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Effect of Oleo-Gum Resin of *Commiphora wightii* Fumes on Microbes of Air

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ABSTRACT

Generally chemical fumigants *viz.,* formaldehyde gas, hydrogen peroxide, chlorine dioxide, *etc.* are used to manage/ reduce the level of microbial contamination/ air pollution. Despite the fact, that chemical fumes possess lots of hazardous side effect and causes serious illness some leads to death. To explore the potential of as bio-fumigant to reduce micro-flora indoors, experiments were conducted with the fumes of oleo-gum resin of *Commiphora wightii.* The experiment antimicrobial activity was done by the Petri plate exposure method. The Petri plates were placed at different distances (1 to 4 meters) and exposed before and after the experiment (30min) and incubated at ambient temperature regimes and colony count was taken after incubation. The study indicates a significant reduction in bacterial colonies after fumigation compared to the control (before fumigation). The percentage of colonies in three seasons varied from 7.03 to 19.79 % (reduction of colonies varied from 88.55 to 91.72%), observed after seven days of incubation.

INTRODUCTION:

Chemical fumigation is used generally to reduce the level of microbial contamination. Various chemical fumigants *viz.*, formaldehyde gas, hydrogen peroxide, chlorine dioxide,*etc.* are used to manage air pollution. However, these chemical are effective, but possess severe hazardous effect and causes serious illness some lead to death also. Fumigation with formaldehyde causes sulphydryl poisoning, protein aggregation, and cancer. Acute renal and liver injury can also develop in cases of severe intoxication by chemical fumigation [3].

Plants produce many bio-chemical compounds for biological functions, including defense against insects, fungi, and herbivores mammals. The use of different plant fumes for air purification or kill to germs is well documented in our ancient literature [4,16,17,18].The smoke produced from natural substances has been used extensively in many cultures and famous ancient ayurvedic physicians have recommended for the ailment of several diseases or purification of air in indoor or outdoor areas. Considering the hazardous effect of chemical fumigation, plant biochemicals may be the most lucrative alternative to combat the notorious microorganism present in the air.

An oleo-gum resin exuded by the plant *Commiphora wightii (Burseraceae* is of medicinal use. Guggul was used as medicine about 5000 years ago. Its medicinal and therapeutic properties are reported in Atharva Veda and as a drug is given in the treaties of Charaka (B.C.1000), Sushruta (B.C.600), Vagbhata (17 century AD), and various Nighantus written in India during 12 to 14 centuries. The resin has a fragrant aroma because of the presence of essential oils. It varies in color from transparent golden brown to dark greenish brown depending upon the season, mode of collection, and impurities found therein. Pure oleo gum-resin collected in the optimum season hardens slowly, retaining its golden color and transparency.

It occupies an important place in the Indian System of Medicine (Ayurveda) and is highly efficacious in the treatment of several diseases- obesity, arthritis, inflammation, cardiovascular, skin diseases, and disorder of lipid metabolism [5,14,15,20]. Besides, it is also used in incense, lacquers, varnishes, ointments as a fixative, and perfumes.

MATERIALS AND METHODS:

Collection of sample

Oleo-gum-resin of *C. wightii* (Fig.1a) was collected from the naturally growing plants in the forest of Bhuj (Gujarat).

Determination of quality of oleo-gum-resin

Determination of moisture % on drying

The moisture % was determined using a Remi make oven at 105±2°C for 5 to 6 hours to constant weight.

Calculation of moisture percent was done using the following formula:

$$\text{Moisture \%} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh Weight}} \times 100$$

Organoleptic properties

Organoleptic properties were evaluated including appearance, size, color, taste, and odor.

For determining the odor of resin material, a small portion of the sample was placed in a beaker of 250 ml size and examined by slow and repeated inhalation of the air over the material. The sample was crushed between the thumb and index finger, between the palms of the hands, using gentle pressure and observed smell. The taste was distinctively classified as aromatic, pungent, sweet, sour, astringent, mucilaginous, or bitter.

