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EFFECT OF POST HARVEST PROCESSING TECHNIQUES ON QUALITY AND ANTIMICROBIAL ACTIVITIES OF OLEO GUM RESIN OF *COMMIPHORA WIGHTII*

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Abstract:
In recent times, there has been increasing interest among researchers relating to critical aspects that affect the development of the herbal product based industries, with storage techniques/practices and their effect on the efficacy of produce. The unscientific collection, processing and storage methods/facilities and contamination by unwanted material as well have also contributed to the negative portrayal of natural plant products globally. The secondary metabolite compounds produced by herbal plants are widely used for medicinal purposes. The roles of the secondary metabolites are mainly for protection purposes in response to specific environmental stimuli such as attacked by pathogens or harmful microbes, insects and herbivores. The common examples of secondary metabolite groups are phenolic compounds, alkaloids, terpenoids and glycosides. Some of these groups of compounds are important medicinal bioactive. Present study clearly showed that drying method, storage containers and conditions significantly affect different parameters of oleo-gum-resin of *C. wightii* like moisture %, essential oil content, total guggulstrone, guggulstrone E & Z% contents and biological activities. Significant deterioration in oleo-gum-resin was observed in sun dried samples stored at room temperature in jute bags under both light and dark conditions. Glass bottles followed by plastic bottles and poly bags, kept at dark conditions in controlled temperature i.e. 4^0C or 10^0C was found best storage container and condition to avoid severe deterioration of biologically active constituents as well as bioactivities of Guggul.
Key words: herbal product, secondary metabolite, processing and storage

Introduction:

Medicinal plants have been cultivated for decades, where there is an awareness of the value of good collection and post-harvest practice, ensuring quality in term of anti-microbial potential.

In recent times, due to the adverse effect of synthetic chemical based medicines, there has been increasing interest among researchers relating to critical aspects that affect the quality as well as potency of medicinal produce. The Indian aurvedic medicinal experts like Vagbhata Charaka, and Sushruta have given detailed description of importance of Guggul as a drug (Kumar & Shankar, 1982; Satyawati, 1988). Traditional practitioners have described the therapeutic potential of many oleo-gum resins in curing many human disorders (Wanner *et al*., 2010; Shuaib *et al*., 2013; Singh *et al*., 2013).

The secondary metabolite compounds produced by herbal plants are widely used for medicinal purposes. The roles of the secondary metabolites are mainly for protection purposes in response to specific environmental stimuli such as attacked by pathogens or harmful microbes, insects and herbivores (Bannet and Wallsgrove, 1994; Mazid *et al*., 2011).The common examples of secondary metabolite groups are phenolic compounds, alkaloids, terpenoids and glycosides (Wink, 2015). Some of these groups of compounds are important medicinal bioactive. The use of herbal medicines have been in traditional practice for the benfit of mankind for centuries (Almagboul *et al*., 1988; Michie and Cooper, 1991; Newall *et al*.,1996; Azaizeh *et al*., 2003; Hawar, 2008; Saadabi and Moglad, 2011; Gadir and Ahmed, 2014).
The quality of Guggul (*Commiphora wightii*) oleo-gum-resin is an important issue which has complex ingredients, Guggulsterone-E and Z. There is no information about practice of the good post-harvest storage information on Guggul oleo-gum-resin after collection from forest. Post-harvest management can affect the commercial quality of the final products. The importance of reducing post-harvest losses by adopting processing technologies is the need of hour. Further, appropriate storage conditions and choice of appropriate types of packaging containers are important aspects for extending the storage life of oleo-gum resin.
The post-harvest handlings at the drying and storage stages are two important operations for maintaining the concentration of bioactive compounds (Razak *et al*., 2014). In actual practice, most of the dried herbs are commonly stored for certain time before processing stages. The selection of proper storage conditions including packaging may be one of the most important criteria to maintain quality of produce. Packaging can directly influence the medicine quality of herbal material by protecting the product from both air and light and also by maintaining it in optimum temperature and relative humidity.
To maintain the quality of medicinally important plant produce, the important factors that should be considered during storage are drying, types of packaging materials used, light intensity, relative humidity, air exposure and temperature. There is meager information on post- harvest storage of *C. wightii* oleo-gum-resin, therefore, more work on this aspect can improve the quality of final product.
Appropriate storage time and choice of types of packaging container are important aspects for extending the life of produce. Extensive studies on harvesting, collection, pharmacological and biological activities of oleo-gum-resin of *C.wightii* have been done; however, no research has yet been done to address the effect of post-harvest conditions on oleo-gum-resin of *C.wightii* under

consumer conditions and associated changes in the specific storage conditons. This knowledge can be adopted by primary collectors to avoid its quality deterioration. Keeping these facts in mind, the present investigation was conducted to study the effect of processing methods, drying of material, type of storage containers, storage conditions, duration of storage and their impact on quality as well as anti microbial activities of *C.wightii*, oleo-gum-resin. Present study was undertaken to standardize post- harvest technique and to evaluate antimicrobial activities of Guggul samples processed in different conditions of Guggul, oleo-gum-resin from *C. wightii.*

