International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2160-2165 www.ijprajournal.com ISSN: 2249-7781

Ethno-pharmacological of Lilium Flower

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Submitted: 15-02-2023 Accepted: 25-02-2023

ABSTRACT

The Lilium flower have been regarded as an excellent source of biological active compounds. The present study reports the control of microbial load in air by open plate methods. The phytochemical analysis of the extract reveals the presence of phenols, flavonoids, tannins, terpenoids, glycosides, coumarins, and quinones. Bacterial identification of the isolates from the study area was confirmed with suitable tests.

Key words: Lilium flower, open plate method, phytochemical, bacteria.

I. INTRODUCTION

Lilium is a genus of herbaceous flowering plants growing from bulbs, all with large prominent flowers. They are the true lilies. India has a great treasure of medicinal plants due to which it is one of the richest nations in terms of a vast collection of genetic resources of medicinal plants in the world [1]. The members of the Liliaceae family have been found to contain phytochemicals such as alkaloids, steroidal saponins, vitamins, and fatty acids, which are responsible for their biological activity [2]. Flowers of various species of lily have been reported to possess broad-spectrum antimicrobial activity [3]. L. longiforum, has been studied and used as an anti-inflammatory agent for the treatment of bronchitis and blood clotting during the surgical procedures [4]. Lipid peroxidation and cholesterol oxidase enzyme inhibitor assays are used to determine the bioactive compounds in L. longiforum flower, which in turn shed light on its anecdotal medicinal use [5]. The Lilium candidum L. is also an ancient plant, which is used as an important edible plant and important biomedicine in China to alleviate the symptoms of various human inflammatory diseases and they are cultivated as an ornamental plant throughout the world [6]. This plant helped from time immemorial in the treatment of inflamed and suppurative wounds, ulcers, skin inflammations, burns and various injuries [7]. It is also used for muscle pain and gynecological problems. After surgery, it speeds up wound healing. Externally used is an alcohol or oil extract [8]. Mechanisms of Lilium anti-inflammatory activity and their bioactive components remain little known, but the therapeutic effects of lilies are confirmed by modern medicine, which has shown its healing, anti-inflammatory, analgesic, antioxidant, and other effects [9]. Many studies have been conducted for the chemical constituents of the genus Lilium, which illustrated their pharmacological effects of anti-tumor, hypoglycaemic, anti-bacterial, anti-inflammatory, hypolipidemic, reducing blood lipid, anti-depression anti-fatigue and hypoxia tolerance. The importance of the genus in the world flower market is due to diversity and large number of hybrid and cultivars commercially available. However, some species are also known for medicinal and food value which increase the economic importance many folds [10]. Outdoor concentrations of airborne bacteria generally were higher than those indoors but similar in summer and winter. Bacterial concentrations indoors showed more seasonal difference, which may be due to changes in occupant dress and activities as well as ventilation patterns during the cooling and heating seasons. The present work was aimed to control the microbes present in the environment with incorparating the Lilium flower extract in medium.

II. METHODOLOGY

Preparation of flower extract

Fresh Lilium flower were collected directly from the farmers and flower sellers of Bengaluru and brought to the laboratory. The flowers were rinsed twice with distilled water and allowed to air dry in shade. It was made into small pieces using sharp sterile scissors. Extraction was done at room temperature by simple extraction method [11]. 10 gm of dried flower material was weighed accurately using digital electronic monopan balance and soaked in 40ml of propylene glycol solvent [12] and kept in a shaker for 48 hours at 37^{o}C. Then the filtration was performed using muslin cloth and the filtrate was preserved for the further studies.

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Phytochemical screening

The aqueous extract of Lilium flower was screened to examine the presence of chemical groups and active compounds such as carbohydrates, saponins, phenols and tannins, coumarins, flavonoids, proteins, glycosides, terpenoids, and quinones. The examination of saponin was performed by the foam test. The flower extract (0.5 g) was vigorously mixed with 2mL of water and observed for the foam formation for more than 10 min as an indication of the presence of saponin [13]. The presence of phenols and tannins were detected by performing the ferric chloride test. Ferric chloride (0.5%) solution was added drop by drop to 2 mL of flower extract and observed for the formation of a bluish-black precipitate of phenols and tannins [13]. For the identification of glycosides, 1 ml of glacial acetic acid, few drops of ferric chloride solution, and concentrated H_2SO_4 (mixed slowly through the sides of the test-tube) were added to the flower extract and observed for the appearance of a reddish-brown ring of de-oxy sugars at the junction of the liquids [13].

