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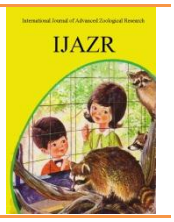

CONTROLLING THE MICROBIAL LOAD IN THE POLLUTED ATMOSPHERE USING DRIED FLOWER EXTRACTS BY MODIFIED OPEN PLATE METHOD

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ABSTRACT

In order to eradicate the microbial load in the air, the proposed study has evolved a modified open plate methodology using different concentration of flower extract. The flower extracts of *Chrysanthemum coronarium, Hibiscus rosa-sinensis, Nerium oleander, Polyanthes tuberosa* and *Rosa centifolia* were tried in different concentration in this study by open plate method. The plates were exposed in a public toilet for 30 minutes and further observations were made. It was concluded that almost all the extracts showed better performance at 15% concentration. Among the five flowers studied the extract of *Rosa centifolia* showed a very good performance even in 5% concentration than the others. The study concluded that among the tested extract the *Rosa centifolia* showed an excellent performance followed by *Nerium oleander, Chrysanthemum coronarium, Polyanthes tuberosa* and *Hibiscus rosa-sinensis.*

INTRODUCTION

There is a growing need for obtaining more information about aerobiology of pathogens. With respect to the experimental area, aerobiology is divided into (a) Intramural (indoor) aerobiology and (b) Extramural (outdoor) aerobiology. Indoor aerobiology deals with the study of indoor environments like dwellings, hospitals, poultry sheds, storage godowns, cinema halls, libraries, fruit and vegetable markets, monuments, caves, etc. and related health hazards of the inhabitants. Microorganisms are found in air and their presence is of considerable importance to public health [1]. Atmospheric pollution is one of the most pressing problems of our age. This pollution has now reached an adverse level that pose a potential threat to the health and well being of the people. The atmosphere consists of different component, which enhance or promote the survival of microorganisms in the air [2]. In the course of days a person inhales over 15 cubic meters of air. Hence the bacterial content of the air one breathes is important particularly when it contains pathogens. The bacterial content of air depends on the location that is whether it is out door or indoor air [3].

Counting microbes in the air is not an easy task. Many different methods are in use, such as the count of colony forming units per cubic meter of air (cfu/m^3); the count of cfu on settle plates; measurement of a chemical component of the microbial cell/m^3 of air; the count under the microscope [4]. Several studies showed that the extracts of plant parts showed good performance in controlling the microbial activity. Extracts of *Bauhinia purpurea, Cardiospermum helicacaum, Cissampelos pareira, Rhinacanthus nastusl* and *Swertia corymbosa* showed best antimicrobial activity against several Gram positive bacteria [5]. Dixit *et al* [6] reported antifungal activity using the extract *Rosa indica.* Using *Nelumbo nucifera* pollen oil [7] showed the antimicrobial activity and its inhibitory effect on the growth of food born pathogens in low concentration. The wound healing activity of *Jasminum grandflorum* was reported by Nayak and Krishna Mohan [8] and *Nymphaea lotus* by Akinjogunla *et al* [9]. Antiparasitic activity of two *Lavandula* essential oils was performed against *Giardia duodenalis, Trichomonas*

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vagginalis by Therese *et al* [10].

The literature survey indicated that most of the studies have focused mainly on the antimicrobial, antifungal, antiparasitic activity of specific pathogens using the extracts of leaves, stem or roots of plants. But in the present study, the microbial load in the polluted air is tried to control or eradicated completely by using the different flower extract with different concentrations using the open plate method.

METHODOLOGY

Preparation of flower extract

Fresh flowers were collected directly from the farmers and flower sellers of Thoothukudi 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.10 gm of dried flower material was weighed accurately using digital electronic monopan balance and soaked in 40ml of propylene glycol solvent and kept in a shaker at 200rpm for 48 hours at 37^{o}C . Then the filtration was performed using muslin cloth and the filtrate was preserved for the further studies.

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. 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^{0}C) for 15 minutes. Then the required flower extract for different concentration were added to the medium, mixed well and poured into the sterile Petri plates.

Detection of air microbes by open plate method

The prepared nutrient agar plates by adding different percentage of flower extract were then exposed in a closed public toilet for 30 minutes which was regularly used by thousands of people daily. 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 [11]. A control plate was separately maintained without the flower extract. All the plates were incubated at 37^{0}C for 24 hours and then the number of colonies formed were counted and tabulated. Triplicates were maintained and mean of the triplicates were taken into consideration for the analysis of results. In order to test the validity of the results obtained the student't' test was employed (Microsoft Eq. version 2.0).

RESULTS

The result of present study (Table – 2) showed an excellent contribution to the field of eradication of pathogenic bacteria in the air medium. Most of the extracts used in the present study prevent the growth of microorganisms even in very low percentage. In almost all the flower extracts studied there was a significant fall in the number of colonies formed in the respective concentration of flower extract than the control plate.

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

% of flower extract	Control	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Nutrient agar (gm)	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46
Vol of distilled water (ml)	20.0	19.8	19.6	19.4	19.2	19.0	18.8	18.6	18.4	18.2	18.0	17.8	17.6	17.4	17.2	17.0
Volume of flower extract (ml)*	-	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4	2.6	2.8	3.0

(*Flower extract added after the medium was sterilized)

Table 2. 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. The 't' value are also incorporated which are significant at 0.05% level.

