ABSTRACT

Last decade have witnessed exceptional rise in demand of plant based medicine and herbal product in International market. Recent data shows that medicinal plants accounts for about 70% by value of the total raw material procured by Ayurvedic pharmacies. Due to excessive demand in the Global market, the rate of extraction of medicinal plants from natural sources is higher than the rate of their regeneration. That directly contributes to the present scarcity of medicinal plants. Moreover the number of Ayurvedic pharmaceutical companies discarding huge quantity of plant residues after preparing Kashaaya form of medicine. Although certainly some Ayurvedic practitioners of different region reuse that residues for preparing medicine again, but there is no scientific base regarding this reuse. Hence on this background the present study was undertaken to analyse the reusability of the residues. In this study a comparative analysis was done on fresh kashaaya with second and third time prepared Kashaaya from the same residues. Amritottaram and Gandharvahasthadi Kashaaya were selected for study as both are very popular and ingredients are easily available. Kashaaya was prepared according to the method mentioned in Shuarrangadhara samhitaa Successive kashaaya was prepared from the same Kashaaya powder and then phytochemical analysis was done for three kashaaya samples (i.e. first time, second time and
third times prepared *kashaaya*) through HPTLC. Study showed that the extract value of successive *kashaaya* was gradually decreasing but the HPTLC profile showed similar banding pattern. Result of the study may provide scientific base regarding the reuse of such residues and also base for further scientific evaluation.

**KEYWORDS:** Residues, Reuse, *Amritottoram kashaaya*, Gandharvahasthadi kashaaya HPTLC analysis.

**INTRODUCTION**

Global market for medicinal plant materials and herbal medicines is estimated to be worth several billion dollars a year. For the past few decades compounds from the natural sources have been gaining importance due to vast chemical diversity they offer.[1] Due to depletion of habitat the genetic diversity of medicinal plants in the world is on the verge of extinction because of ruthless collection and harvesting of medicinal plants for production of medicines, with little or no regard to the future.[2] On the other hand Ayurvedic pharmaceutical companies are discarding several tonnes of plant residues after preparing decoctions or *Kashaaya* form of medicines. Although some Ayurvedic physicians reusing the residues for making medicine again but till now no scientific data is available regarding this concern. Hence it has become obligatory to explore this issue scientifically which may be courteous for conservation aspect of medicinal plants. On this background present study was undertaken to explore the reusability of *kashaaya* residues. A comparative analysis was done on successive three *Kashaaya* prepared from same plants material. *Amritottaram* and *Gandharvahasthadi Kashaaya* were selected for study as both are very popular and ingredients are easily available. Successive *Kashaaya* was prepared from the same *Kashaaya* powder and then phytochemical analysis was done for three *Kashaaya* samples (i.e. first time, second time and third time prepared *Kashaayas*) through HPTLC to assess the qualitative and quantitative parameters of after preparations.

**INGREDIENTS OF AMRITOTTORAM KASHAAYA**[3]

1. *Naagara*: *Zingiber officinale* Roscoe. (Family: Zingiberaceae)
2. *Amrithaa*: *Tinospora cordifolia* (Willd.) Miers (Family: Menispermaceae)
3. *Haritaki*: *Terminalia chebula* Retz.(Family: Combretaceae)

**INGREDIENTS OF GANDHARVAHASTHADI KASHAAYA**[4]

1. *Gandharavahastha*: *Ricinus Communis* L.(Family: Euphorbiaceae)
2. Chirabilva: Holoptelea integrifolia Planch. (Family: Ulmaceae)
3. Huthaasha: Plumbago zeylanica L. (Family: Plumbaginaceae)
4. Viswaa: Zingiber officinale Roscoe. (Family: Zingiberaceae)
5. Pathyaa: Terminalia chebula Retz. (Family: Combretaceae)
4. Punarnavaa: Boerhaavia diffusa Brandegee (Family: Nyctaginaceae)
5. Yavaasaa: Tragia involucrate L. (Family: Urticaceae)
6. Bhumitaala: Curculigo orchioides Gaertn. (Family: Orchidaceae)

MATERIALS AND METHOD

Source of materials: Amritottaram and Gandgarvahasthadi both Kashaaya powder was collected from Pharmacy of V.P.S.V Ayurveda College, Hospital. Materials were supplied from Oushadhi-The Pharmaceutical Corporation (I,M) Ltd, Thrissur. Further authentication of the raw samples were done at CMPR (Centre for Medicinal Plant Research), Arya Vaidya Sala, Kottakkal.