Materials and Methods:

Collection of sample

Oleo-gum-resin of *C. wightii* was collected from the naturally growing plants in forest of Bhuj (Gujarat) during October 2015 to February 2016. Samples were collected once only and all the experimental work was done from those samples.

Drying of samples

In past, it has been observed that quality of oleo-gum resin deteriorates very fast after harvesting. The quality of gum resin is also affected if dried in direct sun light and not stored properly in a suitable container. Therefore, samples were dried only up to six days after harvest. Traditional method was adopted for drying of samples under sunlight and shade for 1, 2, 4, and 6 days at room temperature and outside temperature.

Dried samples were stored in different containers i.e. glass bottles, air tight plastic containers, plastic bags, and jute bags in two conditions – dark and light at room temperature and at different temperature i.e. 4ºC, 10ºC and 20ºC. Samples of each treatment/methods were analyzed bimonthly for quality test.

Determination of moisture % on drying

The moisture % was determined using a Remi oven at 105±2°C for 5 to 6 hours to constant weight. Moisture was determined in fresh and stored sample of the gum resins.

Estimation of essential oil

Extraction of essential oils was done using steam distillation with the help of 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.

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

Estimation of guggulsterone E & Z

Standard solutions were prepared by accurately weighed quantities of Guggulsterone- (1 mg) into 10 ml volumetric flasks, dissolving 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.

Testing of antimicrobial activities:

Assessment of antifungal activities: Poisoned food technique (Nene and Thapliyal, 1979) was employed to assess antifungal activity of Guggul extractives at different dilutions against test fungi- *Fusarium oxysporum*, *Aspergillus niger*, *A. flavus* and *Candida albicans*. Antifungal activity of Guggul extracts of different dilutions was tested against fungi: *Fusarium oxysporum, Aspergillus niger, Aspergillus flavus, Candida albicans.* This technique involves the poisoning of the fungal growth medium using antifungal agent and then measuring the reduction of growth of the organism on the medium. The decrease in mycelial growth indicates the inhibition of fungal growth by the antifungal substance. Experiment was conducted in three replications. The experiments were conducted in completely randomized design (CRD) under laboratory conditions. Data were analyzed using ANOVA test to observe the effect of different treatments using SPSS Version 14.

Results and Discussions:

Post-harvest practice- Effect of drying on quality of oleo-gum resin of *C. wightii*

The drying in shade and sunlight affected the quality of Guggul significantly. The moisture % of the differently dried-sun and shade for 1, 2, 4 and 6 days are presented in Table **1**. The weight of fresh oleogum resin (6.56 g) was gradually lost under sunlight drying, ranged from 5.63 to 1.48 g during 1st to 6th day, respectively while under shade drying weight loss was ranged from 6.07 to 2.6 g during 1st to 6th days, respectively.

Essential oil per cent of fresh oleogum resin was (1.45 g) gradually lost under sunlight drying ranged from 1.23 to 0.68 % during 1st to 6th day, respectively. While, there was no significant variation in essential oil content in samples dried for 1st to 6th days in shade. Essential oil per cent was ranged from 1.62 to 1.56 % during 1st to 6th days, respectively. Minimum quantity of essential oil was observed in those resin samples, which were dried for six days in sunlight while highest quantity was observed in shade dried samples.

The quantity of bioactive constituent of Guggul, Guggulsteron-E&Z also found to be affected significantly due to drying in comparison to fresh sample. The quantity of Guggulsterones E&Z varied 0.49 to 0.0583 and 0.533 to 0.713, respectively.

The observations of room temperature and relative humidity during were noted during the entire storage. The temperature and relative humidity of room varied 16 to 43^{0}C and 12 to 47%, respectively. The fluctuations were observed in room temperature and relative humidity during the experimentation period. Maximum relative humidity % was observed in the month of December while minimum in the month of June and maximum room temperature was observed in June.