Physicochemical analysis

Water-soluble extractive value

Five gm. of the air-dried, coarsely powdered oleo-gum resin was macerated with 100 ml of chloroform water in a closed flask for 24 hours, shaking frequently during the first 6 hours, and allowed to stand for 18 hours. There often filtered rapidly evaporated 25 ml of the filtrate to dryness in a tarred flat bottomed shallow dish and was dried at 105 °C and weighed. The percentage of water-soluble extractives was calculated concerning the air-dried drugs.

Ethanol soluble extractive value

Five gm. of the air-dried coarsely powdered Guggul was macerated with 100 ml of ethanol in a closed flask for 24 hours, shaking frequently during the first 6 hours, and allowed to stand for 18 hours. There after rapidly taking precautions against loss of ethanol, evaporated 25 ml of the filtrate to dryness in a tarred flat-bottomed shallow dish and was dried at 105°C, and weighed. The percentage of ethanol-soluble extractive was calculated concerning the air-dried drug.

Total Ash value

Accurately 2 g of the air-dried oleo gum resin was weighed in a tarred platinum or silica dish and incinerated at a temperature not exceeding 450° until free from carbon and then cold and weighed again. The percentage of ash was calculated concerning the air-dried drug.

Acid insoluble ash value

Accurately 2 g of the air-dried oleo gum resin was weighed in a tarred platinum or silica dish and incinerated at a temperature not exceeding 450°C until free from carbon and then cold and weighed again. Then the ash was boiled with 25 ml of2M hydrochloric acid for 5 minutes. The insoluble matter was collected in a gooch crucible or on an ash-less filter paper, washed with hot water, ignited, cold in desiccators, and weighed. The percentage of acid-insoluble ash was calculated concerning the air-dried drug [21].

Estimation of essential oil

Extraction of essential oils was done using steam distillation with the help of the Clevenger apparatus. Ground gum resin (50 gm) was soaked in 300 ml distilled water and then extracted for 6 hours. The resultant distillate consisted of an emulsion of creamish essential oil and water, which was partitioned with n-hexane. The n-hexane layers were separated using a separating funnel.

The quantity of essential oil was estimated by the following formula:-

$$\text{Essential oil \%} = \frac{\text{Quantity of oil}}{\text{Quantity of resin used for extraction of oil}} \times 100$$

Estimation of bioactive chemicals- Guggulsterone E & Z

Quantification of Guggulsterone

Biologically active ingredients of Guggul was estimated with the help of High-Performance Thin Layer Chromatography (HPTLC) [10].

Standard solutions of Guggulsterones E&Z were prepared by accurately weighing quantities of guggulsterone-E and guggulsterone-Z (1mg) into separate 10 ml volumetric flasks, dissolving each sample in 3 ml of ethyl acetate and diluted to volume with methanol. Accurately weighed resin (50 mg) was placed in a 10 ml volumetric flask, dissolved with 2 ml of ethyl acetate, and the volume adjusted with methanol. A calibration curve was prepared for the estimation of guggulsterone.

Development of High-performance Thin Layer Chromatography Plate

The samples were applied using Linomet 5 (CAMAG) in the form of 7 mm long bands on 20 cm × 10 cm, 200 µm layered silica gel 60 F254 coated aluminum HPTLC plate (Merck) Four different amounts of standards (200, 400, 600 and 800 ng/spot) and 20 µg/spot samples were applied in three replications. After sample application, plates were developed in CAMAG Derivatization Chamber using the mobile phase of toluene: acetone (6:1) solution. The developing chamber was saturated with the mobile phase (20 ml) for 20 min., and the 10 ml mobile phase was allowed to migrate up to 80% distance on the plate at room temperature.

Estimation of guggulsterone E & Z

Standard solutions were prepared by accurately weighing quantities of Guggulsterone- (1 mg) into 10 ml volumetric flasks, dissolving the sample in 3 ml of ethyl-acetate (EtOAc), and diluting to volume with methanol. Accurately weighed resin (50 mg) was placed in a 10-ml volumetric flask, dissolved with 2 ml of ethyl acetate, and the volume was adjusted with methanol.

