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Improvised storage of *Cassia fistula* L. fruit pod with special references to Ayurvedic principles and practices by traditional text: An analytical investigation

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Cassia fistula L. (Sanskrit: *Aragvadha*, family: Caesalpinaceae) is used as a mild laxative in traditional medicine. Ancient texts advocate specific storage of its matured and ripe fruits under a pit filled with sand or soil. The present study was designed to compare the physicochemical, organoleptic and other biochemical parameters of the fruit pulp, stored under usual and specific conditions as mentioned in ancient texts. The sample kept under a pit showed higher total phenolics, flavonoids and anthraquinone levels along with reduced total and reducing sugars. The increased antioxidant activity of the pit-stored sample due to higher total phenolics and flavonoids levels as revealed from the DPPH radical scavenging assay may enhance its medicinal attributes, justifying ancient claim of specific storage of the fruits.

Keywords: Antioxidant assay, *Cassia fistula*, Phytochemical assay, Specific storage
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There is a growing interest on traditional medicines all over the world, because of their efficacy, affordability and perceived non-toxicity to humans[1]. Ayurveda, the Indian traditional system of medicine follows a holistic therapy wherein the herbs, especially the phytoconstituents, play a major role as the raw drugs. However, the phytoconstituent profiles depend on specific collection times and methods and processing and storage procedures of the plants and plant parts. This aspect was well recognized in Indian ancient literature[2,3] and Ayurveda has strongly advocated for adoption of these aspects for optimum therapeutic efficacy and non-toxicity of the raw drugs[4]. Several ancient texts are very specific in storage of raw plant ingredients.

Cassia fistula L. (Golden Shower) is known as *Āragvadha* in Sanskrit text and its different parts show a rich pharmacological profile for potential use against several diseases[5-8]. It is considered as one of the important ingredients of *Aragvadhadi Kvatha Ćurna* in the classical text. The fruit pulp of *C. fistula* is primarily used as a mild laxative[9] (*Mrdu Virećana*) and also as a purgative[10] due to its constituent wax, aloin. However, its efficacy for the treatment of intestinal disorders including ulcer[11] as a broad spectrum antibiotic[12] as well as for neurological activities[13] of the seeds are also reported. More recently, the antimicrobial and anticancer activities of the fruit extracts have also been described[14,15]. A few studies on the phytochemistry of *C. fistula* fruit pulp and seeds have been carried out and presence of flavonoids, anthaquinones, furfural derivatives and a chromone has been reported[10,16-19].

Text of *Charaka Samhita* advocates stringent requirement on the collection and storage of *C. fistula* fruit pulp as shown below from the original literature[20].

फलकाले फलं तस्य ग्राह्य परिणतं च यत। तेषा गुणबता भारं सिकतासु निधापयेत॥ ६॥
सप्त रात्रात समुद्धत्य शोषयेदातपे भिषक। ततो मज्जानमुद्धत्य शुचौ भांडे निधापयेत॥ ७॥

According to this, the matured fruits (Fig. 1a and Fig. 1b) should be collected in a large quantity during appropriate seasons of fruiting and kept covered with sand for 7 days. Thereafter, these fruits should be taken out of the soil and after sun drying, the pulp should be taken out and stored in a clean jar. It is believed that such a storage method may preserve or enhance its medicinal property. This may be due to protection of the constituent phytochemicals from degradation or increased availability of the bioactive principles. However, the cause and effect of putting

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Fig. 1 (a) — flowering tree with matured fruits, (b) broken matured and ripe fruit pods

the pods inside the pit remains unclear. Earlier, the effect of collection methods on the composition of the phytochemicals and reducing as well as total sugars contents of *C. fistula* fruits pulp was studied[21]. The present investigation aimed to compare the different classes of phytochemicals between the ordinarily stored and sand-buried samples (as per *Charaka Samhita*). Oxidative stress is implicated in many human diseases and plant polyphenolic antioxidants are believed to prevent and ameliorate these[22,23]. Hence, the free radical scavenging activities of designated fruits pulp samples were also compared.

Methodology

Plant materials and reagents

The matured fruit pods of *C. fistula* were collected from the natural habitat of Bolpur area, Birbhum District (23°67′70″ N, 87°71′29″ E), West Bengal, in the month of June 2018 and authenticated in the Department of Pharmacognosy, Central Ayurveda Research Institute for Drug Development, Kolkata. A herbarium of the fruit pods was deposited in the department and is available for reference (Reference No. CalP/18708). All chemicals, reagents and solvents used in the work were of Emplura grade (Merck, Mumbai, India).