Post harvest practice- Effect of storage on moisture in different type of containers

Four different type of containers viz., glass bottle, jute bag, plastic bag and plastic bottle in light and dark conditions at room temperature and at 4, 10 and 20 ^{0}C were used for storage of resin samples. Oleo-gum-resin samples collected during 2015-2106 and dried for 1, 2, 4 and 6 days in sun light and shade were stored for two years in different packing materials.

S.No.	Quality parameters	Weight of fresh oleo-gum-resin (gm.)	Drying of oleo-gum-resin							
			Sunlight drying				Shade drying			
			1D	2D	4D	6D	1D	2D	4D	6D
1	Weight loss on drying (g)	**6.56±0.72**	**5.63±0.43**	**3.25±0.35**	**2.14±0.22**	**1.48±0.54**	**6.07±0.45**	**5.67±0.55**	**4.87±0.42**	**2.6±0.61**
2	Essential oil (%)	**1.45±0.30**	**1.23±0.61**	**0.91±0.09**	**0.75±0.22**	**0.68±0.08**	**1.62±0.33**	**1.57±0.32**	**1.53±0.40**	**1.56±0.34**
3	Guggulsterone-E (%)	**0.05±0.01**	**0.055±0.02**	**0.056±0.01**	**0.056±0.01**	**0.0569±0.01**	**0.0561±0.01**	**0.057±0.01**	**0.059±0.01**	**0.058±0.01**
4	Guggulsterone-Z (%)	**0.53±0.07**	**0.651±0.16**	**0.66±0.15**	**0.663±0.15**	**0.666±0.15**	**0.668±0.15**	**0.672±0.15**	**0.67±0.15**	**0.71±0.18**
	CD(P=0.05)	1.251 (Moisture %), 0.152 (Esseential oil%),0.043 (Guggulsteron-E%), 0.012 (Guggulsteron-Z%)								

Table 1: Quality of fresh and dried oleo-gum-resin of *C. wightii*

Values are the mean of three replications, D= days, ± Standard deviation

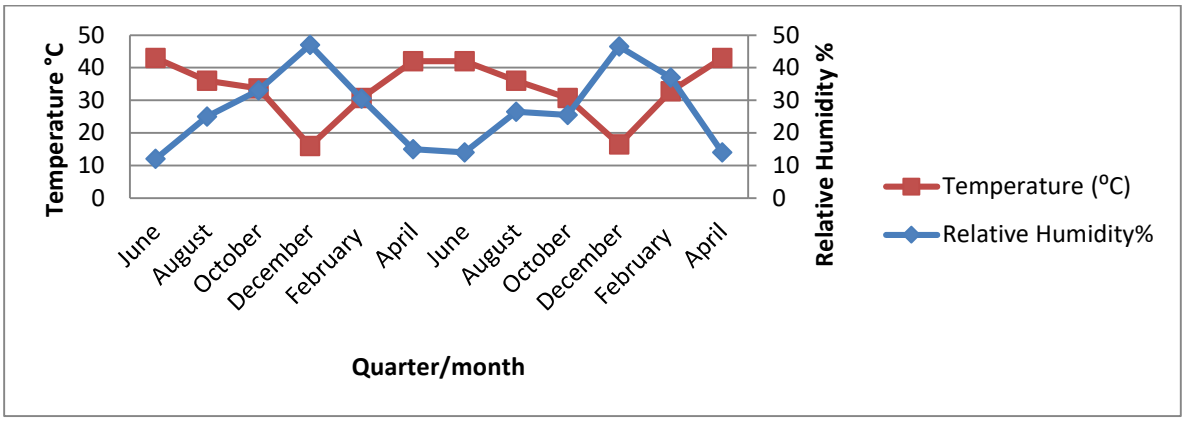

Fig.1Variation in room temperature and relative humidity during different months

Container	Storage period (month)																		
	6 months					12 months					18 months					24 months			
	Room temperature		4° C	10 °C	20 °C	Room temperature		4° C	10 °C	20 °C	Room temperature		4° C	10 °C	20° C	Room temperature		4° C	10 °C	20 °C
	Dark	Light				Dark	Light				Dark	Light				Dark	Light			
GB	2.72±0.23	2.54±0.47	3.79±0.34	3.59±0.13	3.72±0.15	2.64±0.55	2.32±0.38	3.87±0.09	3.8±0.04	3.92±0.41	2.75±0.05	2.15±0.03	3.62±0.09	3.57±0.14	3.24±0.12	2.62±0.05	2.17±0.49	3.42±0.21	3.29±0.12	2.74±0.10
PB	2.73±0.68	2.64±0.40	3.62±0.04	3.45±0.01	3.43±0.02	2.67±0.42	2.48±0.44	3.35±0.01	3.56±0.14	3.58±0.15	2.65±0.11	2.45±0.09	3.68±0.04	3.33±0.05	2.98±0.08	2.5±0.08	2.39±0.09	3.35±0.10	3.41±0.07	3.01±0.02
PoB	2.75±0.50	2.62±0.50	3.43±0.02	3.37±0.04	3.16±0.06	2.68±0.23	2.42±0.42	3.63±0.15	3.27±0.09	3.03±0.01	2.62±0.26	2.4±0.40	3.52±0.03	3.13±0.07	2.87±0.02	2.58±0.41	2.35±0.14	3.35±0.09	2.79±0.08	2.37±0.09