Development of High-Performance Thin Layer Chromatography Plate

The samples were applied using Linomet 5 (CAMAG) in a form of 7 mm long bands on 20 × 10 cm, 200 µm layered silica gel 60 F_{254} coated aluminum HPTLC plate (Merck). Four different amounts of standards (200, 400, 600, and 800 ng/spot) and 20 µg/spot samples were applied in three replications. After sample application, plates were developed in CAMAG

Derivatization Chamber using mobile phase, toluene: acetone (6:1) solution. The developing chamber was saturated with the mobile phase (20 ml) for 20 min and the 10 ml mobile phase was allowed to migrate up to 80% distance on the plate at room temperature (Fig. 1b).

Quantity estimation of Guggulsterone - E&Z

The developed plate was scanned in CAMAG TLC scanner 3 at 254 nm wavelength in a deuterium lamp (D2) with 6.00 × 0.45 mm slit dimension, 10 mm/s scanning speed, and 100 µm/step data resolution. Guggulsterones E&Z peaks were generated through scanning at a wavelength range of 200 to 400 nm with a slit dimension of 6.00 × 0.10 mm. The presence of both Guggulsterones was assessed only in the samples whose scan spectrum was similar to standard. The quantity of Guggulsterone E & Z was estimated through the calibration curve between standards and their peak area using WinCATs Planner Chromatography manager software (CAMAG) Switzerland Excel (Microsoft Office 10) in the USA. The E-GS and Z-GS content (percentage) in the samples was calculated using the following formula:

$$\mathrm{E-GS\ and\ Z-GS\ (\%)} = \frac{\text{Estimated amount (µg)} \times 100}{\text{A dry amount was spotted on the track (µg)}}$$

Quantity of Guggulsterone E & Z were estimated in all samples collected from different tapping treatments in different months.

Testing of microbial activity-

The experimental site used for bio-fumigation was divided into three different locations and labeled as, 1, 1.5, 2, 2.5, 3, 3.5 and 4 feet. Bio-fumigation was carried out by burning 10gm Guggul with fumigation catalyst *viz.*, plants powder, and cow dung cake. The microbial count at each locale before and after fumigation was investigated by exposure of plates for 30 min. The experiment was carried out in triplicates and the results were expressed in terms of mean CFU/30min. The Petri plates were exposed before and after for 30 minutes in the room air.

RESULTS

Physico-chemical properties of Guggul oleo-gum resin

The different physic-chemical properties of the oleo-gum resin are presented in Table 1.The physical nature of resin is viscous, and moist granules look like vermicular pieces. The oleo-gum resin burns in the fire, slightly melt in the sunlight and form a milky emulsion with hot

water. The ash, water-soluble, and alcohol soluble extractive varied 4.47, 25.76 and 10.65%, respectively. Essential oil percent of fresh oleo-gum resin was observed at 0.85 %. The number of bioactive constituents Totals Guggulsterone and Guggulsterone-E&Z was estimated with the help of High-Pressure Thin Layer Chromatography. The quantity of Total Guggulsterone and Guggulsterone-E&Z were quantified at 1.45,0.05 and 0.53%, respectively.

Fig.1a Oleo- gum resin

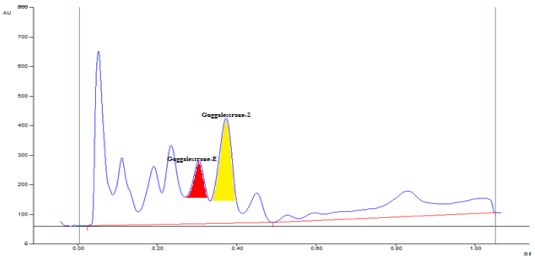


Fig. 1b HPTLC Chromatograph of oleo-gum-resin (Guggulsterone-E (Rf-0.32) and Guggulsterone-Z (Rf-0.38)