Preparation of Kashaaya: Kashaaya was prepared according to the method mentioned in Shaarangadhara Samhitaa. Accurately weighed 50 gm Kashaaya powder was taken in a round bottom flask then to that 16th parts of water i.e. 800 ml water was added and then the materials were boiled and reduced up to 1/8th i.e. 100 ml. Then it was filtered and first time Kashaaya was prepared. Again the filtered plant residue was taken and same way second time Kashaaya was prepared. Likewise third time Kashaaya was also prepared by following the same method. Finally three successive Kashaayas were prepared from same plant material. This same procedure was followed for both Amritottaram and Gandgarvahasthadi Kashaaya.

Preparation of extract: All the Kashaaya samples were taken and filtered rapidly taking care not to lose any solvent. Then the filtered Kashaaya samples were collected in separate dried and pre-weighed beakers. Then the beakers with samples were kept in a water bath to evaporate and dry. After that it was kept on a hot air oven at 105°C for 6 hours. Next it was made cool in a desiccators and weight was taken. Then the percentages of water extractable matter of the samples were calculated. The experiment was repeated twice, and the average values were taken. Same method was followed for both Amritottaram and Gandgarvahasthadi Kashaaya.
HPTLC test: HPTLC analysis and comparison of successive Kashaaya samples of both Amritottaram and Gandgarvahasthadi Kashaaya was done by following the standard method[6] as below:

Stationary phase: Aluminum backed pre-coated Merck silica gel plate 60 F$_{254}$ plate.

Solvent system:
Solvent 1: Toluene: Ethyl acetate: Formic acid (6: 4: 0.5) [for both Amritottaram and Gandgarvahasthadi Kashaaya]
Solvent 2: Toluene: Ethyl acetate: Formic acid (5: 5: 1) [for Amritottaram Kashaaya only]

Procedure: Samples were applied on the plate using Camag automatic TLC sampler 4 attached to camag HPTLC system. The samples (2µl) each were spotted on aluminum backed pre-coated silica gel plate 60$_{F_{254}}$ plate (5×10 cm) in the form of bands with width 8 mm by using Hamilton syringe (100µl). Then the plates were developed in the two different solvent systems in a twin trough chamber to a distance of 9 cm.

Visualization: After making dry in air the plates were examined under UV 254 nm and under UV 366 nm. R$_f$ value and the colour of the resolved bands were recorded. Photographs of the plates were captured using camag TLC visualizer. R$_f$ value was calculated by using the formula (Distance travelled by solute / Distance travelled by solvent)

Scanning: Densitometric scanning of the plates were done by using camag TLC scanner 3 at 254 and 366 nm.

Derivatization of the plates: For solvent system 1 of Amritottaram Kashaaya samples, the plates were sprayed with ferric chloride reagent and visualized for assessment of phenolic compound. Photographs of the plates were captured using camag TLC visualizer.

OBSERVATION AND RESULT:
Extractive value: The water extractive value of kashaaya samples were taken and the result shows that a significant decrease in the extractive values of both the kashaayas. For the first time extraction the extractive values of Amritottaram Kashaaya was 5.23% and it reduced to 3.03 % in the third time. The same pattern of result was observed in the case of Gandharvahasthadi Kashaaya, where the total extractive was 5.96 % for the first time and 2.52% in the third time (Table 1).
HPTLC analysis of Amritottaram Kashaaya: In solvent system 1 [Toluene: Ethyl acetate: Formic acid (6 : 4: 0.5)] HPTLC profile of water extract of successive Kashaaya samples of Amritottaram Kashaaya under UV light at 254 nm showed 2 similar spots with $R_f$ values of 0.35, 0.46 (Fig-1, Fig-2a, Table-2). At UV 366 nm also all the three samples showed similar 2 spots with $R_f$ values of 0.35 (dark colour), 0.83(light colour) (Fig-1, Fig-2b, Table-2). The spots with $R_f$ values 0.35 was observed under both UV 254 nm and UV 366 nm. After derivatization of the plates with ferric chloride reagent all the three samples showed one dark spot at 0.35 $R_f$ (Fig-1,Table-2) value.

In solvent system 2 [Toluene: Ethyl acetate: formic acid (5 : 5: 1)] HPTLC profile of water extract of successive Kashaaya samples of Amritottaram Kashaaya under UV light at 254 nm showed 2 similar spots with $R_f$ values of 0.39, 0.51 (Fig-3, Fig-4a, Table-3). At UV 366 nm also all the three samples showed similar 4 spots with $R_f$ values of 0.43, 0.50, 0.53, 0.60 (Fig-3, Fig-4b, Table-3). The spots with $R_f$ values 0.35 was observed under both UV 254 nm and UV 366 nm.

