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Effects of containers and duration of storage on the guggulsterone and volatile oils content of guggul

Moni Thomas, Niraj Tripathi, Kailash C Meena, JLN Sastry, GP Kimothi, Sakshee Sharma, Jatin Katna, Dhirendra Khare and Niranjan Prasad

Abstract
Guggul - an oleo-resin gum of immense medicinal value is tapped from a bushy shrub *Commiphora wightii* (Arnott) Bhandari distributed in semi-arid and rocky regions in Indian sub continent. *C. wightii* is an IUCN data deficient plant. Increase in the annual demand of guggul gum to the tune of 1000 metric ton, there is an increase in destructive tapping of C. wightii, which is a serious concern. Generally guggul gum from the site of its tapping to the site of processing or value addition being distantly far off, improper post harvest handling, storage and transport results in qualitative loss of its crucial bio-active compounds. Among the four containers (earthen clay pot, plastic jar, polythene bag and jute bag) evaluated for long term storage of guggul the earthen clay pot was the best in terms of Total Guggulsterone (TG) content. In earthen clay pot the TG of guggul gum was maintained over 1% for four months while Total Volatile Oils (TVO) 1% for nine months. In jute bag TG rapidly deteriorated within one month.

Keywords: Data deficient, guggulsterone, containers, deterioration, quality, chromatography

1. Introduction

Guggul- an oleoresin gum, of *Commiphora wightii* (Arnott.) Bhandari, belongs to family Burseraceae is of immense medicinal importance. It is a slow-growing, highly branched shrub or small tree, found in the arid rocky tracts of Rajasthan, Gujarat, Madhya Pradesh and Karnataka States of India and in Sind and Baluchistan provinces of Pakistan (Siddiqui *et al*., 2013) [37]. According to Siddiqui (2011) [35] Guggul comprises resins (61%), gum (29.3%), volatile oils (0.6%), moisture (6.1%) and foreign matter (3.2%). Guggul was first introduced to the scientific world in 1966 as a lipid lowering drug (Satyawati, 1966). However, Guggul is reported to be used since Vedic period for treatment of atherosclerosis, hypercholesterolemia, rheumatism, obesity, respiratory diseases, liver disorders, digestive problems, menstrual irregularities (Siddiqui and Mazumdar, 2012) [36]. Fresh guggul proved to have better effect in lowering serum cholesterol (5.67%), triglyceride (17.7%) and very low-density lipoprotein (18.36%), while one-year old guggulu had mild effect in lowering Tryglyceride (13.64%), VLDL (11.07%) and non-significant increase in serum HDL- cholesterol (0.94%). It also provided significant decrease in body weight (7.69%) and BMI by 7.82% (Vyas *et al*., 2015).

For the past few decades, natural compounds have been gaining value due to their immense element variation, and many of them are being used as a medicine (Sairkar *et al*., 2017). The main bio-active compound of guggul is inter-convertible isomeric form E and Z of guggulsterone that are steroidal in nature (Agrawal *et al*., 2004) [1]. Two different arrangements of CH_3 at C_{20} in three-dimensional space and the hindered rotation about the carbon – carbon double bond at C_{17} and C_{20} classifies guggulsterone into Z-{4,17(20)-cis-pregnadiene-3,16-dione} and E- {4,17(20)- trans-pregnadiene-3,16-dione} isomers.

Volatile oils - the secondary metabolite of plants consisting of a complex mixture of aldehydes, ketones, epoxides, alcohols and esters. Many volatile oils constitute of monoterpenes, diterpenes, and even sesquiterpenes (Rehman *et al*., 2007). However the volatile oils from the resin of *Commiphora* are myrcene, dimyrcene and polymyrcene (Bhati, 1950) [5].

The tapping and collection period of oleo gum-resin from guggul plants varies in different locations. Ideally tapping starts from the last week of February and the yield is about 200-800 g per plant depending on the plant health and ecology.

