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Full Length Research Paper

Comparative study on physicochemical variation for different samples of *Cassia grandis* Linn. leaves

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The present study is intended primarily to confirm changes with respect to quality of the drug. The variability in proper production unit, supply unit, methodology of collection and storage conditions were used to change the crude herbal drugs. The lack of knowledge of proper methodology, availability and more demands of herbal drugs for better treatment are promoting the practices of adulteration and substitution. Therefore, the standardization of the herbal drugs is essential to the identification of their therapeutic efficacy. Comparative studies were carried out to evaluate the physico-chemical standards of *Cassia grandis* with emphasis on botanical sensory evaluation, qualitative analysis of the plant's primary and secondary metabolites, colour produced under ordinary light, ultraviolet-radiation (UV) and thin layer chromatography (TLC) fingerprinting of the drug for chemical identification. Samples were procured from three diverse vicinities to determine the qualitative and quantitative variations. These results indicate the variations in quality and quantity of physicochemical parameters, place and time of collection, analysis procedure, and storage conditions. The study indicates that out of demonstrated samples, self collected sample showed significant results.

Key words: *Cassia grandis* Linn. leaves, % physico-chemical studies, % standardization.

INTRODUCTION

Plants continue to serve as possible sources for new drugs and chemicals. These can be extremely useful as it provides the structure for synthetic modification and optimization of bioactivity. Widespread use of botanicals and herbal products as medicinal products in developing countries are also becoming a part of the integrative health care systems of industrialized nations nowadays. An evaluation of herbs according to their traditional and folk uses in treatment of various diseases can be included in "complementary alternatives system of medicines" (CAM). Safety and efficacy of natural herbal product is therefore a cause for concern in order to promote and rationalize their use (Nadkari, 1954; Kirtikar, 1975). Lag phase for plant medicines is now rapidly changing for a number of reasons.

The problems with drug resistance to micro organisms, side effects of modern drugs and emerging diseases where no medicines are available have stimulated renewed interest in plants as a significant source of new medicines. Considerable research on pharmacognosy, chemistry, pharmacology and clinical therapeutics has been carried out on medicinal plants. There have been impressive successes with plant medicine, most notably quinghaosu and artimesinin from Chinese medicine. The superiority of natural products over synthetic drugs remains undisputed mainly due to compliance that is more favourable and their bioavailability properties. Secondly, metabolic compound of plants using conventional therapy for treatment in phytomedicine

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Table 1. The topographical details of collection of *Cassia grandis* Linn. Leaves.

Parameters of collection	Self collected	Supplied by Nashik distributor (MS)	Market sample
Time of collection	March to May	NP	NP
Location	Town	NP	NP
Climate	Spring	NP	NP
Rainfall average	50-80 cm (Annual)	50-80 cm (Annual)	NP

NP- Not provided by the genuine drug supplier.

yielded unsatisfactory results, although phytomedicine is nutritional. Wonderful cures have been found with the help of its constitutes but these properties have not been properly identified even today.

Extremely vague description about plant has been described (Rastogi, 1991; Mukherjee, 2002; Trease, 1983). *Cassia grandis* Linn. (Leguminoseae) has also been reported by another vernacular name liquorice tree. The extensive literature survey of the plant revealed that no more research work deals with the isolation and characterization of phytochemicals and their pharmacological activities of *Cassia grandis* Linn. leaves. Variation and deterioration of physicochemical constituents in plant drug enhanced its effect on safety and efficacy of drug.

However, one sterol namely β-sitosterol and palmitic acid, two sugars namely glucoside and rhamnose and five basic compounds one of them being kaempferol were reported to be contained in the *Cassia grandis* Linn (Harborne et al., 1970; Jackson et al., 1986). Few studies also claimed the presence of laxative, anti-inflammatory, hepatoprotective and antioxidants properties. The aim of the present study is to provide more information and scientific validation of the *Cassia grandis* Linn. leaves for its acclaimed medicinal use and to determine the quality of the drug based on three samples from different locations.

