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RESEARCH ARTICLE

EFFECT OF STORAGE CONDITIONS AND PACKAGING MATERIALS IN MAINTAINING QUALITY OF *CENTELLA ASIATICA* (L.) URB. RAW DRUG

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Abstract: *Centella asiatica* (L.) Urb. is an important medicinal herb valued for its pentacyclic triterpenoid constituents, particularly asiaticoside, madecassoside, asiatic acid and madecassic acid. However, the raw drug remains vulnerable to quality deterioration during storage. The present study evaluated the influence of storage temperature and packaging material on the retention of important marker compounds and the physical and microbiological quality of *C. asiatica* raw drug over six months. The raw drug was stored under refrigerated (4 °C; M1) and ambient (M2) conditions using six packaging systems: glass jar (S1), plastic jar (S2), vacuum pack (S3), low-density polyethylene (LDPE) bag (S4), aluminium pouch (S5) and cloth bag (S6). Samples were assessed after three and six months for asiaticoside and asiatic acid contents. After three months, refrigerated vacuum packaging (M1S3) retained the highest asiaticoside (0.978%) and asiatic acid (0.097%) contents, while LDPE and cloth-bag samples under ambient storage had spoiled. After six months, the refrigerated vacuum-packed treatment retained 0.86% asiaticoside, equivalent to 88% of the initial value (0.98%), and had the lowest reported total viable count among the surviving treatments (10×10^7 CFU/g compared with 16×10^7 CFU/g initially). Refrigerated aluminium-pouch and cloth-bag samples retained 70% and 74% of the initial asiaticoside content, respectively, whereas the ambient vacuum-packed sample retained 62%. Most other treatments were recorded as spoiled by six months. Overall, the results indicated that combining refrigeration with vacuum packaging was the most effective of the tested storage strategies for maintaining the chemical and sensory quality of *C. asiatica* raw drug for upto six months. The study provides practical information for post-harvest handling and storage of *C. asiatica* intended for medicinal and value-added applications.

Keywords: *Centella asiatica*, Raw drug, Storage stability, Vacuum packaging, Asiatic acid

INTRODUCTION

Centella asiatica (L.) Urb., commonly known as gotu kola or mandukaparni, is a small, creeping herb of the family Apiaceae (Umbelliferae) that has a long history of use in traditional systems of medicine. Its pharmacological and therapeutic interest is largely associated with a group of pentacyclic triterpenoids, asiaticoside, madecassoside, asiatic acid and madecassic acid. These compounds have been investigated for wound-healing, anti-inflammatory, neuroprotective and other biological activities, making *C. asiatica* an important medicinal raw material for pharmaceutical, nutraceutical and cosmeceutical applications (Tomar 2008; Gohil *et al*., 2010).

Maintaining the quality of medicinal plant material after harvest is essential because the chemical composition and microbiological status of the raw drug can change during drying, packaging and storage. Moisture, temperature, oxygen exposure and inadequate protection from environmental conditions may contribute to loss of chemical markers and deterioration of physical quality. For this reason, the World Health Organization recommends systematic quality control of medicinal plant materials, including appropriate assessment of identity, purity, microbial quality and other factors relevant to their intended use (WHO, 1998; WHO, 2011). Stability investigations are particularly important for herbal materials because changes in marker compounds may occur even when the material remains visually acceptable (Kaur *et al.*, 2016).

Storage temperature and packaging are therefore important components of post-harvest management. Temperature influences the rate of chemical and biological reactions, whereas packaging determines the movement of moisture and gases between the material and its surroundings. Vacuum packaging can reduce the amount of oxygen in the package and may also limit moisture exchange when an appropriate barrier film is used. The effectiveness of such systems, however, depends on the characteristics of the raw material, package barrier properties, storage temperature and duration. Evidence from dried food systems shows that microbial survival and growth are strongly affected by temperature, relative humidity and packaging conditions (Hyun *et al*., 2018). Similar principles are relevant to dried medicinal plant materials also, although direct evidence for *C. asiatica* raw drug remains limited.

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Stability studies on *C. asiatica* extracts have demonstrated that marker compounds can decline during storage and that the extent of loss depends on extract composition and storage conditions. Kaur *et al*. (2016) reported reductions in chemical markers and biological activities during six months of accelerated stability testing of dried *C. asiatica* extracts. Puttarak *et al*. (2016) also demonstrated the stability characteristics of a standardized pentacyclic triterpene-enriched *C. asiatica* extract, while Monton *et al*. (2018) showed that the stability of asiaticoside and madecassoside can vary substantially with formulation and storage temperature. These findings support the need to investigate storage conditions at the raw-drug level rather than assuming that stability data generated for extracts or finished formulations will directly apply to raw drug material.

Accordingly, the present study was undertaken to compare two storage temperatures and six packaging systems for maintaining the bioactive quality and overall acceptability of *C. asiatica* raw drug during six months of storage. Particular attention was given to asiaticoside as chemical quality marker, together with sensory observations and, at the end of storage, total viable microbial count of selected best samples.

