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ORIGINAL RESEARCH ARTICLE- EXPERIMENTAL STUDY

A COMPARATIVE HPTLC STUDY OF KUSHMANDA (*BENINCASA HISPIDA* (THUNB.) CONG.) BEEJA CHURNA AND GRANULES

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ABSTRACT:

Background: *Kushmanda* (*Benincasa hispida* Thunb. Cong.) is a well known drug in the texts of Ayurveda. The seeds and their oil is considered as anthelmintic and prescribed in cases of roundworm and tapeworm infestation. **Aims and objectives**: Present study was aimed to observe changes in components during process of preparation of granules from *Kushmanda* (*Benincasa hispida* Thunb. Cong.) *Beeja Churna*. **Methods:** Authentified, matured, seeds were collected, powder and granules were formulated and observe changes in components. **Results:** In chromatographic finger printing and densitometric analysis, three separated components resembles to each other indicating the change in components during process of preparation of granules from *Churna*. **Conclusion:** HPTLC and densitometric analysis showed change in components during process of preparation of granules from *Churna* but the changes observed in minor components as integrated area of major components do not alter after preparation of granules.

Keywords: *Kushmanda*, *Benincasa hispida*, HPTLC

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INTRODUCTION

Kushmand (*Benincasa hispida* thumb. Cong.) is a large climbing or trailing herb with stout, angular, hispid stems, cultivated as a vegetable throughout India up to an altitude of 1200 m. occasionally it is found as an escape (wild).[1] In Vedic prose very few references was observed regarding *Kushmanda. Kushmanda* was used instead of animal in *yajna* for sacrifice [2].

Kushmanda is used as Memory enhancer, tranquilizer, laxative, purgative, helmenthicide, coagulative, cardiac stimulant, diuretic, spermatogenic. The fruit juice is useful in mercurial poisoning. It is also recommended for ailments like epilepsy, constipation, piles, dyspepsia, Syphilis and Diabetes. The ash of fruit rind is applied on painful swelling. The seeds and their oil is considered as anthelmintic and prescribed in cases of roundworm and tapeworm infestation. Syrup of seeds and sweets prepared from the fruits are used to relieve burning sensation and fever. it is also used in the treatment of chronic fever.[3-5] (Indian Materia Medica, Nadkarni; Indian Medicinal Plants, *Kirtikar* & *Basu*; Glossary of Indian Medicinal Plants, L.V.Asolkar)

The seeds consist of 53.3 per cent shell and the remainder kernel. The kernels are rich in fatty oils - palmitic, 10.6; stearic, 5.8; arachidic, 0.3; oleic, 20.0; linoleic, 62.4; and linolenic, 1.0%. The unsapon matter (1.47%) contained ß-sitosterol. In general seeds are very rich in unsaturated fatty acid. Fatty acid components of seed were reported to be linoleic acid, oleic acid and saturated acids.[6]

As kernel of seed is rich in fatty acid, the Powder of *Kushmand* has very short Shelf life so for raise the shelf life, granules were prepared from powder and the comparative HPTLC study was carried out to observe change in components during process of preparation of granules from *Churna*.

MATERIALS AND METHODS

The test drug, seeds of *Benincasa hispida* (Thunb.) Cong. was procured from Agra (U.P.) by Pharmacy of IPGT & RA, GAU, Jamnagar and authenticated in the Pharmacognosy Department of IPGT & RA, G.A.U, Jamnagar.

The granule was prepared from procured seed samples from Agra and was used for HPTLC study.

Preparation of *Kushmanda beeja* granules: *Kushmanda Beeja Churna* (60 mesh size) was prepared. Sugar was taken in equal amount to *K. Beeja Churna* and four times of water was added to it followed by mild heating and *Chasni* (60% sugar solution) was prepared. Then the *K. Beeja churna* was thoroughly mixed with the solution, passed through ten number mesh to prepare the granules and

shade dried before subjecting for further study.

High performance thin layer chromatogrphy (HPTLC)

Detection and identification of a compound from the group of the compounds efficiently in the presence of pure reference compounds, otherwise, efficient separation and establishing the marker compounds is the hall mark of High performance thin layer chromatography.

In these the plates are coated with high performance silica gels, which are of very small and uniform in size (about 5 μm). The high performance silica gel gives more efficient and reproducible separation than conventional grades of silica gel. Small volumes of samples are applied over it, the spots are compact and the spots can be used quantified with the help of the scanner using photo densitometry. The term HPTLC is using for the technique in which substances are accurately assayed using high performance grades of silica gel.