The collection of guggul gum usually extends upto April-May, when the temperature crosses 42 °C and the atmosphere remains hot and dry. Loss of guggulsterone and total volatile oils (TVOs) cannot be ruled out during these hostile climatic conditions. Apart from this loss, qualitative loss of Guggul during its complex trade route and improper packaging as well as storage before it reaches to the processing units or Drug industry, is a matter of worry. Owing to multifarious medicinal and therapeutic values of guggul gum as well as its various other significant bio-activities increasing demand of, guggulsterone in pharmaceutical, perfumery and incense industries requires its quantitative determination in existing natural populations of *C. wightii.*

As the tapping and collection period of *C. wightii* is wide while the trade routes are complex and long, qualitative loss is imaginable but has never been quantified. A scientific post harvest handling of this oleo-resin with volatile oils, bio-active chemicals can minimize the qualitative degradation of Guggul gum during transit from harvesting to processing centre. Thus the present investigation was to analyze the qualitative changes in guggulsterone and volatile oils in different storage conditions in a regular time interval.

2. Materials and Methods

Generally, various chemical fingerprinting tools are used for quantification and also for quality control of herbal remedies including chromatographic, spectroscopic, thermo-gravimetric analysis, capillary electrophoresis and polarography techniques (Choudhary and Sekhon, 2011) [9] but HPLC is a highly efficient, robust and quick analytical method for quantitative estimation of desirable components with optimum resolution.

2.1 Sample details: Fresh guggul gum (Fig. 1) was collected from Morena district in Madhya Pradesh, India. Iimmediately after harvest in the year 2016 guggul gum was brought to the laboratory at Jawaharlal Nehru Agricultural University, Jabalpur, Madhya Pradesh, India. Fresh guggul weighing 250g was stored in the month of June 2016 for 12 months in the four types of containers viz. earthen clay pot (T_1), plastic jar (T_2), polythene bag (T_3) and jute bag (T_4). Each type of containers had 12 units and each unit representing a month of the 12 months of storage (Fig. 1). Fresh guggul filled all the 48 containers with tightly closed mouth were stored in laboratory at room temperature. The samples stored in different containers were analyzed for total guggulsterone (%w/w) and total volatile oils (%v/w) monthly basis starting from July 2016 at Dabur India Limited, Site-IV Sahibabad, India. Dabur India is a leading Industrial House in numerous Ayurvedic products.

2.2 Chemicals & Instruments: All the chemicals used are HPLC grade (Merk) used in extraction and HPLC analysis. Analytical standards used in quantification of guggulusterone E & Z were procured from sigma Aldrich. HPLC analysis was carried out on Alliance Waters e2695 Separation module, 2998 PDA detector along with Phenominex Luna 5µ, C_{18} 250 × 4.6 mm HPLC column.

Fig 1: Illustration of A. fresh B. Old guggul gum C. Containers used For of Fresh Guggul

2.3 HPLC analysis

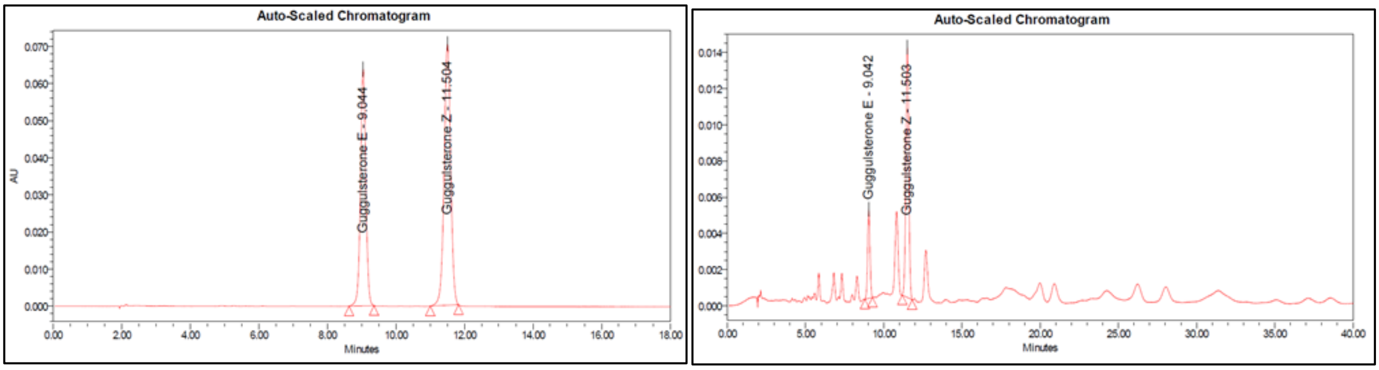
2.3.1 Standard solution: The standard solution for HPLC analysis was prepared with a mixture of 4.80 mg E-Guggulusterone and 3.99 mg Z-Guggulusterone weighed in 50 ml volumetric flask and dissolved in 10 ml Acetonitrile by sonication. Final volume was made upto the mark by Acetonitrile. The injected amount was 20 µL.