HPTLC analysis of Gandharvahasthadi Kashaaya: HPTLC profile of all the three successive Kashaaya samples of Gandharvahasthadi Kashaaya in solvent system 1 [Toluene: Ethyl acetate: Formic acid (6: 4: 0.5)] showed similar colour of spot with similar $R_f$ value. Under UV 254 nm the samples showed 1 similar dark colour spots at 0.33 $R_f$ value (Fig-5, Fig-6a, Table-3). Under UV 366 nm also all the three samples showed similar type of 3 spots with $R_f$ values of 0.24 (Yellow colour), 0.33 (Dark Blue colour), 0.53(Dark Blue colour) (Fig-5, Fig-6b, Table-4). The spots with $R_f$ value 0.33 was observed under both UV 254 nm and UV 366 nm light.

Table 1: Water soluble extractive value of both Kashaaya samples

<table>
<thead>
<tr>
<th>Gandharvahasthadi Kashaaya</th>
<th>Extract value</th>
<th>Amritottaram Kashaaya</th>
<th>Extract value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kashaaya -1</td>
<td>5.96%</td>
<td>Kashaaya -1</td>
<td>5.23%</td>
</tr>
<tr>
<td>Kashaaya -2</td>
<td>4.02%</td>
<td>Kashaaya -2</td>
<td>3.54%</td>
</tr>
<tr>
<td>Kashaaya -3</td>
<td>2.52%</td>
<td>Kashaaya -3</td>
<td>3.03%</td>
</tr>
</tbody>
</table>
Table 2: $R_f$ values of successive Kashaaya samples of Amritottaram Kashaaya in solvent system 1

<table>
<thead>
<tr>
<th>Visualization</th>
<th>Kashaaya -1</th>
<th>Kashaaya -2</th>
<th>Kashaaya -3</th>
</tr>
</thead>
<tbody>
<tr>
<td>254 nm</td>
<td>0.35, 0.46</td>
<td>0.35, 0.46</td>
<td>0.35, 0.46</td>
</tr>
<tr>
<td>366 nm</td>
<td>0.35(Dark),0.83(Light)</td>
<td>0.35(Dark),0.83(Light)</td>
<td>0.35(Dark),0.83(Light)</td>
</tr>
<tr>
<td>After derivatization</td>
<td>0.35</td>
<td>0.35</td>
<td>0.35</td>
</tr>
</tbody>
</table>

Table 3: $R_f$ values of successive Kashaaya samples of Amritottaram Kashaaya in solvent system 2

<table>
<thead>
<tr>
<th>Visualization</th>
<th>Kashaaya -1</th>
<th>Kashaaya -2</th>
<th>Kashaaya -3</th>
</tr>
</thead>
<tbody>
<tr>
<td>254 nm</td>
<td>0.39, 0.51</td>
<td>0.39, 0.51</td>
<td>0.39, 0.51</td>
</tr>
<tr>
<td>366 nm</td>
<td>0.43, 0.50, 0.53, 0.62</td>
<td>0.43, 0.50, 0.53, 0.62</td>
<td>0.43, 0.50, 0.53, 0.62</td>
</tr>
</tbody>
</table>

Table 4: $R_f$ values Successive Kashaaya samples of Gandharvahasthadi Kashaaya in Solvent system 1 [Toluene: Ethyl acetate: formic acid (6: 4: 0.5)]

<table>
<thead>
<tr>
<th>Visualization</th>
<th>Kashaaya -1</th>
<th>Kashaaya -2</th>
<th>Kashaaya -3</th>
</tr>
</thead>
<tbody>
<tr>
<td>254 nm</td>
<td>0.33(Dark)</td>
<td>0.33(Dark)</td>
<td>0.33(Dark)</td>
</tr>
<tr>
<td>366 nm</td>
<td>0.24(Yellow)0.33(Blue) 0.53(Blue)</td>
<td>0.24(Yellow), 0.33(Blue), 0.53(Blue)</td>
<td>0.24(Yellow), 0.33(Blue), 0.53(Blue)</td>
</tr>
</tbody>
</table>

(Track-1: 1st time prepared Amritottaram Kashaaya; Track-2: 2nd time prepared Amritottaram Kashaaya; Track-3: 3rd time prepared Amritottaram Kashaaya)

Fig.-1: HPTLC profile of successive Kashaaya samples of Amritottaram Kashaaya in solvent system 1.
Fig.-2a HPTLC densitometry at 254 nm (Track-1: 1\textsuperscript{st} time prepared Amritottaram Kashaaya; Track-2: 2\textsuperscript{nd} time prepared Amritottaram Kashaaya; Track-3: 3\textsuperscript{rd} time prepared Amritottaram Kashaaya)

Fig.-2b HPTLC densitometry at 366 nm (Track-1: 1\textsuperscript{st} time prepared Amritottaram Kashaaya; Track-2: 2\textsuperscript{nd} time prepared Amritottaram Kashaaya; Track-3: 3\textsuperscript{rd} time prepared Amritottaram Kashaaya)

Fig.-2: HPTLC densitometry of successive Kashaaya samples of Amritottaram Kashaaya in solvent system 1.