MATERIALS AND METHODS

The leaves of *Cassia grandis* Linn. used for present study were self collected, supplied by Nashik distributor (MS) and also purchased from market (Table 1). Transverse section of leaves were taken, stained and mounted following the usual plant micro-techniques. Representative diagrams were sketched through Lucida camera. For the study of isolated cells and tissues, small pieces of leaves were macerated with Schultz's fluid, washed and mounted in glycerin. Physio-chemical test were carried out adopting standard procedure.

Ash-value, solubility of (maceration, cold percolation and graded soxhlate extraction) extracts in the various organic solvents values, screening of thin layer chromatography (TLC), effect of different chemical reagents and florescence analysis under ultra-violet radiation (UV), which are considered to be immense help in detection of adulterants, were also carried out. Qualitative analysis for the identification of phyto-constiuents like alkaloids, steroids terpenoids, phenols, tannins, saponins and flavinoids etc, were also carried out (Jackson et al., 1986; Ambasta et al., 1996; Verma et al., 1995; Chase, 1949).

TLC analysis

TLC analysis of various extracts of leaves of *Cassia grandis* Linn. was subjected to TLC on silica gel-G (manually coated on glass plate in laboratory). Various combinations of the solvents of different polarity were adjusted to find out the suitable TLC pattern of the phyto-constituent. The resolution pattern was detected in iodine-chambers and Lieberman-reagent (Stahl, 1969) (Table 8).

RESULTS AND DISCUSSION

The leaves were 4 cm long, 1.5 cm wide and 0.5 - 1.0 cm thick, compound and alternate. Leaves were odourless with slight taste; dorsal surface was smooth and greenish yellowish in colour. They were covered externally with white papery membrane. Organoleptic characters of leaf have been summarized (Table 2).

Microscopic examination of the leaves showed cutical, epidermis, palisade cells, collenchymatous and parenchymatous cells in single or in groups. Fibers, vessels and trachoids were single or in groups. Transverse section of leave was circular in outline. Epidermal cells were barrel shaped having cuticle in outline. The epidermis below was large ground tissue consisting of parenchymatous cells. Parenchymatous cells towards the epidermis were smaller in size while those towards the center were bigger. Parenchymatous cells had sufficient inter-cellular spaces. Phloem was encircled by xylem. Vascular elements showed scalariform and spiral thickening (Figure 1).

The powder of *Cassia grandis* Linn leaves. was non-hygroscopic in nature. The qualitative analysis of three samples (self collected, supplied and marketed) showed that most of the major plant metabolites are common, except for alkaloids and flavonoides, which are less or negative in the marketed sample (Table 3). The difference in foaming index of the samples (self collected, supplied and marketed are >500, 365 and 234, respectively) indicates that the foaming ability of the drug decoction is governed by the nutrient, chemical nature of the constituents, region, season, climatic conditions and storage. Comparative lower value of swelling index (self collected 1.13, Market 1.02) reveals that the self collected sample have appreciably more amounts of mucilage, pectin and carbohydrates compared to the market sample (Table 4). The comparison of total ash value

Table 2. Organoleptic identification of *Cassia*

Parameters	Observations		
	Self collected sample	**Supplied by Nashik distributor (MS)**	**Market sample**
Botanical sensory evaluation			
Visual macroscopy	Curved	Curved	Curved
Size (in lenght)	4.4 cm	4.1 cm	3.9 cm
Shape	Broadly oblong	Slightly oblong	Slightly oblong
Touch	Dorsal smooth and Ventrical rough	Dorsal smooth and Ventrical rough	Rough
Odour	Odourless	Odourless	Odourless
Taste	Slight	Slight	Slight
Colour	Pale green	Greenish brown	Yellowish brown
Foreign Organic Matters	No adulterants have been found	No adulterants have been found	Slightly adulterants have been found

grandis Linn. leaves.

