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Standardization of Ayurvedic formulation- Kutajadi Kashay Curna

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ABSTRACT

The present study deals with the scientific evaluation and standardization of the Ayurvedic compound formulation Kutajadi Kashay Curna (KKC) following quality control procedures recommended for the finished product. The parameters studied for standardization are physicochemical parameters, phytochemical parameters and HPTLC profiles. The values obtained for physico-chemical parameters, HPTLC profile will be useful for maintaining batch to batch consistency and quality of the formulation. HPTLC studies revealed the presence of bands belonging to the ingredients in the compound formulation. These diagnostic spots could be utilized for standardization purposes.

Keywords: Kutajadi Kashay Curna, standardization, quality control, HPTLC profile.

INTRODUCTION

Use of herbal remedies is on rise globally. According to an estimate of World Health Organization (WHO) an approximately 85–90% of the world's population consumes traditional herbal medicines. The reason for this seems to be their better tolerance and negligible adverse drug reactions [1]. WHO has considered phytotherapy in its health programme because these drugs are safe, cost effective and most importantly people have faith in them. WHO has also evolved guidelines for the validation of plant based drugs for developing countries like India [2-4]. In spite of these various efforts by WHO, there are not many studies supporting their scientific evaluation. The parameters to be followed at the time of preparation are not well defined and in several instances they are not clear to the manufacturers.

Plant material varies in its photochemical content and therefore in its therapeutic effect depending on the place and time of collection. Plant material collected from the same place but in different time period with different environmental conditions influencing the field conditions will naturally show variation in their chemical constituents. In several instances herbal medicine is a combination of more than one plant. The absence of an ingredient or addition of different part or plant will certainly affect the therapeutic value of the medicine. This emphasizes the need for standardization and quality control of Ayurvedic products.

The present study is aimed to lay down pharmacopoeial standard for Kutajadi Kashay Curna an Ayurvedic polyherbal formulation extremely useful in Raktãtisãra (Diarrhea with blood), Aamatisara, Sashulatsara, Atisar [5-6]. The formulation consists of *Holarrhena antidysenterica* (Kutaja - stem bark), *Woodfordia fruticosa* (Dhataki - flower), *Aegle marmelos* (Bilva - fruit pulp), *Cyprus rotundus* (Nagarmotha - rhizome), *Punica granatum* (Dadima - fruit rind), *Symplocos racemosa* (Lodhra - stem bark), *Pterocarpus santalinus* (Raktachandan - heart wood),

Cissamples parriera (Pathamool - root) and *Pavonia odorata* (Sugandhabala - rhizome). There are several parameters which have been recommended to standardize and scientifically validate Ayurvedic preparations as safe drugs [7-8]. The parameters studied for the present study for the standardization of test formulation include organoleptic characters, physicochemical parameters, phyto-chemical analysis and development of HPTLC finger print profiles as recommended by WHO and Ayurvedic Pharmacopoeia Committee [9].

EXPERIMENTAL SECTION

Plant materials

The constituent drugs of the Kutajadi Kashay Curna such as *Holarrhena antidysenterica* (Kutaja – stem bark), *Woodfordia fruticosa* (Dhataki - flower), *Aegle marmelos* (Bilva – fruit pulp), *Cyprus rotundus* (Nagarmotha or Musta - rhizome), *Punica granatum* (Dadima – fruit rind) were collected from the forest of Chitrakoot, Satna district of Madhay Pradesh. On the other hand *Symplocos racemosa* (Lodhra – stem bark), *Pterocarpus santalinus* (Raktachandan - heart wood), *Cissamples parriera* (Pathamool - root) and *Pavonia odorata* (Sugandhabala or Netrabala - rhizome) were procured from an Ayurvedic pharmacy located in Arogyadham, Deendayal Research Institute, Chitrakoot, Satna, M.P. All the ingredients were tested for quality before using them in the formulation [10].

Pharmacognostic Evaluations

Physico-chemical analysis

Air dried material was used for the quantitative determination of loss on drying, total ash, acid insoluble ash, alcohol and water soluble extractive values according to standard procedure of Indian Pharmacopoeia and WHO/QCMMPM [11-12].

Phytochemical analysis

Phytochemical analysis was carried out using chloroform, aqueous ethanol and water (for qualitative) and aqueous ethanol extract for quantitative analysis as per the standard methods [13].