To test the presence of flavonoids in the flower extract, a 10% lead acetate solution was added in the extract. The formation of yellow precipitate confirmed the presence of flavonoids [13]. To detect the presence of carbohydrates, the flower extract was dissolved in 5 mL distilled water and filtered. The filtrate was hydrolyzed with dilute HCl and further neutralized with alkali and subsequently heated with Fehling's solution A and B and observed for the formation of a red precipitate of reducing sugars [13]. The flower extract was treated with a few drops of concentrated H_2SO_4 and observed for the formation of yellow color as an indication of the presence of quinones compound [13]. For terpenoids identification in flower extract, 2 ml of chloroform was added in 5 ml of the flower extract and thereafter 3 ml of concentrated H_2SO_4 was added slowly and observed for the appearance of the reddish-brown color of terpenoids [13]. The presence of amino acids in flower extract was checked by employing a ninhydrin test. Few drops of ninhydrin solution were added to the flower extract and the appearance of blue color indicated the presence of amino acid [14]. About 0.5 g of the moistened flower extract was taken into the test-tube. The mouth of the test-tube was covered with the filter paper treated with 1N NaOH solution. The treated test-tube was placed in boiling water for a few minutes and examined for the formation of yellow color as an indication of the presence of coumarins [15].

Preparation of Nutrient agar plates

In open plate culture, the media are exposed for a specific period and then incubated at 37^0 C for 24 hrs and the number of colonies formed was counted [16]. But in the present study a new approach of open plate method was performed in which the researcher has prepared the medium by incorporating the flower extracts in different percentages. The preparation of the media with different concentration used in this study is clearly indicated in the table 1. The required nutrient agar (Hi-media) was weighed and it was dissolved in the distilled water. The distilled water quantity was planned in such a way that it will meet out the required percentage. The medium was sterilized at 15 lbs pressure (121^0C) for 15 minutes. Then the required Lilium flower extract for different concentration were added to the medium, mixed well and poured into the sterile Petri plates.

Table 1: The ingredients used in the preparation of different concentrations of Lilium flower extract medium.

% of flower extract	**Control**	**4**	**8**	**12**	**16**	**20**	**24**
Nutrient agar (gm)	0.46	0.46	0.46	0.46	0.46	0.46	0.46
Volume of distilled water (ml)	20.0	19.2	18.4	17.6	16.8	16	15.2
Volume of flower extract (ml)*	-	0.8	1.6	2.4	3.2	4	4.8

(* Lilium flower extract added after the medium was sterilized)

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Study Area

A leading IT Industry established in the Bengaluru, Karnataka was used for the study. Everyday more than five hundred employers visit the workplace. During all the study time the office was fully occupied by the office workers. The samples for the study were collected.

Air Sampling and Microbiological Examination

The prepared nutrient agar plates of flower extracts with different percentages were exposed in the study area [17], leaving the Petri dish open to air for 1 hour and positioning it 80 – 100 cm above the floor and at 100 – 150 cm from the wall to obtain the average and useful value for the microbial fallout from the air in the environment [18]. The microbiological samples were collected two times a day that is, in the morning between 8.00 – 9.00am and in the evening 8.00 – 9.00 pm. A control plate was separately maintained without the Lilium flower extract. After exposure, the plates were transported in a clean container to the laboratory for microbiological examination. All the plates were incubated at 37^0 C for 24 hours and then the number of colonies formed were counted and tabulated. The total number of colony forming unit (cfu) was enumerated and converted to organisms per cubic meter of air [19]. Triplicates were maintained and mean of the triplicates were taken into consideration for the analysis of results.

Bacterial Identification

According to Cheesbrough (2009) procedures the identification of bacterial isolates were initially characterized by morphology and using staining techniques (Gram staining) and identified further by biochemical tests. Biochemical tests such as Catalase, Oxidase, Indole, Methyl Red test (MR), Coagulase, Voges Proskauer (VP) and Citrate utilization were carried out on the isolated bacteria.