This has been utilized on Methanolic extract of the raw drug *Kushmanda beeja churna* and *Kushmanda beeja* granule for establishing the fingerprints and to study for the presence of identical chemical constituents

Chromatographic conditions:

Adsorbent Layer: Precoated TLC aluminium sheets of Silica gel 60 F_{254} GLP plates (Merk KGaA, Germany)

Sample Application: By Auto-sampler CAMAG Linomat V

Mobile Phase: *1- Dichloromethane*

Detection: *1- Viewing the TLC chromatogram at 254 nm under UV*

2- Viewing the TLC chromatogram at 366 nm under UV after

Procedure:

Analysis were performed on 10 cm and 20 cm high performance silica gel GF 60_{254} plates (Merck) of 0.5 mm thickness. The plates were precleaned by development to the top with HPLC grad methanol and dried in a fumehood before used and sample solutions were applied to the plate by means of CAMAG Linomate V automated spray on band application equipped with a 100 μL syringe then the plates were developed in different systems as mobile phase in vapour-equlibrated Camag twin-through chamber containing a saturation pad. The development time was approx. 30 minutes after development the mobile phase was evaporated from the plate by drying in a fume hood for 10 min and detection was done under 254 and 366 nm U.

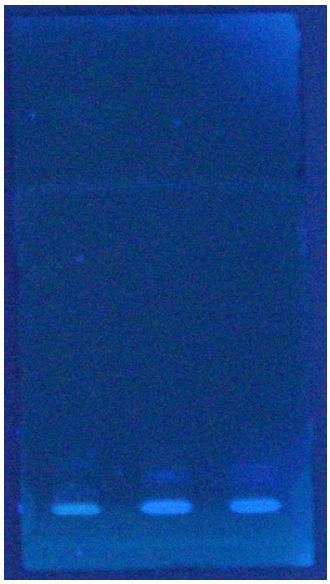


Fig. 1 HPTLC plate observed under long UV light

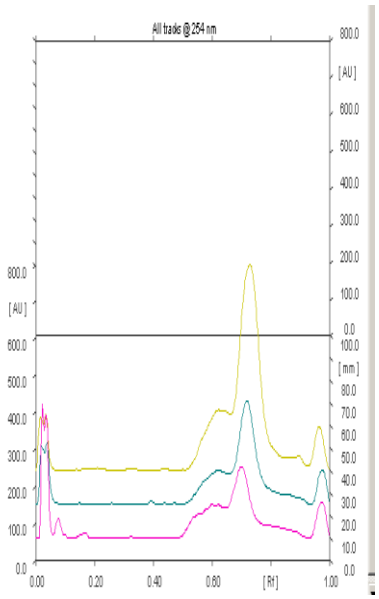


Fig.2 Line diagram after scanning with short U.V.

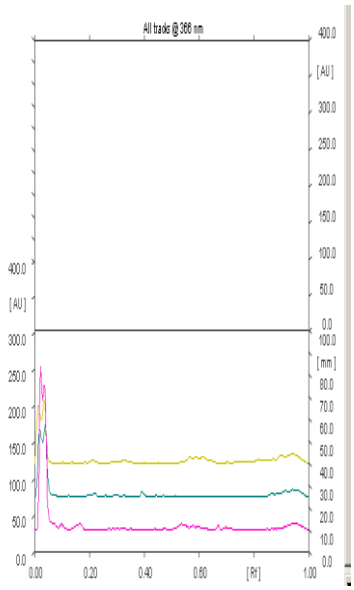


Fig.3 Line diagram after scanning with Long U.V.

Densitometric evaluation of TLC plate

Densitometric scanning was performed with a Camag T.L.C. scanner III in reflectance absorbance mode at 254 nm and 366 nm under control of CATS software (V 3.15 Camag). The slit dimensions were 6 mm x 0.45 mm and the scanning speed was 10 mm^{-1}

OBSERVATION AND RESULTS

TABLE-1

COMPARATIVE HPTLC OF KBC & KBG

Extract	Solvent system	Visualization UV	KBC		KBG		KBG	
			No. of spots	R_f	No. of spots	R_f	No. of spots	R_f
Methanol extract	Dichloromethane	254 nm	07	0.02, 0.08, 0.17, 0.60, 0.70, 0.82, 0.97	06	0.02, 0.04, 0.39, 0.62, 0.72, 0.97	06	0.01, 0.04, 0.62, 0.73, 0.90, 0.96
		366 nm	01	0.02	03	0.02, 0.04, 0.94	03	0.01, 0.04, 0.94

KBC = *Kushmanda beeja Churna* KBG = *Kushmanda beeja granules*

Chromatographic profile is shown in Table- 1 The results obtained from densitometric scanning using Camag scanner III.