JB	6.37±0.19	5.93±0.23	5.07±0.07	5.9±0.89	6.03±0.07	6.19±0.16	6.05±0.13	6.67±0.14	6.7±0.07	5.17±0.08	6.12±0.31	5.85±0.60	5.98±0.15	5.74±0.18	5.07±0.05	5.51±0.24	6.8±0.55	6.09±0.40	5.62±0.18	4.98±0.06
CD (0.05)	Container*storage conditions 0.062 Container*storage conditions*months 0.054																			

Table 2 Effect of post-harvest practice-storage on moisture % of oleo-gum-resin of *C.wightii*

Values are the mean of three replications± Standard deviation**,** GB=Glass bottle, PB=Plastic bottle, PoB=Poly bag and JB=Jute bag

The post-harvest handlings at the drying and storage stages are two important operations for maintaining the concentration of bioactive compounds (Razak *et al*., 2014). In actual practice, most of the dried herbs are commonly stored for certain time before processing stages.

Quality of *C. wightii* oleo-gum-resin is an important issue which has complex ingredients, Guggulsteron-E and Z. Lack of the good post-harvest storage information on Guggul, oleo-gum-resin during collection from forest, can affect the commercial quality of the final products. Appropriate storage conditions and good choice of types of packaging container are important aspects for extending the storage life of oleo-gum resin.

Table 3 Effect of post-harvest practice-storage on essential oil % of oleo-gum-resin of *C. wightii*

D Different containers	Essential oil% in different storage period (month)																			
	6 months					12 months					18 months				24 months					
	Room temperature		4° C	10 °C	20 °C	Room temperature		4° C	10 °C	20° C	Room temperature		4° C	10 °C	20 °C	Room temperature		4° C	10 °C	20 °C
	Dark	Light				Dark	Light				Dark	Light				Dark	Light			
GB	1.42±0.3	1.34±0.08	1.43±0.01	1.42±0.02	0.95±0.03	1.32±0.05	1.29±0.04	1.24±0.02	1.16±0.18	0.9±0.04	1.15±0.06	1.09±0.07	1.05±0.04	0.98±0.04	0.74±0.31	1.09±0.07	1±0.04	0.92±0.02	0.87±0.13	0.65±0.01

PB	1.36±0.4	1.23±0.05	1.4±0.02	1.28±0.05	0.83±0.01	1.24±0.07	1.18±0.07	1.29±0.28	1.15±0.03	0.94±0.23	1.12±0.03	1.05±0.04	1.21±0.02	0.94±0.03	0.78±0.03	0.98±0.02	0.92±0.04	0.85±0.02	0.67±0.02	0.58±0.02
PoB	1.32±0.07	1.22±0.08	1.35±0.02	1.27±0.01	0.98±0.02	1.19±0.05	1.12±0.08	1.38±0.01	1.05±0.04	0.9±0.01	1.06±0.08	1±0.02	1.34±0.02	0.9±0.01	0.79±0.03	0.96±0.06	0.87±0.02	0.82±0.03	0.59±0.02	0.5±0.01
JB	0.57±0.05	0.35±0.03	0.98±0.02	0.92±0.01	0.72±0.01	0.45±0.08	0.41±0.08	0.9±0.03	0.79±0.03	0.58±0.01	0.4±0.03	0.34±0.04	0.87±0.03	0.71±0.01	0.69±0.02	0.32±0.02	0.29±0.07	0.67±0.01	0.47±0.01	0.32±0.01
CD (0.05)	Container*storage conditions 0.023 Container*storage conditions*months 0.014																			

Values are the mean of three replications, ± Standard deviation, GB=Glass bottle, PB=Plastic bottle, PoB=Poly bag and JB=Jute bag

Effect of storage at room temperature (dark condition) on Guggulsterone-E and Z content (May 2015-April 2016)

