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Evaluation of the antimicrobial Property of Selected Flower Extracts when exposed in a Hospital Environment

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Abstract: This study was carried out to assess the performance of the flower extracts on the airborne microflora that are prevailed in the dental hospital of Thoothukudi City, Tamil Nadu. Samples were collected using the settled plate techniques for the enumeration of bacterial isolates. Every day, the air samples were collected four times and the bacterial volume was assessed. The bacterial population was completely arrested in 15% concentration of all the flower extracts studied. Two different locations were chosen for the study namely the doctor's diagnosis room and patient's waiting room. Among this the high bacterial population was observed in the patient's waiting room. The total microbial population was calculated by counting the total colonies developed over the medium and expressed as colony forming unit (cfu). The bacterial population was completely vanished in all the study area of the hospital using 15% concentration of all the studied flower extract.
Key words: Dental hospital, bacteria, flower extracts of *Tagetes erecta*, *Hibiscus rosa-sinensis*, *Nerium oleander*, *Rosa centifolia* and *Polyanthes tuberose*.

Introduction

Nature has been a source of medicinal agent for thousands of years and an impressive number of modern drugs have been isolated form natural sources, many based on their use in traditional medicine. Various medicinal plants have been used for years in daily life to treat disease all over the world. They have been used as a source of medicine[1]. Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it. The effect of plant extracts on bacteria has been studied by a very large number of researchers in different parts of the world[2-4]. With the rising prevalence of microorganism showing resistance to antibiotics, there is an urgency to develop new antimicrobial compounds. Since antiquity, plants have been used to treat common infectious diseases. The healing potential of many plants have been utilized by Indian traditional medicines like Siddha, Ayurvedha and Unani. Being nontoxic and easily affordable, there has been resurgence in the consumption and demand for medicinal plants[5]. Bacterial resistance to currently used antibiotics is becoming a concern to public health[6].

The hospital is a complex environment, traditionally considered at high infectious risk, for both the patients and the health care workers; they can contract diseases, because of the exposure for relatively long times to various dangerous chemical, physical and biological factors. The biological contamination in the Doctor's operating rooms is mostly imputable to airborne and blood borne microorganism, whose primary source represent the staff, patients and operating team, while its secondary sources contaminate the air in the surrounding environment[7]. Apart from this the general atmospheric pollution is one of the most pressing problems of our age. This pollution has now reached an advanced level that pose a potential threat to the health and well being of the people. The polluted atmosphere consists of different components, which enhance and promote the survival of several microorganisms in the air[8]. In general the health and well being of the public are affected to a greater extent by the physical, chemical

biological properties of the indoor environment of the hospital. The quality of the indoor environment, however, is not easily defined or controlled and can potentially place human occupants at risk[9]. In order to eradicate the microbial population to a greater extent, this study was planned. Using the flower extracts of several flowers, complete eradication of microbes was achieved in most of the extracts used.

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].10 gm of dried flower material was weighed accurately using digital electronic monopan balance and soaked in 40ml of propylene glycol solvent[11] 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.

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[12]. 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.

Study Area

A leading Dental hospital established in the Great Cotton road of Thoothukudi City was used for the study. Everyday more than five hundred patients visited the hospital and get treatment for their dental problems. During all the study time the hospital was fully occupied by the patients. The samples for the study were collected from two different locations of the hospital, namely the doctor's diagnosis room and the patient's waiting room.

Air Sampling and Microbiological Examination:

The prepared nutrient agar plates of flower extracts with different percentages were exposed in the study area[13], 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[14]. The microbiological samples were collected four times a day that is, in the morning between 10.00 - 11.00am, in the afternoon 2.00 – 3.00 pm, in the evening 6.00 - 7.00 pm and in the night 9.00 - 10.00 pm. A control plate was separately maintained without the 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[15]. 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).

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
Volume 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 Open plate method performed inside the doctor's room at different time intervals to observe the number of colonies formed using different concentration of flower extracts in the medium

TIME	CONTROL	*Tagetes erecta*			*Hibiscus rosasinensis*			*Nerium oleander*			*Rose centifolia*			*Polyanthes tuberosa*		
		5%	10%	15%	5%	10%	15%	5%	10%	15%	5%	10%	15%	5%	10%	15%
10-11 AM	183	114.33±3.0 (37.52) -38.93	40.66±2.5 (77.77) -97.96	Nil (100)	284±3.0 (55.19) 58.31	88.66±3.5 (51.54) -46.52	Nil (100)	103.66±2.5 (43.35) -54.60	85.33±4.0 (53.36) -41.85	Nil (100)	9±2.0 (95.08) -150.68	Nil (100)	Nil (100)	107.33±8.3 (41.34) -15.73	88±2.6 (51.91) -62.19	Nil (100)
2-3 PM	167	102.33±4.0 (38.72) -27.71	33.33±2.0 (80.03) -111.21	Nil (100)	197±3.6 (17.96) 14.41	58.33±2.5 (65.06) -74.78	Nil (100)	95±3.0 (43.11) -41.56	72.33±3.5 (56.68) -46.68	Nil (100)	8±2.0 (95.20) -137.69	Nil (100)	Nil (100)	93.66±2.5 (43.91) -50.47	63.66±2 (61.87) -85.97	Nil (100)
6-7 PM	158	77.33±2.0 (51.05) -67.11	14.66±3.0 (90.71) -81.26	Nil (100)	81.66±1.5 (48.31) -86.55	26.66±2.5 (83.12) -90.38	Nil (100)	81.66±3.5 (48.31) -37.64	63.33±3.0 (59.91) -53.67	Nil (100)	6±2 (96.20) -131.63	Nil (100)	Nil (100)	72.33±2.5 (54.21) -58.95	20.66±3.0 (86.91) -77.86	Nil (100)
9-10 PM	195	138.33±1.5 (29.05) -64.25	58.66±4.0 (69.91) -58.42	Nil (100)	295.33±3.7 (51.45) 45.90	95.66±2.5 (50.94) -68.36	Nil (100)	128.33±3.5 (34.18) -32.87	96.66±2.5 (50.42) -67.67	Nil (100)	11.66± 2.5 (94.01) -126.17	Nil (100)	Nil (100)	128±3 (34.35) -38.68	97±2.6 (50.25) -64.15	Nil (100)

