone of *Brugmansia suaveolens* flower extract against *Penicillium atramentosum*



B. Discussion

1) Phytochemical Analysis

Chloroform extract of the plant showed the positive results for Steroids, Terpenoids and Saponins. Acetone extract of the plant showed the positive result for Steroids, Saponins, Taninns and Glycosides. Ethanol extract of the plant showed the positive result for Steroids, Terpenoids, Flavonoids, Phlobatannins and Glycosides. Distilled water extract of the plant showed the positive result for Alkaloids and Tannins.

2) Antimicrobial Activity

The antimicrobial activity of *Brugmansiasuaveolens* was assayed invitro by agar well diffusion method against two bacterial strain and one fungal strain. The table summarized the bacterial growth and fungal growth inhibition zone of Chloroform, acetone, Ethanol and distilled water extract of *Brugmansiasuaveolens*.

V. CONCLUSION

The traditional uses, phytochemicals, and toxicity of the plant *Brugmansiasuaveolens*, which shows interesting chemical constituents with different biological activities. The presence of antimicrobial substance in the higher plants is well established. Plants are the potential bio factories of chemical compounds which are serving for the benefits of mankinds. From the above investigation study we can conclude that *Brugmansiasuaveolens*has proved to be a good antimicrobial agent. Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health.

The plant showed the positive results of different secondary metabolites such as steroids, saponins, tannins, terpenoids, glycoside, Flavonoids,

Phlobatannins and Alkaloids. Further, *Brugmansiasuaveolens* seems to be held great potential for in depth investigation for various biological activities and the obtained through this work may be useful in developing new formulation with more therapeutic value. So, the plant flower extract could be used as drug for various elements ,which can be studies in future studies

VI. ACKNOWLEDGMENT

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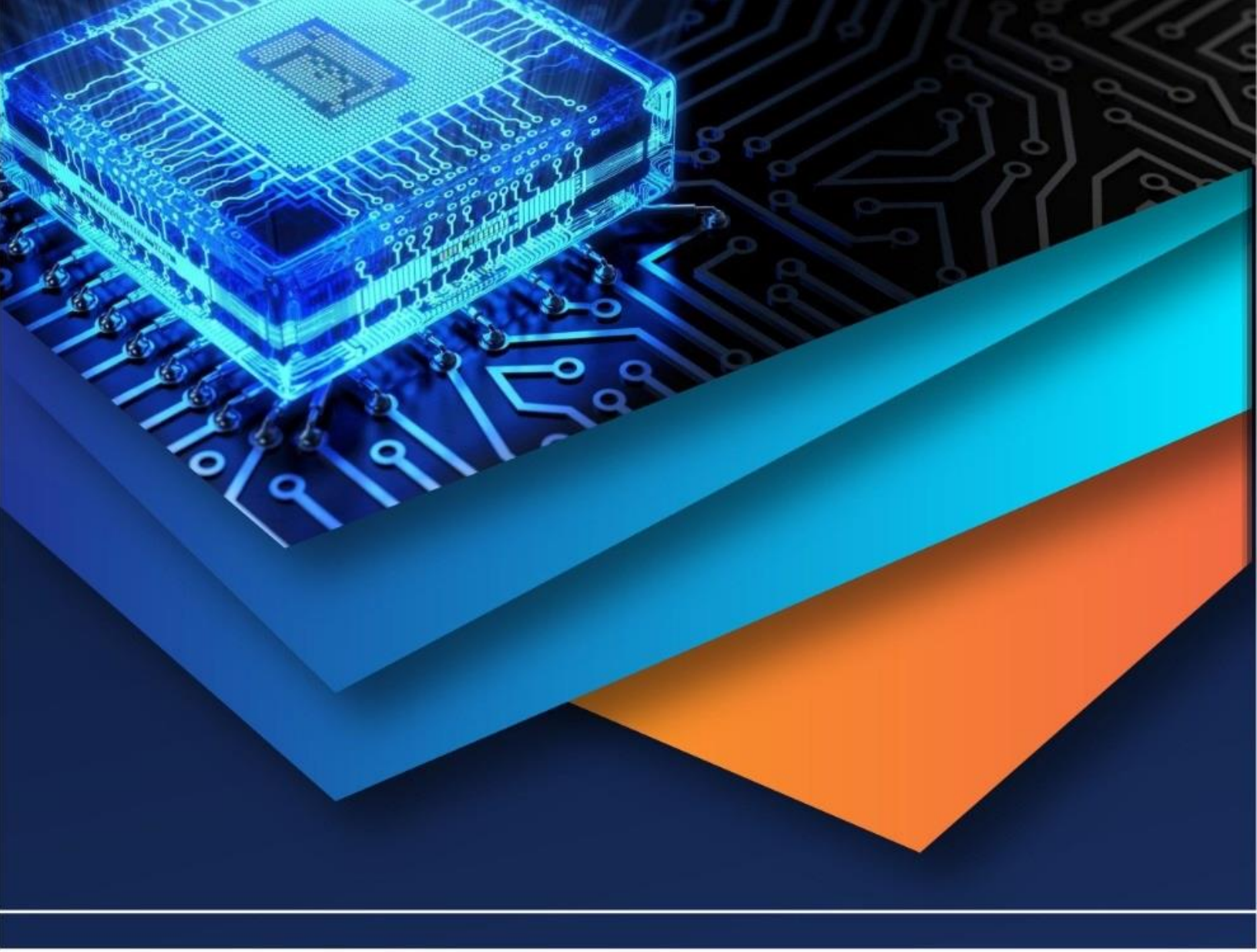

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