Table-1 Physico-chemical properties of Guggul oleo gum resin

S.No.	Parameters	Observations
1	Physical State	Viscous, moist, granules
2	Shape	Vermicular pieces
3	Ocular observation	Pale yellow or brown color, Fresh resin is viscid and golden in color
4	Taste	Bitter astringent taste
5	Moisture %	4.65±0.45
6	Water soluble extractive	25.76±0.15
7	Alcohol soluble extractive	10.65±0.24
8	Ash	4.47±0.78
9	Acid- insoluble ash	1.03±0.50
10	Alcohol soluble extractive	29.42±0.43
11	Water-soluble matter	54.65±0.76

12	Essential oil%	0.85±0.21
13	Gum%	25.98±0.71
14	Total Guggulsterone	1.45±0.30
15	Guggulsterone-E (%)	0.05±0.01
16	Guggulsterone-Z (%)	0.53±0.07

Values are the mean of three observations ± Standard deviation

Effect of oleo-gum-resin fumes on air quality of rooms

The experiment was conducted in different seasons. The effect of fumes on microbes-bacteria was evaluated in three different seasons- summer (March), winter (December), and also rainy (July).The observations were recorded regularly for up to 7 days.

The observations regarding the efficacy of oleo-gum-resin on-air quality- microbes (bacterial population) are depicted in **Table 2.** Significant variations in colony-forming units (CFU) were observed at different distances. Burning 10 g of Guggul resin for 30 min, decreased the CFU of the environmental microbes-bacterial population significantly (Fig.2).

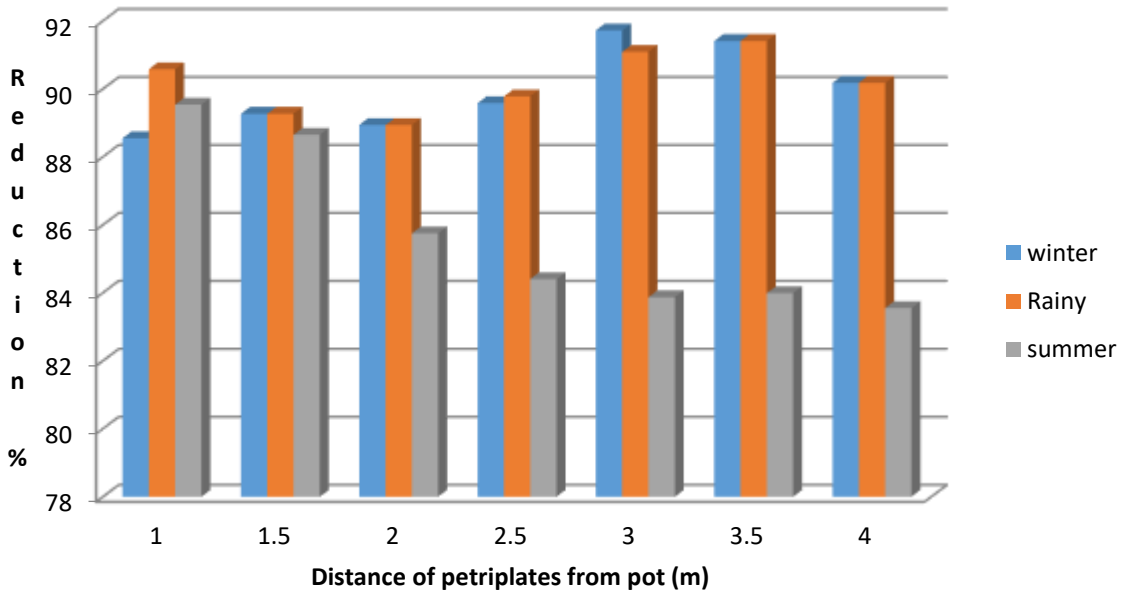
Medium plates were open in the room before and after the burning of resin and incubated for 7 days to grow a bacterial colony. The number of CFU in the room decreased significantly at different distances, only 7.43 to 13.43% (reduction rate of 88.55 to 91.72%) colonies was observed after fumigation in the winter season. These results show that the bacteria population reduced virulence when fumigated. Also, the reduction rate in the room in the summer and rainy seasons remarkably increased after fumigations. In the room, the % of CFU after exposure to the Guggal fumes in the summer season and rainy season varied from 7.07 to 19.79 % and 7.03 to 16.67, respectively. The burning of Guggul reduced bacterial count significantly compared to the control. The percentage of colonies in three seasons varied from 7.03 to 19.79%, observed after seven days of incubation.