The values indicated are the mean of 3 observations with ± SD. The values indicated inside the parenthesis are the percent decreases in the number of colonies than the control. The value of't' also indicated which are significant at 0.05% level.

Table 3 Open plate method performed in the patient's waiting room at different time intervals to observe the number of colonies formed using different concentration of flower extracts in the medium

TIME	CONTROL	*Tagetes erecta*			*Hibiscus rosasinensis*			*Nerium oleander*			*Rosa centifolia*			*Polyanthes tuberosa*		
		5%	**10%**	**15%**	**5%**	**10%**	**15%**	**5%**	**10%**	**15%**	**5%**	**10%**	**15%**	**5%**	**10%**	**15%**
10-11 AM	296	168.66±2.5 (43.01) -87.63	65.66±2.5 (77.81) -158.52	Nil (100)	141.33±2.5 (52.25) -106.44	76.66±2.5 (74.09) -150.95	Nil (100)	164.33± 2.5 (44.48) -90.61	119±2 (59.79) -153.28	Nil (100)	6±1 (97.97) -502.29	Nil (100)	Nil (100)	133.33±2.5 (54.95) -111.95	79±3.0 (73.31) -125.28	Nil (100)
2-3 PM	187	103±2 (44.91) -72.74	47.33±2.0 (74.68) -116.21	Nil (100)	128.33±2.0 (31.37) -48.81	55.33±3.0 (70.40) -74.64	Nil (100)	156.33± 2.5 (16.39) -21.10	105±4 (43.85) -35.50	Nil (100)	4.33±1.5 (97.68) -207.12	Nil (100)	Nil (100)	106.66±2.0 (42.95) -66.84	59±2.6 (68.44) -83.79	Nil (100)
6-7 PM	141	72.33±2.5 (48.69) -47.25	32.66±1.5 (76.83) -122.83	Nil (100)	112.66±2.5 (20.09) -19.50	42.66±2.5 (69.73) -67.67	Nil (100)	143.66± 2.5 (1.89) 1.83	97±2 (31.20) -38.10	Nil (100)	Nil (100)	Nil (100)	Nil (100)	93.33±2.0 (33.80) -39.66	42.33±2.5 (69.97) -67.90	Nil (100)
9-10 PM	346	185.33±2.5 (46.43) -110.57	78.33±2.0 (77.36) -222.71	Nil (100)	153±2 (55.78) -167.14	86.33±1.5 (75.04) -294.43	Nil (100)	177.66± 1.5 (48.65) -190.87	128±2 (63.00) -188.79	Nil (100)	10±1 (97.10) -581.96	Nil (100)	Nil (100)	148.33±1.5 (57.12) -224.13	95.66±2.5 (72.35) -172.29	Nil (100)

The values indicated are the mean of 3 observations with ± SD. The values indicated inside the parenthesis are the percent decreases in the number of colonies than the control. The value of't' also indicated which are significant at 0.05% level.

Result and Discussion

The total airborne microbial population of the two different locations studied in the Dental hospital varied from units to units (Table 2 and 3). The concentrations of the total airborne microorganisms varied with time of investigations. The concentration of airborne bacteria obtained during the study period from the different locations of the hospital was high during night time than the other time of the study period. This was mainly because during this time more people visited the doctor[8&16]. Generally the hospital environment may be loaded with microorganisms such as *Staphylococcus aureus*, *Proteus mirabilis, Escherichia coli, Streptococcus micrococcus Bacillus* sp and so on[8&9]. The control plate of the present study showed very high number of colonies during the (140-346 cfu) entire study period in both the study localities[17]. In the present study also a very high percentage of microbial loads were observed in 5% concentration of all flower extract medium except *Rosa centifolia* both in the doctor's room and patient's waiting room. As the concentration of the extracts increased to 10 and 15% there was no growth of microbes were observed both in the doctor's room as well as in the patient waiting room[18-21]. In general in all the percentage of the extracts of different flowers the different flowers the formation of the microbial colony was minimum than the control plate even in 5% of the concentration[22-24]. Among the five flower extracts used in the present study the extract of *Rosa centifolia* showed a very good performance in both the exposed area. In the concentration of the 10 and 15% there was no symptom of growth of microbes were recorded both in the doctor's room and in the patient's room. At 5% concentration, the formation of the microbial colony observed was in single digit during all the study time in both the doctor's room and in the patient's room[25-27]. From this study it was concluded that the extract of *Rosa centifolia* has a very good microbial controlling property and hence that can be used for cleaning and sterilization purpose in the public places such as hospitals, toilets, theatres, public enterprises and so on.

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