High Performance Thin Layer Chromatography (HPTLC)

1gm powdered plant material was extracted with 10ml of methanol for 10 minutes. The extract was filtered and evaporated. The residue was re-dissolved in 1ml methanol and 10µl extract was spotted through Camag Linomet 5 applicator. The sample consisting of the alcoholic extract of each ingredient and test formulation was spotted on silica gel pre-coated plates (E. Merck). The plate was developed in the solvent system of Toluene: Ethyl acetate (7:3). When the solvent front reached the edge of the plate (8cm) the run was stopped. The plate was then air dried and the chromatogram was visualized under long wavelength (366nm). After spraying with 5% methanolic-sulphuric acid followed by heating at 110^0C for 5-10 min [14-16].

RESULTS AND DISCUSSION

The formulation used for the present study was chocolate brown in colour with spicy odour and kasaila taste. It was observed that approximately 95% of each ingredient passed through a sieve of 80mesh. The formulation prepared after mixing equal portion of each constituent drug was passed through a sieve of 44 mesh.

Table 1: Physico-chemical analysis of Kutajadi Kashay Curna(KKC)

Parameters	KKC
Total ash value	Not more than 5.30%
Water soluble ash value	Not more than 3.40%
Acid in soluble ash value	Not more than 1.00%
Moisture content	Not more than 7.00%
Alcohol soluble extractive	Not less than 14.00 %
Water soluble extractive	Not less than 25.00%
Hexane soluble extractive	Not less than 6.00%
Chloroform soluble extractive value	Not less than 3.00%
Volatile oil content	Not less than 0.80%

Physicochemical analysis

The results of the physicochemical analysis of test formulation have been given below in Table 1. The analytical parameters such as total ash, water soluble and acid insoluble ash value studied for Kutajadi Kashay Curna have been presented in the table below. The formulation was when subjected to successive sohxlet extractions the ethanol soluble extractive for forest sample was 14%. The hexane soluble extractive was 6% whereas chloroform soluble extractive value was observed as 3%. The value observed for loss on drying was found to be 5.3%. Standard protocols based on WHO guidelines were followed to carry out physicochemical analysis.

Phytochemical analysis

Preliminary phytochemical analysis of the extract (aqueous and ethanol) of Curna of KKC revealed the presence of various bioactive components which include alkaloid, flavonoid, carbohydrate, protein, saponin, resin, volatile oil and tannin. The results of phytochemical test (qualitative) has been summarized in Table 2.

Table 2: Phytochemical qualitative analysis of Kutajadi Kashay Curna(KKC)

Sl.no.	Plant Species and KKC formulation	Alkaloid (Wagner's test)	Carbohydrate (Fehling's test)	Flavonoid	Protein (Biuret test)	Resin	Saponin	Tannin	Volatile oil
1	*Holarrhena antidysentrica*	++	+	-	-	+	++	-	-
2	*Wood fordiafruticosa*	++	+	+	-	-	-	++	-
3	*Symplocos racemose*	++	+	-	-	+	-	+	-
4	*Aegle marmelos*	+	+	-	+	++	-	+	-
5	*Cyperus rotundus*	-	+	-	-	-	-	+	++
6	*Cissam pelospareira*	-	+	-	-	-	+	+	-
7	*Pterocarpus santulinus*	-	+	-	-	-	-	+	++
8	*Punica granatum*	-	++	-	+	++	-	++	-
9	*Pavonia odorata*	-	+	-	-	-	-	+	+
10	KKC	+	++	-	-	+	-	+	+

HPTLC Identity Test Performed on Kutajadi Kashay Curna (KKC)

Comparison of the HPTLC profile of Kutajadi Kashay curna with its nine ingredients has been made from track A-J (Figure 1). The track A belonging to the formulation showed 11 bluish bands at R_f 0.04, 0.10, 0.14, 0.22, 0.24, 0.30, 0.35, 0.40, 0.45 and 0.55 at 366nm and. The track B (Kutaj- stem bark) showed three prominent bands at Rf 0.14, 0.30 and 0.45. The same bands because of Kutaj (R_f 0.14, 0.30 and 0.45) could also be seen in track A of KKC at R_f 0.14, 0.30 and 0.45. The track C (Dadima- fruit rind) showed two bands at Rf 0.10 (yellowish) and 0.30 (blue). The same bands because of Dadima at R_f 0.10 and 0.30 could also be seen in KKC. Two prominent bands were present in track D as Dhataki (flower) at R_f 0.10 (yellowish) and 0.30 (blue) which were also present in the formulation. The track E (Lodhra-stem bark) showed three bluish bands at R_f 0.10, 0.45 and 0.84. The same bands because of Lodhra (R_f 0.10, 0.45 and 0.84) could also be seen in KKC at R_f 0.10, 0.45 and 0.84. The track F (Nagarmotha or Musta) showed two prominent bands at R_f 0.30 and 0.45. The same bands because of Musta could also be seen in KKC lane. Pathamool (root) (Track G) showed one prominent blue coloured band at R_f 0.30. The same bands because of Patha could also be seen in the KKC at R_f 0.30. The track H as Raktachandan(heart wood) showed three bands at Rf 0.04 (yellowish), 0.35 and 0.55 (blue) which were also present in the KKC. Bilva-fruit pulp (Track I) showed three bluish bands at R_f 0.10, 0.30 and 0.55. The same bands because of Bilva could also be present in the KKC at R_f 0.10, 0.30 and 0.55. The track J (Sugandhabala or Netrabala-rhizome) showed three prominent bands at R_f 0.16, 0.30 and 0.40. The same bands of Netrabala (R_f 0.16, 0.30 and 0.40) were also present in the compound formulation at R_f 0.16, 0.30 and 0.40.