Plant samples processing and storage

The fruit pods were divided into two groups. One group of pods was buried under soil in a pit of 24 inches, while the other group was kept in an airtight glass jar. The individual samples were taken out of the pit and jar exactly after 7 days in twilight and the fruit pods were broken to obtain the respective pulp and seeds. The samples stored in the pit and the glass jars were designated as PS and GS, respectively and were separately stored in airtight glass jars. The photographs of PS and GS are shown in Fig. 2.

Fig. 2 — Fruit pulps from GS and PS

Soil analysis

Soil in which the pods were buried was taken for analyzing the moisture content by gravimetry, while pH of 10% aqueous suspension of the soil was measured[24]. For analyzing heavy metals in it, the soil sample was digested[25] in a microwave using a dilute nitric acid and hydrochloric acid (1:1) mixture, filtered into a 50 mL volumetric flask and the volume made up with distilled water. The mercury (Hg), lead (Pb), cadmium (Cd) and arsenic (As) contents were assayed by atomic absorption spectroscopy (AAS) using an Agilent instrument (model 240 AA).

Phytochemical screening of the plant samples

Each of the plant materials (5 g) was extracted separately with petroleum ether, chloroform, ethyl acetate, acetone, methanol, ethanol, water and equi-volume aqueous ethanol (each 150 mL) following the WHO guidelines[26]. The individual extracts were filtered and evaporated to obtain the respective solid extracts, which were used for screening the presence of secondary metabolites using standard protocols[27].

Organoleptic characters and physico-chemical evaluation

Organoleptic properties of the plant samples were assessed in terms of senses like touch, taste, sight and

smell. The physicochemical constants like ash values, loss on drying (LOD), extractive values and pH values of the sample plant materials were determined using crushed samples containing mixtures of pulp and seeds[28]. For extractability studies, the plant materials were extracted with different solvents like ethyl acetate, ethanol, water and with equi-volume aqueous ethanol under both cold and hot conditions[28].

Biochemical assay

The total phenolics contents (TPC) of the plant samples were determined using Folin-Ciocalteu reagent by spectrophotometry at λ_{max} = 760 nm[29]. Gallic acid was used as the standard and TPC is expressed as gallic acid equivalent in mg/g of dry sample. The total flavonoids contents (TFC) were measured using ferric chloride reagent by spectrophotometry at absorption maxima[30]. Quercetin was used as the standard and the TFC is expressed as quercetin equivalent in mg/g of dry sample. Total sugar was determined using dimethylphenol by spectrophotometry (λ_{max} = 510 nm)[31]. The reducing sugar was determined using 3, 5-dinitrosalicylic acid by spectrophotometry (λ_{max} = 575 nm)[32]. For estimating total anthraquinones, the free and *O*-glycosides were extracted with diethyl ether and hydrochloric acid plus glacial acetic acid, respectively. These were quantified using 1,8-dihydroxy-anthraquinone as the standard by spectro-photometry (λ_{max} = 520 nm) and total anthrquinones were calculated[33]. All the assays were performed in triplicates and the values are expressed as mean ± SD.

Heavy metals analysis

The plant samples were analyzed for the presence of heavy metals by a standard method as described in Ayurvedic Pharmacopoeia of India[34].

DPPH radical scavenging activity

The free radical scavenging activity was checked by the DPPH assay[35]. Butylated hydroxytoluene (BHT) solution in ethanol was used as the reference. A measured aliquot of 0.1 mM solution of DPPH was used as control by diluting with required quantity of ethanol. Different concentrations (50-500 µg/mL) of GS and PS in ethanol were added to the DPPH solution, the mixtures were incubated for 30 min at room temperature and their absorbance at λ_{max} = 520 nm measured with a Shimadzu UV-1800 spectrophotometer.

Results and Discussion

Characters of the soil wherein the plant drug was buried are noted in (Table 1). It had almost neutral pH value of 6.87 with 28% moisture content. Presence of lead (6.3 ppm) and arsenic (1.2 ppm) were noted whereas the cadmium and mercury levels were <0.02 ppm. The organoleptic characters of the plant samples are noted in (Table 2). The strong aroma and dark brown color in PS may be due to the enhanced phenolic contents (*vide infra*).

The physico-chemical data in (Table 3) revealed higher extractive values of PS in hydroalcohol and

Table 1 — Soil characters[a]

Soil Parameters	Observation (s)
Moisture content	28.05±0.06%
pH	6.87±0.02
Hg	< 0.02 ppm
Pb	6.3±0.02 ppm
As	1.2±0.02 ppm
Cd	< 0.02 ppm

[a]Values are expressed as Mean ± S.D.