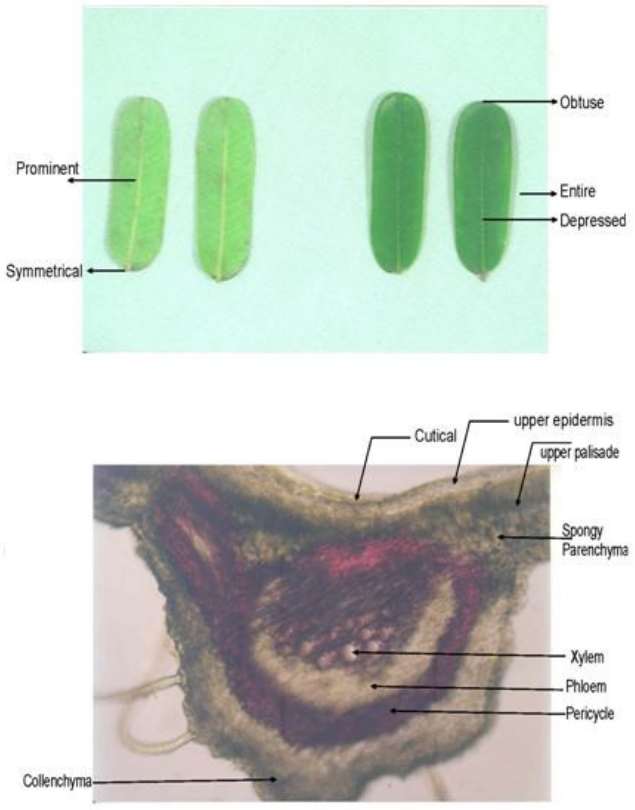

Figure 1. Diagramatic representation of leaf (T.S.) of *Cassia grandis* Linn.

suggests that the total physiological and non-physiological ash is higher in self collected sample compared to the market sample (Table 4). But the non-physiological ash (acid insoluble value), clearly indicate that the market sample is contaminated with silica or siliceous matters.

The chemical assay of the *Cassia grandis* Linn. leaves are yet to be instituted. The variation of extractable matter in various solvents is suggestive of the fact that the formation of bioactive principles of medicinal plants is influenced by a number of intrinsic and extrinsic factors. This leads to strange qualitative and quantitative changes making such plants totally unfit for the prescribed purpose even of same species. This highlights the importance of chemical mapping of the drug species. Behavior of different drug powder samples in different reagents was observed under ordinary light and UV radiation (254 and 365nm) (Tables 5, 6 and 7). Few reactions showed the diagnostic colour under either ordinary or UV radiation. The TLC patterns were recorded and the results tabulated (Table 8).

Following the instruction of Indian Herbal Pharmacopoeia and material medica, the preliminary TLC studies revealed that the solvent system was ideal and gave the well-resolved spot of the fractions. The chemical nature of various extracts of *Cassia grandis* Linn. leaves were identified by thin layer chromatography and chemical reaction. Thus, the quality of the chemical constituents of *Cassia grandis* Linn. varies with the different factors.

Conclusion

The present study indicates the variability of drug constituents and physicochemical nature due to various exogenous and endogenous factors temperature, rainfall, light length, age of plant, drying procedure, moisture and storage of sample. From results, it concluded that the self collected sample is more qualitative and quantitative.

Table 3. Qualitative analysis of the primary and secondary metabolites of *Cassia grandis* Linn. leaves.

S/No.	Metabolites	Self collected sample	Supplied by Nashik distributor (MS)	Market Sample
1	Carbohydrates	+ve	+ve	+ve
2	Proteins	-ve	-ve	-ve
3	Alkaloids	+ve	+ve	-ve
4	Steroles	++ve	+ve	-ve
5	Anthraquinones	+++ve	++ve	++ve
6	Resins	-ve	-ve	-ve
7	Saponins	+ve	-ve	-ve
8	Flavonoids	++ve	++ve	+ve
9	Glycosides	++ve	++ve	+ve
10	Tannins	+ve	-ve	-ve
11	Carotenoids	-ve	-ve	-ve
12	Triterpenoids	+ve	-ve	-ve

Table 4. Physicochemical characterization of *Cassia grandis* Linn. leaves.