2.3.2 Sample solution: The sample solution of guggul samples stored in different containers was prepared by dissolving 500 mg of guggul gum in a 150 ml iodine flask and refluxed with 25 ml HPLC grade Acetonitrile thrice. Extract was filtered and final volume was adjusted to 50 ml in a volumetric flask for use as test solution.

2.3.3 Chromatographic conditions and procedure: Quantification of guggulusterone was done on Luna column using Acetonitrile and water as mobile phase in the ratio of 60: 40 using PDA detector at wavelength 242 nm for 30 min at 1ml/min flow rate and a constant column oven temperature of 25 °C. Determination of compounds was done by comparing the peak of the specific compound in the chromatogram with that of the respective standard peak.

3. Results and Discussion

Better packaging, storing and handling of guggul so as to maintain its Total guggulsterone (TG) content is a set of essential to achieve best effeicacy of the drug prepared from it. Guggul gum majorly consists of steroidal compounds commonly known as guggulsterones. Amongst all the reported guggulsterones, E and Z are the compounds majorly studied for quality assessment of guggul gum. Amongst all the samples studied Guggulsterones E and Z elutes in 9 and 11 minutes respectively, showing a persistent lower concentration of Guggulesterone E in comparison with Guggulesterone Z (Fig 2).



A. Standard **B. Sample**

Fig 2: HPLC chromatogram of A. standard and B. guggul gum sample

3.1 Total guggulsterone

Monthwise Total guggulsterone (TG) content (%w/w) present in the guggul gum samples stored in the four different containers for 12 months (Table 1) was analyzed. TG of guggul stored varied with the age of the storage and containers used for storage indicating qualitative degradation of the sample. This is a serious concern as this valuable plant product tapped from the IUCN data deficient plant is used for medicinal and therapeutical use. Fresh guggul sample of Chambal ravines with TG value of 1.5 percent, is a good indicator of its premium quality.

Guggul being an oleo-gum resin, is a complex mixture of Resin, Gum and Essential oils. The resin factor contains medicinally important bio-active guggulsterones, guggulsterols, cambrene and mukulol (Patil *et al*., 1972).The role of Guggulsterone isomers (E&Z) as hypo-lipidemic blood cholesterol reduction and cardio protection is widely acknowledged (Bajaj and Dev, 1982, Chander *et al*., 2003; Shishodia *et al*., 2008; Jasuja *et al*., 2012) [4, 7, 34, 18].

The sample stored in earthen clay pot (T_1) had highest (1.5 %) guggulsterone while it was lowest (1.2 %) in the sample stored in plastic jar (T_2) after 30 days of storage i.e. July, 2016. Reduction in the TG though was found in the samples stored in all the containers, but it was lowest (0.01%) in the samples stored in clay pots for the initial 60 days. The TG of guggul stored in clay pots though reduced to 1.19 percent from 1.5 percent by the end of four months of storage, yet it maintained the TG above 1 percent before sliding to 0.91 percent by the end of fifth month of storage. The TG of guggul samples stored in both plastic jar (T_2) and polythene bag (T_3) also more than 1 percent but only upto three months. Rapid degradation of TG in the guggul sample stored in jute bag reaching 1percent just after one month after its storage, was an eye opener as gums, gugguls as well as medicinal plant materials are generally packed and transported in jute bags.

Reduction in TG in storage is also reported by Singh *et al*. (2013), but it was less in the guggul stored in glass vial (GL) as compared to prolypropylene vial (PP) and polystrene vial (PS).Rapid deterioration of TG of Guggul in GL and PP occured only after 6 months of storage while in PS it was as earlier as 4 months from the date of storage. Guggul stored in dark and at 10^0 C had the least (10.35%) loss of TG. When Guggul was of guggul stored at 20, 30 and 40 °C for 2 months, the loss of TG was 2.12, 2.55 and 3.13 times more than that (10.35%) at 10 °C. Storing and transportation of large quantity of guggul gum in glass vessel is practically not possible given the fragile nature of glass. Secondly, glass being transparent may also contribute to qualitative degradation. Separation of glass pieces sticking to guggul gum in the event of breaking of glass container will be a serious issue.