Table 2 — Organoleptic characters of GS and PS

Organoleptic Characters	GS	PS
Colour	Brownish black	Dark brown
Odor	Aromatic	Strong aromatic
Touch	Smooth	Smooth but very sticky
Taste	Sweet	Sweet

Table 3 — Physico-chemical evaluations of GS and PS[a]

Parameters	GS		PS	
	Hot Extraction	Cold Extraction	Hot Extraction	Cold Extraction
Extractive value in ethyl acetate	0.6109±0.04	0.7301±0.06	0.6103±0.07	0.7203±0.05
Extractive value in hydroalcoholic (1:1) solvent	28.604±0.05	30.267±0.08	30.477±0.04	37.246±0.03
Extractive value in Water	29.340±0.11	33.730±0.09	29.479±0.11	39.520±0.11
Total ash	5.324±0.031		5.391±0.024	
Sulphated ash	2.931±0.017		2.892±0.015	
Acid insoluble ash	0.34±0.01		0.29±0.01	
pH (10 % aq. suspension)	5.19±0.01		4.98±0.01	

[a]Values are expressed as Mean ± S.D.

Table 4 — Phytochemical screening of GS and PS[a]

Phytochemical class	Hexane		Chloroform		Acetone		Ethyl Acetate		Methanol		Ethanol		Water	
	GS	PS	GS	PS	GS	PS	GS	PS	GS	PS	GS	PS	GS	PS
Anthraquinones	-	-	-	-	-	-	-	-	+	+	+	+	+	+
Alkaloids	-	-	+	+	+	+	+	+	+	+	+	+	-	-
Flavonoids	-	-	-	-	-	-	-	-	-	-	+	+	-	-
Glycosides	-	-	+	+	+	+	-	-	+	+	+	+	+	+
Polyphenols	-	-	-	-	-	-	-	-	+	+	+	+	+	+
Phenolics	+	+	-	-	-	-	-	-	+	+	+	+	+	+
Oil	+	+	+	+	+	+	-	-	-	-	-	-	-	-
Steroids	+	+	+	+	+	+	+	+	+	+	+	+	-	-
Terpenoids	-	-	+	+	+	+	-	-	+	+	+	+	-	-
Fatty esters	-	-	-	-	+	+	+	+	-	-	-	-	-	-
Free acids	-	-	-	-	-	-	-	-	-	-	+	+	+	+

aqueous solvents *vis-à-vis* GS, both by cold and hot extraction protocols. Appreciable hike in LOD in PS indicated that the natural moisture content of the plant sample was retained when kept under soil. The LOD results are consistent with a previous report, but the observed trend in the water extractive values was oposite[21]. There was no significant change in the ash values of PS and GS. From the pH values of the aqueous suspensions of the samples, it was noted that GS was slightly more acidic than PS. This may be due to release of free acids from some esterified phytochemicals. The phytochemical analysis data mentioned in (Table 4) did not reveal any qualitative difference, both GS and PS showed identical phytochemical array. Presence of alkaloids, terpenoids, steroids, phenolics, glycosides, free and esterified acids etc. was noted in the samples. The anthraquinones were present in the alcohol and water-soluble fractions, while the hexane-soluble fractions primarily contained oils and steroids. Interestingly the constituent phenolics were found in hexane, alcohol and water, suggesting that these may be highly in non-polar or present as glycosides.

The proximate biochemical data are shown in (Table 5). There was an appreciable reduction in the total sugar as well as reducing sugar contents due to storage in the pit. The total sugar and reducing sugar levels in GS were 51.3% and 12.15%, respectively, while the corresponding values in PS were 39.47% and 8.31% only. The higher temperature, maintained in the pit may degrade sugar, accounting for the results[36].

The TPC value of a plant extract is conveniently determined by absorption spectroscopy of the blue color complex formed between its constituent phenolics and the Folin-Ciocalteu reagent. Likewise,

Table 5 — Biochemical results of GS and PS[a]

Parameters	GS	PS
Total sugars	51.3±0.11%	39.47±0.15%
Reducing sugars	12.15±0.13%	8.31±0.12%
Total phenolics[#]	47.23±0.11	53.12±0.12
Total flavonoids[$]	14.20±0.09	16.10±0.05
Total anthraquinones[@]	63.21±0.07	67.53±0.07

[a]Values are expressed as Mean ± S.D.
[#]Expressed as mg of gallic acid equivalent/g of dry extract
[$]Expressed as mg of quercetin equivalent/g of dry extract
[@]Expressed as mg of 1, 8-dihydroxyanthraquinone equivalent/g of dry extract

the complexation between aluminum trichloride and the hydroxy-keto group-containing constituents of an extract can be used to estimate its TFC by absorption spectroscopy. Presently, the TPC values (mg gallic acid/g of dry sample) of GS and PS were 47.23 and 53.12, respectively. The TFC values (mg of quercetin equivalent/g of dry sample) of GS and PS were 14.20 and 16.10, respectively. The total anthraquinones (free and *O*-glycosidic anthraquinones) contents were found to be 67.53 and 63.21 for PS and GS, respectively. The increased phenolics and flavonoids contents of PS *vis-à-vis* GS may be due to more hydrolysis of the corresponding glycosides at a slightly higher temperature in the pit. This would increase these compounds in free forms along with sugars, which, however, may be degraded due to the temperature effect as observed in this study.