Guggulsterone- E & Z (%) after storage												
Type of container	(2nd Month)		(4th Month)		(6th Month)		(8th Month)		(10th Month)		(12th Month)	
	% E	% Z	% E	% Z	% E	% Z	% E	% Z	% E	% Z	% E	% Z
Glass Bottle	0.055	0.664	0.048	0.610	0.046	0.589	0.044	0.551	0.038	0.523	0.035	0.508
Poly Bag	0.054	0.626	0.046	0.522	0.042	0.484	0.041	0.448	0.043	0.419	0.038	0.396
Plastic Bottle	0.055	0.687	0.046	0.589	0.044	0.558	0.042	0.539	0.042	0.508	0.037	0.480
Jute Bag	0.054	0.575	0.044	0.442	0.035	0.381	0.033	0.321	0.029	0.278	0.026	0.219
CD(P=0.05)	CV(%)-0.1802(E), 0.2325(Z) CD(P=0.05) 0.031(E), 0.0327(Z)											

Container × months	

Values are the mean of three replications; ± Standard deviation

Effect of storage at room temperature (dark condition) on Guggulsterone-E and Z content (May 2016-April 2017)

Guggulsterone- E & Z (%) after storage												
Type of container	(14th Month)		(16th Month)		(18th Month)		(20th Month)		(22st Month)		(24th Month)	
	% E	% Z	% E	% Z	% E	% Z	% E	% Z	% E	% Z	% E	% Z
Glass Bottle	0.031	0.434	0.028	0.397	0.024	0.375	0.020	0.337	0.017	0.300	0.015	0.263
Poly Bag	0.034	0.329	0.030	0.301	0.025	0.277	0.019	0.220	0.014	0.181	0.009	0.141
Plastic Bottle	0.035	0.418	0.032	0.410	0.028	0.368	0.022	0.284	0.017	0.241	0.012	0.197
Jute Bag	0.024	0.108	0.021	0.086	0.018	0.055	0.014	0.034	0.012	0.012	0.009	0.011
CD(P=0.05) Container × months	CV(%)- 0.0580(E),0.5640(Z) 0.015(E),0.075(Z)											

Values are the mean of three replications; ± Standard deviation

Efficacy of fresh oleo-gum-resin in different solvent on *Escherichia coli* and *Staphylococcus aureus*

Solvent	Dilution	*E. coli*	*S. aureus*
Methanol	100	1.80±0.47	1.63±0.34
	200	2.23±0.55	2.13±0.98
	300	2.53±0.70	2.03±0.56
	400	2.80±0.98	2.43±0.43
	500	3.10±0.66	3.40±0.12
Ethyl-acetate	100	1.00±0.41	0.80±0.32
	200	1.20±0.27	1.20±0.34
	300	1.40±0.45	1.70±0.91

	400	2.10±0.36	1.80±0.39
	500	2.23±0.57	2.13±0.98
Water	100	0.20±0.06	0.20±0.10
	200	0.70±0.21	0.50±0.22
	300	1.00±0.27	0.60±0.26
	400	1.21±0.64	0.90±0.23
	500	1.50±0.25	1.00±0.30
SEM±		0.101	0.116
CV(%)		0.169	0.213
CD(0.05)		0.433	0.495

Values are the mean of three replications; ± Standard deviation

The result reveals that methanol extract 500 mg/ml of resin exhibited maximum inhibition of *E. coli* with a diameter of inhibition 3.1cm while maximum concentration of aqueous extract showed only 1.0 cm. The results of antibacterial activity showed significant variation at all tested dilutions of different extractives against *E.coli.* Different extractives of fresh sample of oleo-gum-resin inhibited the gram positive bacteria, *S. aureus* at different degree. Significant higher inhibition was recorded by methanol extract followed by ethyl-acetate and aqueous extractives. Methanol extract of resin was found effective against *S. aureus* at all tested dilutions. The zone of inhibition varied 1.18 to 2.54 cm tested at 100, 200, 300, 400 and 500 mg/ml dilutions, respectively.

Conclusion:

From the above results it was concluded that the post-harvest handlings at the drying and storage stages are two important operations for maintaining the concentration of bioactive compounds. In actual practice, most of the dried herbs are commonly stored for certain time before processing stages.

It was also concluded that the Quality of *C. wightii* oleo-gum-resin is an important issue which has complex ingredients, Guggulsteron-E and Z. Lack of the good post-harvest storage information on Guggul, oleo-gum-resin during collection from forest, can affect the commercial quality of the final products. Appropriate storage conditions and good choice of types of packaging container are important aspects for extending the storage life of oleo-gum resin.

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