In our present study, the guggul samples kept in the clay pots were covered with clay lids to maintain darkness and coolness, the TG reduced from 1.5 percent to 1 percent till 4 months of its storage while in the 5th month the TG was 0.91 percent (i.e loss of TG by 39.33%). The reduction in TG and TVO has been illustrated in Fig. 2 and Fig. 3. The percentage of TG in Guggul from Udaipur was 1.138 ± 0.02 percent (Soni *et al*., 2010). Concentration of bioactive components are influenced by many factors like season, collection area, annual rainfall, geographical variation, planting, harvesting practices, solvent system, mobile phase etc (Kulkarni *et al*., 2015).

We agree the fragile nature of earthen clay pot, but it is comparatively cheaper, cooler and provide darkness that may slow the natural degradation process of Guggul gum in storage and transportation.

Earlier workers have used clay pots to increase the shelf life of biologically active products. Jute seeds stored in earthen pots for 12 months had highest (14.80%) moisture content (Haque *et al* 2014) [16] while Paranjape and Kulkarni (2018) reported that food cooked in unglazed earthen clay pot have better shelf life. Al Shaibi *et al*. (1999) [2] reported that storing of insulin in a clay pot in dessert caused no loss of its activity as the mean temperature of the pot was 8.7 ^{0}C cooler than that was stored in shade. Gill *et al*. (2002) [15] found a reduction of only 0.8 ^{0}C from a clay pot used a storage for insulin in hot climates of South Africa with an ambient temperature of 23.4 ^{0}C. Allen (1992) [3] found water maintained a temperature of 15-17 ^{0}C despite increase in ambient temperature upto 41 ^{0}C in Uganda when spherical clay porous clay pot filled with 7-10 litres of water was kept in well ventilated position in shade. Pendsey (2006) reported maintenance of a temperature of 21-25 ^{0}C for storage of insulin in Nagpur pot within pot design when the ambient temperature was 42-48 ^{0}C.

Earthenware is an eco-friendly, non toxic, less expensive, and bio-degradable pot made up of readily available natural resources.

Table 1: Effect of storage age and containers on guggulsterone (%w/w) & total volatile oil (%v/w) in the guggul stored in different containers

Month	Total guggulsterone and volatile oil in relation to storage age and containers							
	Total guggulsterone (%w/w)				Total volatile oil (%v/w)			
	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
Jul-16	1.50	1.20	1.49	1.21	2.40	2.95	1.99	2.39
Aug-16	1.48	1.11	1.33	0.86	1.97	2.38	1.97	1.98
Sept-16	1.29	1.01	1.10	0.82	1.90	2.05	1.95	1.94
Oct-16	1.18	0.79	0.91	0.82	1.89	2.00	1.73	1.85
Nov-16	0.91	0.75	0.72	0.73	1.65	1.87	1.48	1.36
Dec-16	0.81	0.75	0.56	0.61	1.55	1.47	1.45	1.24
Jan-17	0.72	0.63	0.53	0.55	1.46	1.32	1.31	1.18
Feb-17	0.66	0.50	0.48	0.51	1.39	1.22	1.19	1.11
Mar-17	0.50	0.49	0.47	0.45	1.09	0.98	0.93	0.89
April-17	0.48	0.46	0.41	0.40	0.88	0.81	0.79	0.71
May-17	0.41	0.39	0.36	0.33	0.65	0.58	0.55	0.51
Jun-17	0.37	0.24	0.31	0.27	0.32	0.29	0.22	0.18