S. No.	Parameters	Observations		
		Self collected sample	Supplied by Nashik distributor (MS)	Market sample
		Physicochemical		
	Ash values (% w/w)			
	Total ash value	3.76±0.56	2.54±0.35	2.61±0.49
	Acid insoluble ash	2.07±0.21	2.87±0.06	3.62±0.30
	Water soluble ash	3.69±0.42	3.72±0.43	3.75±0.40
	Extractive values (% w/w)			
	Cold maceration method			
	PE(60-80°)	3.21±0.48	4.87±0.65	5.37±0.49
	CHL	3.12±0.50	4.22±0.42	5.17±0.47
	ME	4.67±0.58	5.91±0.60	7.43±0.43
1	Cold percolation method			
	PE(60-80°)	4.53±0.39	5.17±0.38	5.87±0.47
	CHL	4.08±0.41	4.96±0.51	5.59±0.46
	ME	6.81±0.29	7.07±0.45	6.45±0.48
	Soxhlet extraction method			
	PE(60-80°)	3.72±0.43	6.95±0.40	7.76±0.65
	CHL	3.11±0.38	4.17±0.41	6.54±0.42
	ME	8.49±0.53	7.51±0.51	9.76±0.54
	Loss on drying (LOD)	18.48±0.89	7.62±0.56	7.86±0.45
		By Hot air method	By Hot air method	By Hot air method
		17.59±0.34	7.37±0.41	7.53±0.43
		By Azeotropic method	By Azeotropic method	By Azeotropic method
	Volatile oils (% v/w)	0.47±0.23	0.13±0.12	NI
		Physicochemical		
2	Swelling Index	1.13±0.15	0.89±0.12	1.09±0.12
	Foaming Index	More than 500	365	234

Values presented are mean of triplicate (Mean±S.D.), NI: Not identified

Table 5. Behaviour of powder of self collected *Cassia grandis* Linn. leaves with different reagents observed under ordinary light and UV-radiation.

S.No.	Reaction of self collected sample with different reagents	Colour produced under ordinary light	Colour produced under UV radiation	
			Short (254 nm) wavelength	Far (365 nm) wavelength
1	Drug (P) such as	Greenish	Greenish yellow	Pinkish-yellow
2	P+Amyl acetate	Brown	Rose pink	Green
3	P+1N.NaOH+Water	Light brown	Violet	Light green
4	P+1N.NaOH+Amyl acetate	Greenish brown	Dark brown	Brown
5	P+1N.HCl+Amyl acetate	Redish brown	Dark brown	Dark brown
6	P+1N.NaOH in methanol	Grey	Red	Green
7	P+50%+KOH+Methanol	Blue violet	Bluish	Violet
8	P+1N.HCl+Ethanol	Magenta	Crimson	Pink
9	P+50%H_2SO_4+Anisaldehyde	Yellow	Pink	Blue
10	P+50%HNO_3	Violet	Dark brown	Brown
11	P+Conc.HNO_3	Cherry red	Brown	Light brown
12	P+Acetic acid	Blue	Green	Violet
13	P+Conc.H_2SO_4	Light brown	Dark red	Brown
14	P+50%$FeCl_3$+Methanol	Yellow	Pink	Violet
15	P+Iodine water	Brown	Brown	Dark brown

Table 6. Behaviour of powder of supplier sample of *Cassia grandis* Linn. leaves with different reagents observed under ordinary light and UV-radiation.