MATERIALS AND METHODS

Plant material and preparation of raw drug

Fresh, disease-free *C. asiatica* plants were harvested at physiological maturity from the herbal garden of the AICRP on Medicinal and Aromatic Plants & Betelvine (MAP & B), Kerala Agricultural University, Vellanikkara, Thrissur, Kerala, India. The plant material was authenticated by qualified botanist. The harvested material was cleaned and processed under hygienic conditions to obtain a uniform raw drug. The material was shade-dried for 24 hours and then used for the storage experiment.

Experimental design and storage conditions

The storage experiment comprised two storage conditions and six packaging materials, with three replications. The treatments were as follows:

Factor	Treatment
Storage condition (Factor A)	M1 – Refrigerated storage (4 °C) M2 – Ambient storage (room temperature)
Packaging material (Factor B)	S1 – Glass jar S2 – Plastic jar S3 – Vacuum pack S4 – Low-density polyethylene (LDPE) bag S5 – Aluminium pouch S6 – Cloth bag

A known quantity (1 kg) of raw drug was packed in each packaging system and stored under the respective temperature condition. Samples were evaluated at 0 (initial), 3 and 6 months. Because the sampling was destructive, separate packages were used for each scheduled sampling point.

Estimation of asiaticoside and asiatic acid

Asiaticoside and asiatic acid were quantified by high-performance thin-layer chromatography (HPTLC) (Abhishek *et al.*, 2014). Samples were analysed on silica gel 60 F254 plates using mobile phase of toluene: ethyl acetate: formic acid (5:5:1) for asiatic acid and n-butanol: ethyl acetate: water (4:1:5) for asiaticoside. Methanolic solution of samples and standard compounds were applied to the silica plates. Detection was performed using anisaldehyde–sulphuric acid derivatization and densitometric scanning on a CAMAG TLC Scanner. Asiaticoside and asiatic acid reference standards were obtained from Natural Remedies Pvt. Ltd. and Sisco Research Laboratories, respectively. Results were expressed as percentage content (% w/w) on a dry-weight basis.

The percentage retention of asiaticoside was calculated as follows:

Retention (%) = (Asiaticoside content at a given storage interval / Initial asiaticoside content) × 100.

Total viable microbial count

Microbial load of selected samples was assessed as total viable count (TVC) using a standard plate-count dilution method and expressed as colony-forming units per gram (CFU/g) of raw drug (WHO, 1998; WHO, 2011).

Sensory and physical quality assessment

Stored samples were examined for colour, odour and general appearance by a semi-trained panel. Samples showing visible mould growth, caking, pronounced off-odour or marked discoloration were classified as spoiled. Once a sample was classified as spoiled, it was not used for subsequent chemical or microbiological evaluation. Quantitative data were analysed using analysis of variance appropriate to the experimental design, and treatment means were compared at the 5% probability level using the critical difference (CD) wherever applicable.

RESULTS AND DISCUSSION

Effect of storage condition and packaging on asiaticoside and asiatic acid after three months

After three months, both asiaticoside and asiatic acid contents were lower in most stored samples than in the initial raw drug, although the magnitude of change differed among storage treatments (Table 1).

The initial asiaticoside and asiatic acid contents were 0.980% and 0.110%, respectively. Among the refrigerated treatments, vacuum packaging (M1S3) showed the highest recorded asiaticoside content (0.978%) and asiatic acid content (0.097%). The refrigerated cloth-bag treatment (M1S6) also retained a high asiaticoside content (0.973%). Refrigerated LDPE packaging (M1S4) recorded the lowest asiaticoside content among the refrigerated treatments (0.790%).

Under ambient storage, the retention of the marker compounds was generally lower than under refrigeration. The LDPE-bag (M2S4) and cloth-bag (M2S6) treatments were recorded as spoiled by three months and were therefore unavailable for chemical analysis. This pattern suggests that storage temperature and package characteristics jointly influenced the preservation of the raw drug. The superior performance of vacuum packaging under refrigeration is consistent with the broader principle that temperature and moisture/oxygen management are important determinants of stability in dried biological materials (Hyun et *al*., 2018).

Table 1. Effect of three-month storage on asiaticoside and asiatic acid contents of C. asiatica raw drug.

Storage condition	Packaging material	Asiaticoside (%)	Asiatic acid (%)
Initial	—	0.980	0.110
M1 (Refrigerated)	S1 – Glass jar	0.932	0.077
	S2 – Plastic jar	0.890	0.080
	S3 – Vacuum pack	0.978[a]	0.097[a]
	S4 – LDPE bag	0.790	0.083
	S5 – Aluminium pouch	0.950	0.087
	S6 – Cloth bag	0.973	0.093
M2 (Ambient)	S1 – Glass jar	0.893	0.073
	S2 – Plastic jar	0.873	0.073
	S3 – Vacuum pack	0.947	0.083
	S4 – LDPE bag	Spoiled	Spoiled
	S5 – Aluminium pouch	0.830	0.080
	S6 – Cloth bag	Spoiled	Spoiled
CD (0.05)		0.018	NS

Effect of storage on quality after six months

By six months, differences among the storage treatments had become more pronounced (Table 2). The refrigerated vacuum-packed treatment (M1S3) was the best-performing treatment in the experiment, retaining 0.86% asiaticoside, equivalent to 88% of the initial concentration. It also recorded the TVC 10 × 10^7 CFU/g, compared with 16 × 10^7 CFU/g in the initial material. Refrigerated cloth-bag (M1S6) and aluminium-pouch (M1S5) samples retained 74% and 70% of the initial asiaticoside content, respectively, and were observed as in usable condition at the end of six months.