Seven components were depicted when the plate observed under short U.V. (254 nm) amongst them only one component produce fluorescent under long U.V. (360 nm). (Fig 4 & 5)

After preparing granules the short U.V. responding number of components reduce to six and in long U.V. responding increase 3 from one indicating presence of Chromophore. (Fig 6 & 7)

Densitometric peaks and peak areas after scanning

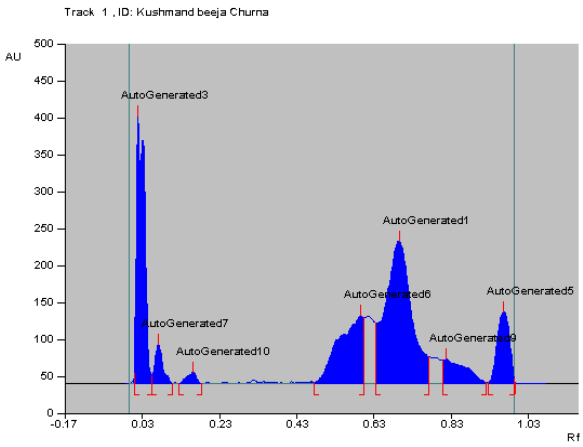


Fig. 4

KBC (Short UV)

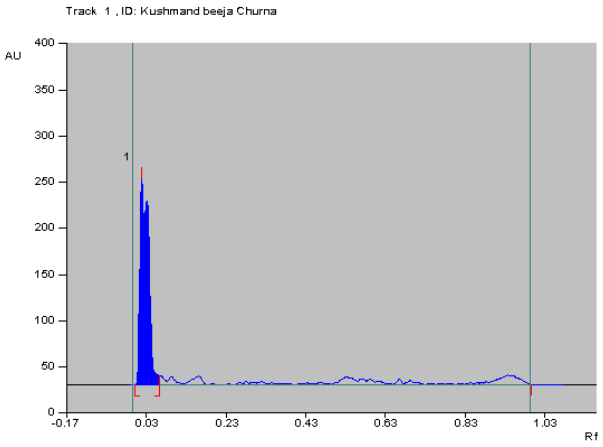


Fig.5

KBC (Long UV)

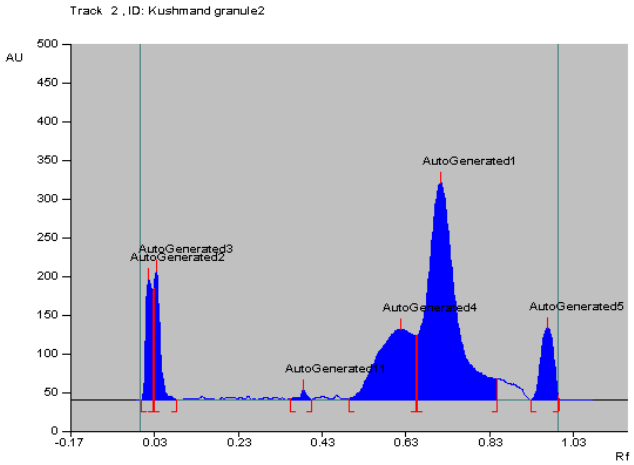


Fig. 6

KBG (Short UV)

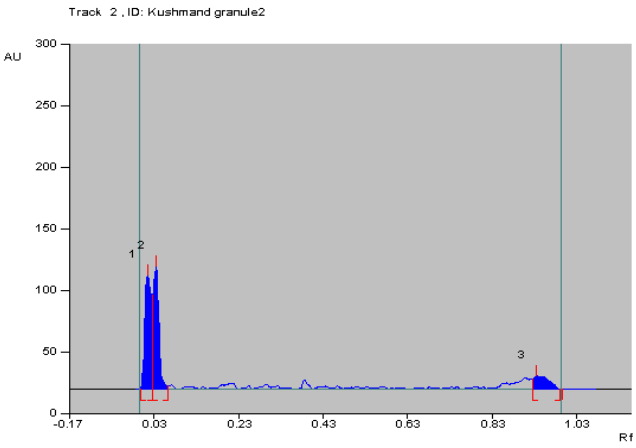


Fig. 7

KBG (Long UV)

Densitometric analysis:

Densitometric evaluation of developed plate was carried out using adsorption mode of T.L.C. scanner III.