S. No.	Reaction of supplied sample by distributor with different reagents	Colour produced under ordinary light	Colour produced under UV radiation	
			Short (254 nm) wavelength	Far (365 nm) wavelength
1	Drug (P) such as	Greenish	Greenish yellow	Pinkish-yellow
2	P+Amyl acetate	Brown	Rose pink	Green
3	P+1N.NaOH+Water	Light brown	Violet	Light green
4	P+1N.NaOH+Amyl acetate	Greenish brown	Dark brown	Brown
5	P+1N.HCl+Amyl acetate	Reddish brown	Dark brown	Dark brown
6	P+1N.NaOH+Methanol	Grey	Red	Green
7	P+50%KOH+Methanol	Blue	Bluish	Violet
8	P+1N.HCl+Ethanol	Magenta	Crimson	Pink
9	P+50%H_2SO_4+Anisaldehyde	Yellow	Pink	Blue
10	P+50%HNO_3	Violet	Dark brown	Brown
11	P+Conc.HNO_3	Cherry red	Brown	Light brown
12	P+Acetic acid	Bluish	Light green	Violet
13	P+Conc.H_2SO_4	Light brown	Dark red	Brown
14	P+50%$FeCl_3$+Methanol	Yellow	Pink	Violet
15	P+Iodine water	Brown	Brown	Dark brown

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Table 7. Behaviour of *Cassia grandis* Linn. leaves powder of marketed sample with different reagents observed under ordinary light and UV-radiation.

S.No.	Reaction of marketed sample with different reagents	Colour produced under ordinary light	Colour produced under UV radiation	
			Short (254 nm) wavelength	Far (365 nm) wavelength
1	Drug (P) such as	Greenish grey	Greenish yellow	Brownish-yellow
2	P+Amyl acetate	Brown	Pinkish brown	Yellowish-green
3	P+1N.NaOH+Water	Light brown	Violet	Light green
4	P+1N.NaOH+Amyl acetate	Greenish brown	Dark brown	Brown
5	P+1N.HCl+Amyl acetate	Redish brown	Light brown	Light brown
6	P+1N.NaOH+Methanol	Grey	Red	Green
7	P+50%KOH+Methanol	Brown	Light blue	Dirty violet
8	P+1N.HCl+Ethanol	Magenta	Crimson	Pinkish
9	P+50%H_2SO_4+Anisaldehyde	Brown	Pink	Bluish
10	P+50%HNO_3	Violet	Dark brown	Brown
11	P+Conc.HNO_3	Brick red	Brown	Light brown
12	P+Acetic acid	Grey	Light grey	Violet
13	P+Conc.H_2SO_4	Light brown	Redish	Brown
14	P+50%$FeCl_3$+Methanol	Light blue	Brownish green	Yellowish green
15	P+Iodine water	Light brown	Light Brown	Brown

Table 8. Thin layer chromatographic pattern of extract of *Cassia grandis* Linn. (powder) of samples different.

S.No.	Stationary phase	Mobile phase	Loading extract	Visualization/ Detection	R_f Value
1	Silica gel-G	Toluene: EthAc : MeOH (20:4:1:2drop)	MeOH (Soxhlet) self collected	I_2	0.99, 0.73, 0.53, 0.49
2	Silica gel-G	do	MeOH (Soxhlet) Marketed	I_2	0.51, 0.42, 0.19, 0.05
3	Silica gel-G	Toluene : EthAc (3:1)	PE (cold maceration) self collected	LB	0.81, 0.74, 0.59, 0.47
4	Silica gel-G	do	PE (cold maceration) Marketed	LB	0.58, 0.32, 0.19, 0.08
5	Silica gel-G	MeOH: $CHCl_3$: Hexane (1:1:4)	MeOH (Soxhlet) self collected	I2	0.90, 0.85, 0.65, 0.44
6	Silica gel-G	do	PE (cold maceration) Marketed	I2	0.39, 0.29, 0.12, 0.09
7	Silica gel-G	PE: $CHCl_3$ (1:2)	MeOH (Soxhlet) Marketed	LB	0.64, 0.40, 0.23, 0.12
8	Silica gel-G	do	PE (Soxhlet) self collected	LB	0.70, 0.55, 0.50, 0.39
9	Silica gel-G	Hexane: $CHCl_3$: MeOH (4:1:1)	PE (Soxhlet) Marketed	I2	0.63, 0.41, 0.28, 0.15
10	Silica gel-G	do	MeOH (cold maceration) Marketed	I2	0.65, 0.38, 0.29, 0.14

Methyl acetate, MeOH = Methanol, P = Powder, PE = Petroleum ether, LB = Laboratory, I_2 = Iodine

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