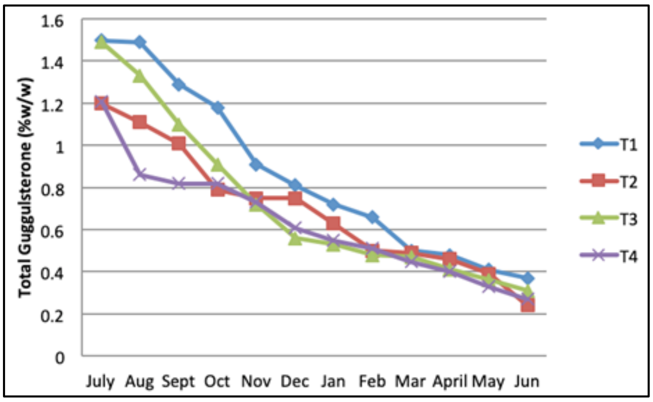


Fig 3: Changes in guggulsterone content with storage age and containers

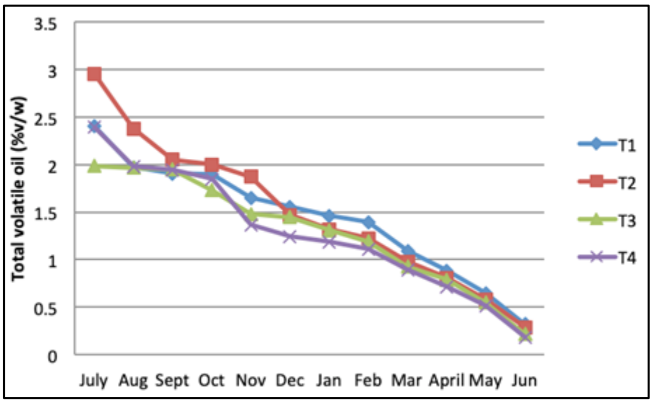


Fig 4: Changes in volatile oil content with storage age and containers

3.2 Total volatile oil

There is effect of container and age of guggul on total volatile oil (TVO) as evident from the qualitative analysis. (%v/w) The highest level of TVO was observed in the guggul samples stored in plastic jar (T_2) after 30 days of storage i.e. July, 2016. The guggul sample stored in jute bag (T_4) rapidly lost its TVO. Lowest reduction rate of TVO was observed in the guggul stored in polythene bag (T_3). By extending storage period in all packaging methods, TVO content significantly decreased. But, earthen clay pot was found to be better among all four containers used in the study because it maintained TVO upto 1% till 9 months of storage. In the present experiment all containers were able to maintain total volatile oil more than 1% upto 8 months. The TVO of guggul remained above 2% after few months of storage in polythene jar, while in all others it was below 2% from the second month of storage onwards. The TVO of the guggul samples after twelve months was 0.32 percent (earthen pot), 0.29 percent (polythene jar), 0.22 percent (polythene bag) and 0.18 percent (jute bag). Changes in TVO with storage age and containers are illustrated in fig. 2 and 3.

The change in the TVO composition during storage depends on the type of compound, the plant species, and the storage conditions (Mahmoodi Sourestani *et al.*, 2014). Volatile compounds reported to be present in guggul are myrcene, dimyrcene, and polymyrcene (Bhati 1950) [5]. Other components of the oil are eugenol, d-limonene, α- pinene, (±) linalool, cineole, α-terpineol, d-α-phellandrene, methylheptanone, bornyl acetate, (±) geraniol, and some other unidentified compounds (Saxena and Sharma, 1998). These compounds can migrate into the packaging material, which will produce changes in their levels. Misharina (2001) imagined terpene bio-transformation as a reason for these changes. He explained: terpenes are able to bind or release a water molecule, to isomerize or rearrange, and TVO components themselves, or trace contaminants, may catalyze or initiate these reactions. It has been observed that the composition of TVOs readily changes upon processing and storage, whereby factors such as temperature, light, and oxygen availability have a crucial impact on alteration processes (Díaz-Maroto *et al.*, 2009) [11]. Masand *et al.* (2014) suggested airtight HDPE container, HDPE carboys, and cardboard box with polyethylene linen for protecting volatile contents.