Table no 2 Effect of fumes on the Number of bacterial colonies obtained on NA plates exposed in different seasons

Distance/locations (meter)	% of colonies after fumigation in July (Rainy) over the initial stage	% of colonies after fumigation December (Winter)	% of colonies after fumigation March (Summer)
1.0	4.08	12.5	7.07
	7.92	13.43	10.11
	6.25	9.6	13.26
	12.14	10.15	12.36
	16.67	11.51	9.52
Mean	9.41	11.43	10.46
1.5	10.77	10.77	9.54
	11.94	11.94	12.94
	8.75	8.75	14.40
	10	10	9.25
	12.12	12.12	10.56
Mean	10.71	10.71	11.33
2.0	11.11	11.11	11.62
	9.04	9.04	16.25
	12.43	12.43	16.60
	10.86	10.86	13.08
	11.79	11.79	13.67
Mean	11.04	11.04	14.24
2.5	11.55	11.55	14.09
	11.96	11.96	19.71
	7.69	7.69	13.47
	10.90	10.90	14.37
	8.92	8.92	16.29
Mean	10.20	10.20	15.58
3.0	7.86	7.86	17.44
	10.41	10.41	14.55
	10.15	10.15	15.28
	8.67	8.67	17.33
	7.43	7.43	15.98
Mean	8.90	8.90	16.11
3.5	8.11	8.11	15.46
	9.05	9.05	16.43
	7.03	7.03	19.79

	9.40	9.40	15.71
	9.28	9.28	15.60
Mean	8.57	8.57	16.59
4.0	8.36	8.36	15.86
	10.48	10.48	18.93
	11.32	11.32	15.86
	9.22	9.22	14.06
	9.66	9.66	18.49
Mean	9.80	9.80	16.64
CD (P=0.05)	0.45	1.05	1.98

Fig. 2 Effects of fumes on reduction of bacterial colonies in different season viz., Summer, Rainy, Winter



DISCUSSION

The results indicated that Guggul fumes are effective to reduce the microbial population significantly in different seasons. The reduction in the microbial load in the air due to fumes might be due to the presence of medicinal volatiles or antimicrobial chemicals released.

The observation of the present study is in accordance of the findings of several workers, who suggested using herbal products as an ingredient of hawan material to create a pure, hygienic, and healing atmosphere. Mono and multi-ingredient herbal remedies administered as smoke

were documented [1, 11]. The effect of burning natural substances to eliminate environmental microbes has been confirmed by different studies ([9, 19].

The positive effect of benzoin resin fume on microbes has been observed in other studies that used different types of incense extracts on environmental microbes [1, 2, 6, 7].

Natulyal*etal*.(2007) observed that burning 500 g of wood and a complex mixture of odoriferous and medicinal herbs for one hour caused over 94% reduction in aerial bacterial population counts [13].The inhibitory effect of benzoin resin fumes may be attributed to the volatile active ingredients, which have confirmed antioxidant and antibacterial effects [8], reported the antibacterial activity of leaf extract and essential oils against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Salmonella gallinarum*.

CONCLUSION

From the present study, it can be concluded that *Commiphora wightii,* oleo-gum resin fumes has the potential to reduce microbes significantly. This product's antibacterial activity was confirmed by the measurement of the efficacy of fumes on several colony-forming units. These findings may address the use of Oleo-gum resin of Guggal for formulations as fumigants.

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