III. RESULT AND DISCUSSION

In the Lilium flower extract the phytochemical studies shows the presence of phenols, tannins, glycosides, flavonoids, quinones, terpenoids and coumarins (table – 2) which act as a bioactive compounds in suppressing the growth of microorganisms. The bacterial identification of the isolates confirms Staphylococcus aureus, Escherichia coli and Bacillus subtilis (table – 4). Staphylococcus aureus was the dominant isolated organism and this bacterium is a common causative agent of various human diseases, it is responsible for many gastrointestinal tract infections, respiratory tract infections and skin disorders [20]. Escherichia coli is a leading cause of urinary tract infections and intra abdominal infections in which the extent of the disease can range from cystitis to life threatening sepsis [21]. Exposure to these airborne particles can result in respiratory disorders and other adverse health effects such as infections, hypersensitivity pneumonitis and toxic reactions. In addition, long-term contact of people with bioaerosols can influence a person's mental power and learning ability [22].

Different environmental conditions such as temperature, UV light, dryness and humidity, play a role in controlling the growth of airborne particles. Nevertheless the microbes manage to reach new hosts through the air for its survival. Poor ventilation, crowded conditions and increase in number of air conditions inside building nowadays can facilitate the spreading and the survival rates of airborne particles and also can increase the chance of people at risk of airborne infections. Among dust particles present in the indoor environment, fungus which reproduce by forming spores, some bacteria especially gram positive bacteria and some viruses can survive for a long time in the air [23].

In the present study, the open plate method was employed with slight modification, showed a significant good result that eradicate or prevent the growth of all types of microbes than the other methods employed. The result conducted at different concentration of Lilium flower extract have control the growth of microbes in the indoor environment. The microbial load was more in evening time (8-9pm) when compared to the morning time (8-9am) due to the workers present. The control of microbes was able to see in all the plates according to the increase in the contraction of the flower extract (table – 3). This result indicated that the extracts of flowers showed better performance in eradicating or preventing the growth of microorganisms in total than the extracts of other parts of plants which prevents specific microbes to certain extent in a specific concentration.

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Table 2: Phytochemical analysis of Lilium flower

S.No	Compounds	Aqueous Extract
1	Saponins	-
2	Phenols	+
3	Tannins	+
4	Glycosides	+
5	Flavonoids	+
6	Carbohydrates	-
7	Quinones	+
8	Terpenoids	+
9	Proteins	-
10	Coumarins	+

Table 3: Shows the number of colonies (cfu/m^3) observed in different percentage of flower extracts used. The values represented are the mean of three observations and ± SD. The values indicated in the parenthesis are the percent decrease in the number of colonies than the control.

Time	Control plate	Flower extract					
		4%	8%	12%	16%	20%	24%
8 – 9 am	278±8	211±5 (24)	158±4 (43)	115±5 (59)	67±4 (76)	9±2 (97)	0 (100)
8 – 9 pm	356±5	293±5 (18)	249±2 (30)	183±4 (49)	128±6 (64)	65±4 (82)	14±4 (96)

Table 4: Shows the confirmation test for the isolated organisms form the study area.

S.No	Test / Staining	Staphylococcus aureus	Escherichia coli	Bacillus subtilis
1	Gram staining	Gram-positive bacteria, cocci-shaped.	Gram-negative bacteria, rod shaped.	Gram-positive bacteria, rod shaped.
	Biochemical Test			
1	Catalase Test	Positive	Positive	Positive
2	Oxidase Test	Negative	Negative	Negative
3	Indole Test	Negative	Positive	Negative
4	Methyl Red Test (MR)	Positive	Positive	Negative
5	Voges Proskauer Test (VP)	Positive	Positive	Positive
6	Coagulase Test	Positive	Negative	Negative
7	Citrate utilization Test	Positive	Negative	Positive

IV. CONCLUSION

Using the open plate method it is proved that the flower extract prevents the growth of microbes present in the environment. In the control plate we have observed Staphylococcus aureus, E. coli and Bacillus subtilis. And these organisms can

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cause several infections to the workers. In order to develop the quality of indoor air in working place overcrowding has to be avoided, good ventilation systems has to be designed and good hygiene practice must be observed. Flower extract can also be used as an air sprayers or air fresheners to control the overload of microbes in indoor environments.

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