Flower Extract	Concentrations						
	1%	2%	3%	4%	5%	6%	7%
Rose centifolia	12±2 (95.94) -245.95	10±1 (96.62) -495.36	7.67±1.5 (97.40) -326.93	6±1 (97.97) -502.29	4±1 (98.64) -505.75	Nil (100)	Nil (100)
Nerium oleander	183.67±4.5 (37.9) -43.14	169.67±2.1 (42.68) -105.11	154.67±3.1 (47.7) -80.12	7.67±1.5 (54.72) 50.39	116.67±2.5 (60.58) -123.42	85.67±2.5 (71.05) -144.76	74.67±4.7 (74.77) -81.12

Chrysanthemum coronarium	196.67±2.1 (33.55) -82.65	188.33±3.1 (36.37) -61.04	166.33±2.5 (43.80) -89.24	142.33±2.5 (51.91) -105.76	127±2 (57.09) -146.35	103.33±1.5 (65.09) -218.46	78.33±2.1 (73.53) -181.10
Polyanthes tuberosa	205.67±4.2 (30.51) -37.58	192±5.7 (35.13) -32.35	173±3.6 (41.55) -59.08	164.67±3.1 (44.36) -74.45	157.67±3.1 (46.73) -78.42	112.33±2.5 (62.04) -126.40	76±3.6 (74.32) -105.68
Hibiscus rosainensis	233.33±3.1 (21.17) -35.52	212.33±2.5 (28.26) -57.58	197.67±2.5 (33.22) -67.67	187.33±5.7 (36.71) -33.10	178.33±3.5 (39.75) -58.03	150.67±2.5 (49.09) -100.02	98±3 (66.89) -114.31

Control plate – 296±2

Continued…

Flower Extract	**Concentrations**							
	8%	**9%**	**10%**	**11%**	**12%**	**13%**	**14%**	**15%**
Rose centifolia	Nil (100)	Nil (100)	Nil (100)	Nil (100)	Nil (100)	Nil (100)	Nil (100)	Nil (100)
Nerium oleander	63±10.6 (78.71) -38.13	34.67±3.1 (88.28) -148.16	24.33±3.5 (91.77) -133.98	23.33±5.7 (92.11) -83.05	13.67±2.1 (95.38) -234.91	11.33±2.3 (96.17) -213.5	6.33±2.5 (97.86) -199.36	Nil (100)
Chrysanthemum coronarium	52.33±1.5 (82.31) -276.29	38±3 (87.16) -148.95	23.67±1.5 (92.00) -308.79	19±2 (93.58) -239.88	13.67±1.5 (95.38) -320.13	11±2 (96.28) -246.81	7.33±1.5 (97.52) -327.31	Nil (100)
Polyanthes tuberosa	61.67±6.0 (79.16) -67.33	44.67±4.0 (84.90) -107.71	27.67±4.5 (90.65) -103.06	20±3.0 (93.24) -159.34	15.67±1.5 (94.70) -317.86	11±2.0 (96.28) -246.81	6.33±1.5 (97.86) -328.45	Nil (100)
Hibiscus rosasinensis	79.33±3.5 (73.19) -106.85	56±4 (81.08) -103.92	35±3.6 (88.17) -125.38	25±3.6 (91.55) -130.18	18±3 (93.91) -160.50	14±2 (95.27) -244.21	9±2 (96.95) -248.54	Nil (100)

Control plate – 296±2

As the concentration of the extract used increased the eradication / prevention of growth of microorganisms were also significantly decreased to a greater extent. Among the extracts used the *Rosa centifolia* showed the excellent performance even in 1% level. The percent decrease of colony observed was 95.9, 96.6, 97.4, 97.9, 98.6 and 100 in the extract concentrations of 1, 2, 3, 4, 5 and 6% respectively than the control. In the remaining flower extracts, the 100% prevention of microbial growth was observed in 14 or 15% of concentrations. Except the *Rosa centifolia* extract in all other extracts 50% microbial growth prevention was achieved in 5% concentration. Next to *Rosa centifolia* extract, the best performance was observed in various extracts in the order of *Nerium oleander*, *Chrysanthemum coronarium*, *Polyanthes tuberosa* and *Hibiscus rosa-sinensis.*

DISCUSSION AND CONCLUSION

Many studies on microbial contaminants in indoor air have been recorded by several investigators in different environments such as hospitals, residential buildings, agricultural settings, industries and institutions and so on. The evaluation of the level of air microbial contamination in places at risk is considered to be a basic step towards prevention. However, there are still problems to be solved relating to methodology, monitoring, data interpretation and maximum acceptable levels of contamination.

In the present study the open plate method was performed to asses the microbial load in the atmosphere but in which the extract of flower was directly incorporated in the medium. Usually the specific medium or the specific microbial growth in a medium was assessed using the extracts of different plants or its parts. But in the present study the flower extract of various flowers in different concentration were used in open plate method, completely eradicated / prevented the growth of any microorganisms even in very low concentration. 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.

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 flower extracts with low concentration of all extracts (<5%) in the present study showed a significant good result in controlling the microbes in air than the same plants other part extracts which were already studied and reported by several workers by invitro studies. From this it is concluded that the flower extracts of plants are more prominent and promising one in controlling the growth of microbes in the general atmosphere. Hence it may be used as one of the ingredient in the cleaning agents, air sprayers, and air fresheners and so on.

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