Fig.-3: HPTLC profile of successive Kashaaya samples of Amritottaram Kashaaya in solvent system 2.
Fig. 4a: HPTLC densitometry at 254 nm
(Track-1: 1st time prepared *Amritottaram Kashaaya*; Track-2: 2nd time prepared *Amritottaram Kashaaya*; Track-3: 3rd time prepared *Amritottaram Kashaaya*)

Fig. 4b: HPTLC densitometry at 366 nm
(Track-1: 1st time prepared *Amritottaram Kashaaya*; Track-2: 2nd time prepared *Amritottaram Kashaaya*; Track-3: 3rd time prepared *Amritottaram Kashaaya*)

Fig. 4: HPTLC densitometry of successive *Kashaaya* samples of *Amritottaram Kashaaya* in solvent system 2

254nm

366nm

(Track-1: 1st time prepared *Gandharvahasthadi Kashaaya*; Track-2: 2nd time prepared *Gandharvahasthadi Kashaaya*; Track-3: 3rd time prepared *Gandharvahasthadi Kashaaya*)

Fig. 5: HPTLC profile of successive *Kashaaya* samples of *Gandharvahasthadi Kashaaya* in Solvent system 1 [Toluene: Ethyl acetate: formic acid (6: 4: 0.5)]:

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DISCUSSION

Result of the present study showed that extract value of all the successive Kashaaya samples were gradually decreased. In case of Amritottaram Kashaaya samples extract value of first time prepared Kashaaya was 5.23%, after preparing second time kashaaya from the filtered plant residues of first procedure, extract value was found 3.54%(Table 1). Again similarly proceeded in third time prepared Kashaaya extract value was found 3.03%. Similar kind of result was observed in case of Gandharvahasthadi Kashaaya also. In first time prepared Gandharvahasthadi kashaaya extract value was found 5.96 % and in second time and third time prepared successive kashaaya it was observed  4.02 %, 2.52 % respectively(Table 1). Hence that indicates concentration of successive kashaaya samples is gradually down in second and third time. That may due to loss of starch, carbohydrate etc compounds in successive kashaaya. HPTLC analysis (in solvent system Toluene: Ethyl acetate: Formic acid 6: 4: 0.5) of the successive kashaaya samples of Amritottaram Kashaaya showed similar pattern of chemical compounds presents in three samples (Fig.-1, Fig.-2, Table-2). The spots with $R_f$ value 0.35 was observed under both UV 254 nm and UV 366 nm and also after derivitization with ferric chloride reagent (Fig.1, Table-2). That indicates, similar type of phenolic compound presents in all the samples. Again to analyse the different type of chemical compounds different solvent system [Solvent system 2- Toluene: Ethyl acetate: formic acid = 5: 5: 1] was selected and HPTLC was done. There also all the samples showed presence of similar pattern of chemical compound (Fig-3, Fig-4, Table-3) and the spots with
$R_f$ values 0.35 was observed under both UV 254 nm and UV 366 nm (Fig.-3, Table-3). By analysing the HPTLC profile in *Gandharvahasthadi Kashaaya* also similar type of result was noticed. Study [In Solvent system 1 Toluene: Ethyl acetate: formic acid 6: 4: 0.5] showed Under UV 254 nm 1 similar dark colour spots (Fig-5, Fig-6a, Table-4) and under UV 366 nm 3 spots with same colour and $R_f$ values (Fig-5, Fig-6b, Table-4). The spots with $R_f$ value 0.33 was observed under both UV 254 nm and UV 366 nm light. Hence the study indicates the presence of active compounds in the fresh as well as in successive Kashaaya residues. Further marker based quantification studies is required to identify the exact amount of chemical compounds present in the first and third Kashaaya and a pharmacological evaluation is also required to prove the efficacy of second and third Kashaaya.

**CONCLUSION**

From the present study it can be concluded that concentration of successive *Kashaaya* gradually drop off but their active chemical pattern is remaining same. This same result was observed in different analysis of both *Amrithottaram* and *Gandharvahasthadi Kashaaya*. As the extractive values are decreasing but similar chemical composition is presenting in successive *Kashaayas*, hence it can be suggested in higher doses for therapeutic indication. Adding of plant residues with fresh plants materials can also be thought off. But further scientific evaluation is needed to ensure these probabilities of application. Presence of exact quantity of active compound in successive *Kashaaya* should be evaluated and along with the comparative efficacy of the successive *Kashaaya* should be confirmed by further pharmacological and clinical trial.

**ACKNOWLEDGEMENT**

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**REFFERENCES**