The vacuum-packed storage under ambient conditions, (M2S3) was the only ambient treatment that remained unspoiled after six months. However, asiaticoside content had declined to 0.61%, corresponding to 62% retention. All other ambient treatments were observed as spoiled. Results indicated that vacuum packaging alone did not provide the same level of chemical preservation as the combination of vacuum packaging and refrigeration.

Decline in asiaticoside content during storage is in consistent with earlier stability studies on *C. asiatica* derived materials. Kaur *et al*., (2016) reported reductions in chemical markers during six months of accelerated stability testing of dried *C. asiatica* extracts. Puttarak *et al*., (2016) reported stability changes in a standardized pentacyclic triterpene-enriched extract, while Monton *et al.*, (2018) demonstrated that the stability of asiaticoside and madecassoside can depend strongly on the storage temperature and formulation matrix. These studies were performed on extracts or formulations rather than raw drug, but they support the interpretation that the triterpenoid profile of *C. asiatica* is sensitive to storage conditions.

The present findings also agree with the importance of maintaining suitable environmental conditions for medicinal plant materials. WHO quality-control guidance emphasizes the need to control contamination and deterioration in medicinal plant materials, while studies on dried biological products have shown that temperature, humidity and packaging can influence microbial behaviour during storage (WHO, 1998; WHO, 2011; Hyun *et al*., 2018). In the present study, the combination of refrigeration and vacuum packaging was associated with the longest observed period of acceptable quality among the tested treatments.

Table 2. Effect of six-month storage on asiaticoside retention and total viable count of *C. asiatica* raw drug.

Storage condition	Packaging material	Asiaticoside (%)	Retention (%)	TVC (CFU/g)
Initial	—	0.98	100	16×10^7
M1 (Refrigerated)	S1 – Glass jar	Spoiled	Spoiled	Spoiled
	S2 – Plastic jar	Spoiled	Spoiled	Spoiled
	S3 – Vacuum pack	0.86[a]	88	10×10^7
	S4 – LDPE bag	Spoiled	Spoiled	Spoiled
	S5 – Aluminium pouch	0.68[c]	70	
	S6 – Cloth bag	0.73[b]	74	
M2 (Ambient)	S1 – Glass jar	Spoiled	Spoiled	Spoiled
	S2 – Plastic jar	Spoiled	Spoiled	Spoiled
	S3 – Vacuum pack	0.61[d]	62	—
	S4 – LDPE bag	Spoiled	Spoiled	Spoiled
	S5 – Aluminium pouch	Spoiled	Spoiled	Spoiled
	S6 – Cloth bag	Spoiled	Spoiled	Spoiled
CD (0.05)		0.025		

Combined influence of temperature and packaging on shelf-life extension

The overall pattern of results suggests that the refrigerated vacuum-packed treatment provided the most favourable environment for preserving *C. asiatica* raw drug. Refrigeration slows down many chemical processes, whereas vacuum packing reduces the oxygen in the package and, depending on the material used, it will provide a barrier against the moisture exchange. The apt material and vacuum in combined form thus give protection to materials stored.

Vacuum packing under ambient conditions preserved the sample avoiding visible spoilage for six months, but the marker-compound retention was lower than the sample stored under refrigerated vacuum condition. Refrigeration alone did not guarantee chemical preservation, as some refrigerated packages were noted as spoiled.

The decline in asiaticoside observed in this study even in the best treatment agrees with the reported literature on *C. asiatica*. Puttarak *et al*., (2016) reported stability changes in a standardized triterpene-enriched extract, and Monton *et al*., (2018) found temperature-dependent changes in asiaticoside and madecassoside in a film-forming formulation. Kaur *et al*., (2016) likewise demonstrated measurable losses of chemical markers during accelerated stability testing of dried extracts. These observations support the present finding that refrigeration can reduce, though not completely prevent, chemical changes during prolonged storage.

From a practical perspective, the findings are relevant to medicinal plant growers, processors and raw-material suppliers who need to maintain quality between harvest and downstream processing. The refrigerated vacuum-pack system identified here may be particularly useful where the raw drug is intended for longer-term storage or transport.

CONCLUSION

The present study demonstrates that storage temperature and packaging material are important determinants of the quality of *C. asiatica* raw drug during extended storage. Among the combinations evaluated, refrigerated storage at 4 °C together with vacuum packaging provided the best overall preservation of the raw drug for six months. This treatment retained 88% of the initial asiaticoside content. The findings indicated that combining temperature control with suitable packaging is more effective than relying on either measure alone.

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