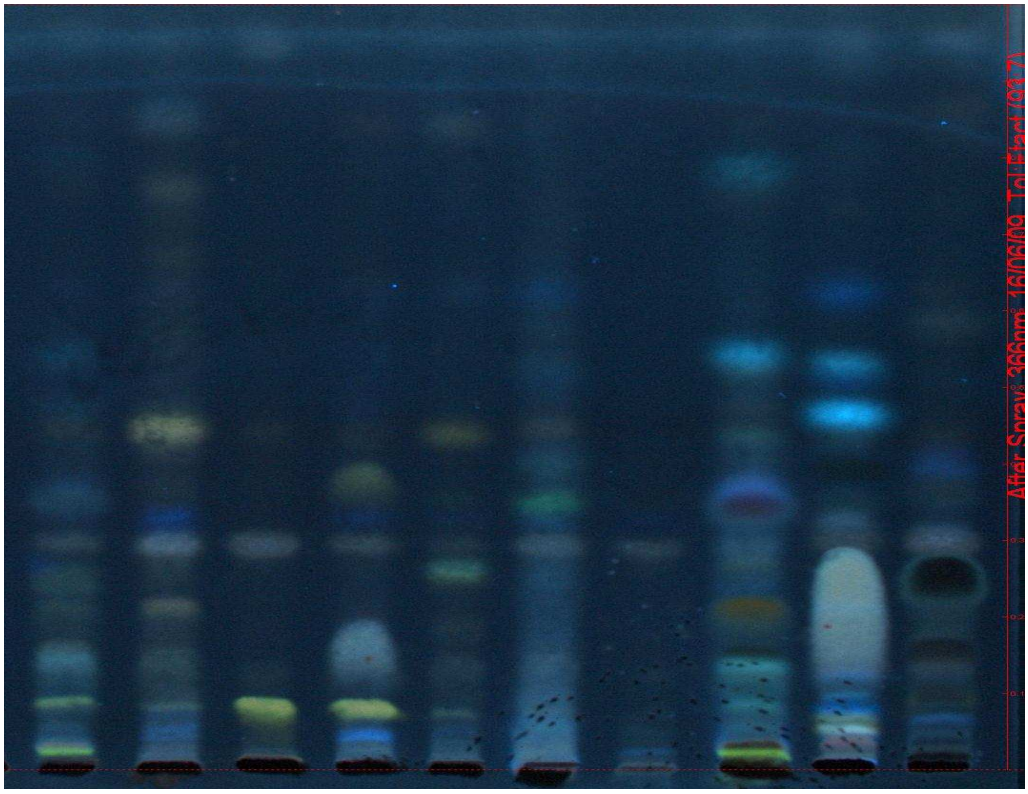

Figure 1.TLC profile of Kutajadi Kashay Curna after derivetization with 5% methanolic sulphuric acid reagent observed under 366 nm

A - Kutajadi Kashay Curna formulation, B- Holarrhena antidysenterica (Kutaja - stem bark), C- Punica granatum (Dadima - fruit rind), D- Woodfordia fruticosa (Dhataki - flower), E- Symplocos racemosa (Lodhra - stem bark), F- Cyprus rotundus (Nagarmotha - rhizome), G- Cissamples parriera (Pathamool - root), H- Pterocarpus santalinus (Raktachandan - heart wood), I- Aegle marmelos (Bilva – fruit pulp) and Pavonia odorata (Sugandhabala - rhizome).

CONCLUSION

Standardization of Kutajadi Kashay Curna was carried out using physicochemical, phytochemical studies and HPTLC finger print profiles. These parameters were also studied for the quality control of raw material, processed powder. The results obtained through this study were quick, reproducible and could be used for routine monitoring of raw material. Looking at the growing demand for the herbal drugs in the global market it would be a good idea to use this protocol for other drugs too. It could help in maintaining the quality and batch to batch consistency of many important Ayurvedic medicines.

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