Guggul is used in numerous Ayurvedic preparations is widely known. Tomar *et al.* (2014) tested 61 guggul based Ayurvedic formulations. Guggul based drugs and formulations in trade make a further lengthy list further longer. The resin fraction of guggul gum contains medicinally important bio-active molecules of guggulsterones, guggulsterols, cembrene and mukulol etc (Patil *et al.*, 1972). Guggulsterones exhibit ant-inflammatory activity and potent inhibitors of nuclear factor kβ, a key regulator for inflammatory responses (Shishodia and Aggrawal, 2004). Pure guggulsterone isomers possess pronounced hypo-lipidemic activity, reduced blood cholesterol level significantly and possess cardio-protective properties (Bajaj and Dev, 1982; Chander *et al.*, 2003) [4, 7]. Guggulsterone –Z reported to mediate by its interaction with an array of nuclear receptor. It has been identified as an antagonist at the nuclear receptor farnesoid x receptor, a key regulator for the maintenance of cholesterol and bile acid homeostasis (Wu *et al.*, 2002; Cai and Boejer, 2006) [6]. Guggulsterone inhibits osteoclstogensis induced by receptor activator of nuclear factor-kappa β legend and by tumor cells by suppressing nuclear factor-kappa β activation (Ichikwana and Aggrawal, 2006). Plant sterol guggulsterone inhibits nuclear factor kappa β kinase and ameliorates acute murine colitis (Cheon *et al*., 2006) [8]. Guggulsterone –Z has been reported to inhibit the proliferation of a number of human cancer cell types like leukemia, head and neck carcinoma, ovarian carcinoma including drug resistant cancer cells like bleevac resistant leukemia, dexamethasone-resistant multiple myeloma and doxorubicin-resistant breast cancer cells (Shishodia *et al*., 2007; Marte and Downward,1997) [33, 21]. The given the bio-active compounds in guggul gum and its importance in human health, qualitative degradation cannot be afforded at any level. Especially when we know that the valuable gum is tapped from C. wightii which is an IUCN data deficient plant.

It may be for the first time, we have tried to bring the issue of qualitative decline of guggul in storage in an exclusively convincing way. Any qualitative loss will have a negative impact on the therapeutic value of the Ayurvedic formulation or drug, needs no elaborative explanations. According to Ayurvedic Pharmacopeia of India (API) as per GMP 1.1(F), stores should have proper ventilation free from dampness, adequate space for different types of material while GMP 1.1(F) (A) different stores may be used for herbs. Temperature for storage of raw herbal drugs may be between 8-25 ^{0}C, raw herbs must be protected from moisture, freezing, light and excessive heat for preventing decomposition.

Bhavaprakasha, one of the classical Ayurvedic texts mentioned two types of guggul i.e. fresh and old which is based on its storage time. Characteristics of both varieties of guggul are described in classics. Guggul that is golden yellow colour with balsamic odour and sticky is considered as fresh while guggul with dry texture, bad odour and discoloured is considered as old (Mishra and Viasya, 2002). Based on the colour of guggul, Bhavmishra and Kaidev classified guggul into Mahishaksha (Krishna), Mahaneel (Neel), Kumud (Kapish), Padm (Rakt) and Kanak (Peat) types. Kanak type guggul having brightness, good smell and stickness is considered as best quality and used for human (Chunekara *et al*., 2009) [10]. All these properties, guggul gum looses during existing practice of collection, storage and trade route. Decrease in active constituent of any medicinal product commonly reduces its efficacy. Therefore, it is important to determine their optimum storage condition and time, to know the period up to which the drug may be stored without losing much of their efficacy.

It has to be bear in mind that *C. wightii* is naturally distributed in the semi-arid and arid regions (Dixit and Rao, 2000) [12]. These regions are hostile in terms of adverse climatic factors (El-Beltagy and Madkour, 2012) [14] besides being remotely located from the processing centres or Ayurvedic industries. The tapping of *C. wightii* and collection of guggul gum is carried out during summer season, when the temperature in these regions crosses 42 °C. Majority of the volatile oils loose their liquid stage to gaseous nature at temperature 43 °C (Rodrigues and Carvalho, 2001). Thus chances of loss of TVO in field cannot be ruled out.

4. Conclusions

The time lag between collection of guggul from the plants and it arrival to the processing plant (drug industry) is often more than 12 months in India and other countries where it is tapped. During this transit period, guggul packed in polythene bag or jute bag is stored in open places. The temperature of these makeshift, stores are often above the normal temperatures, further susceptible to qualitative loss. Small traders after procuring guggul from gum collectors sell off the large traders. Large traders in the region, deal with the drug industry. The guggul packed in the polythene or jute bag is transported in open trucks; such transportation further degrades the guggul quality in terms of TVO. Finally on the arrival of guggul in the drug industry again it remains in the store or open space before the production unit of the industry indents it for processing.

In such a complex collection, trade and transportation, the qualitative degradation is an important issue which needs to be attended. Given the fact that guggul is extracted from Red listed data deficient plant *C. wightii*.

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