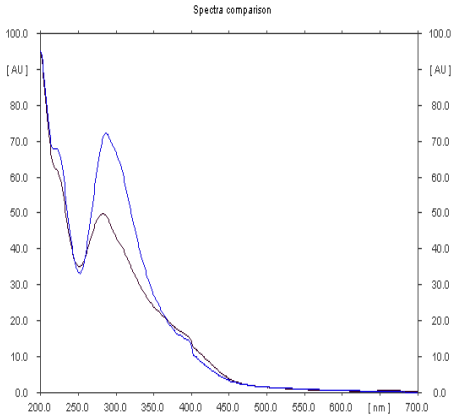
The data obtained shows that under short U.V. light, out of seven components, three components cover major integrated area, 39.49%., 23.31% and 17.54% for detection factor 0.07, 0.02 and 0.6 respectively. On Track 2, major integrated areas under curve are 54.63% and 22.77% for Rf 0.72 and 0.62 while others are not major.

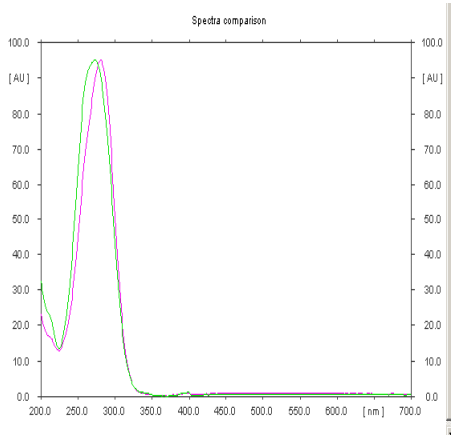
Under long U.V. only single spot found on Track-1, with area of 100% at 0.02 Rf value but on Track-2, three major i.e. 0.04 for 39.63%, 0.01 for 39.24% and 0.9 for 21.08% observed.

Assigning peaks:

Densitometric analysis followed by UV-VIS spectrum for the components having same Rf value. The scan was performed using TLC scanner III. Component one at Rf 0.70 on Track-1 and Rf at 0.72 at Track-2 seems common, next on Track-1 Rf 0.02, 0.72 and Rf 0.97 are comparable with track-2 component. (Fig. 8,9 & 10)

UV- Vis. Spectrum of assigned peaks





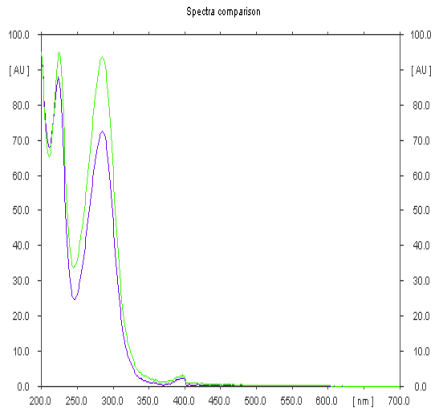


Fig. 8: KBC & KBG spot at 0.02 Fig.9: KBC & KBG spot at 0.72 Fig.10: KBC&KBG spot at 0.97

DISCUSSION

When the plate observed under short U.V. (254 nm), seven components were showed amongst them only one component produce fluorescent under long U.V. (360 nm). After preparing granules the short U.V. responding number of components reduces to six. In long U.V. responding increase 3, from one indicates presence of Chromophore. The increase can be due to adding of Sugar in

granule preparation. In chromatographic finger printing and densitometric analysis, three separated components resembles to each other (Fig. 8,9 & 10) indicating the change in components during process of preparation of granules from *Churna* but the changes observed in minor components as integrated area of major components do not alter after preparation of granules.

CONCLUSION

As the seeds consist of 53.3 per cent shell and the remainder kernel and the kernels are rich in fatty oils. Due to presence of fatty oil it is very tough to make *churna* or *vati* of *Kushmand Beeja* as it is rancid soon. In chromatographic finger printing and densitometric analysis HPTLC and densitometric analysis showed change in components during process of preparation of granules from *Churna*. But the changes observed in minor components as integrated area of major components do not alter after preparation of granules. So for therapeutic use of *Kushmanda beeja* and for long shelf life, granules can be prepared as major components are not changed during preparation process.

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