Soil contains various metals and their salts and the heavy metals are toxic. Hence, the heavy metals levels of PS and GS were compared and the results are shown in (Table 6). The levels of heavy metals like As, Hg, Pb and Cd in both the samples were identical and below 0.02 ppm which is far below the permissible limit prescribed by the Ayurvedic

Table 6 — Heavy metals levels in GS and PS[a]

Heavy Metals	GS	PS
Hg	< 0.02 ppm	< 0.02 ppm
Pb	< 0.02 ppm	< 0.02 ppm
As	< 0.02 ppm	< 0.02 ppm
Cd	< 0.02 ppm	< 0.02 ppm

[a]Values are expressed as Mean ± S.D.

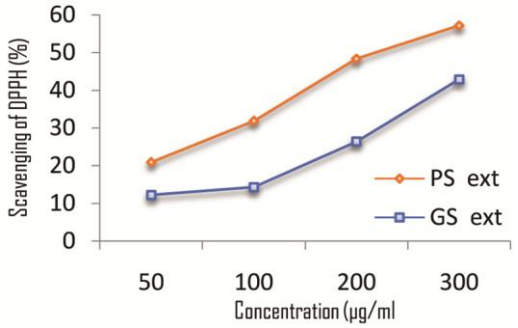

Fig. 3 — DPPH scavenging activities of GS and PS ethanol extracts

Pharmacopoeia of India. Hence, it may be assumed that there is a rare chance of contamination of heavy metals due to this type of specialized storage.

Given that many plants or herbs provide health benefits through their antioxidant action, we also assessed the effect of the specific storage protocol on the antioxidant property of the test samples. The bleaching of DPPH absorption at 517 nm by a test sample represents its capacity to scavenge free radicals and is often used for assessing its antioxidant property[35]. Hence, we assayed concentration-dependent DPPH scavenging activities of the ethanol solutions of PS and GS. Since the sample solutions were deep in colour, the data obtained beyond 300 µg/mL of sample were omitted. As shown in Fig. 3, PS showed higher scavenging activity than GS with an IC_{50} value 206.6 µg/mL. On the other hand, even at the highest test concentration (300 µg/mL) GS provided only ~43% DPPH scavenging and its IC_{50} value could not be ascertained.

Earlier, the effect plant maturity on the antioxidant activity of *C. fistula* bark, stem, leaf and root extracts was evaluated[37]. In separate investigations, the antioxidant activities of aqueous methanol and aqueous ethanol extracts of *C. fistula* stem bark, leaves, flowers and fruit pulp were also reported[38,39]. However, this is the first report on the augmented antioxidant activity of *C. fistula* fruits pulp due to storage of the fruits under soil, as mentioned in ancient Ayurvedic literature. The enriched total phenolics and flavonoids may account for the increased antioxidant activity of PS over GS. The data also revealed lesser total and reducing sugars levels in PS than GS. Hence consumption of PS may not adversely affect the blood sugar levels.

Conclusions

Overall, based on the results of the comprehensive study, it was found that the pulp obtained from *C. fistula* fruits, stored under a soil pit for 7 days, as prescribed in *Charaka Samhita* is enriched with total phenolics, flavonoids and anthraquinones, but contains lesser total and reducing sugar levels compared to that stored in a glass jar. The soil-buried storage does not alter the secondary metabolites contents of the pulp qualitatively. Given the broad spectrum of biological activities of the anthraquinones, the increase in their level due to storage under soil may provide better health benefit. The augmented TPC and TFC levels in the specially stored *C. fistula* fruits pulp (PS) *vis-à-vis* the conventionally stored sample (GS) may also be beneficial against oxidative-stress related diseases. The higher extractive values of PS in hydrophilic solvents would assist in better circulation of the bioactive principles of *C. fistula* fruits pulp. The underground storage did not lead to any toxic heavy metal contamination. Taken together, these findings may emphasize the need of storing the designated sample as per *Charaka Samhita* for optimizing its health benefits[20].

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Conflict of Interest

Authors declare that there is no conflict of interest associated with this manuscript.

Authors' Contributions

K H has designed and conceptualized the research work. He also contributed in performing analytical work, writing and editing of the manuscript. S D has contributed in writing and analytical work related to botanical identification.

A M and R K R has conceptualized the issue on reviewing the traditional text. M M R has the responsibilities of overall mentoring during all along the work.

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