



Research Article

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COMPARATIVE ANALYSIS OF TRADITIONAL KASHAYAM & KASHAYAM TABLET THROUGH PHYTOCHEMICALS & EFFICACY STUDY ON ANIMALS

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ABSTRACT

The titled study is an attempt to explain the traditional Ayurvedic formulations and its modern method of preparations in terms of its active components and efficacy comparison. Explaining Ayurvedic drugs on basis of its own principles and language is not sufficient today. One of the major processing methods in Ayurveda that have been modified in different ways is that of kashayam being stored and used as spray dried powders. It is challenging to shift from the traditional proven methods of processing to other methods without any way affecting the efficacy. Pointing out the active compound in a kashayam with multiple ingredients is not easy. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Plant extracts also contain those phytochemicals, which can be assessed quantitatively. Thus, 7 traditional kashayams were analyzed for its phytochemical quantity and compared with that of spray dried powder of kashayam. The results indicated that they are similar with each other and even though the presence of some phytochemicals is not shown in screening, quantitative assay indicated their presence. To further ascertain the above said effect, Raasnerandaadi kashayam in liquid form and its spray-dried powder in tablet form were tested in Carrageenan induced paw oedema on Rats. The results have shown closer resemblance of the tablet in relation with the liquid kashayam, ascertaining its efficacy. These results also call for further researches in this area that will improve the acceptance of new methodologies among Ayurveda and other medical fraternities.

Keywords: Ayurveda, Carrageenan, Kashayam, Phytochemicals, Spray dried powder

INTRODUCTION

Plant-derived substances recently are of great interest owing to their versatile applications. Medicinal plants are plants which contain substances that could be used for therapeutic purposes or which are precursors for the synthesis of useful drugs.¹ They are the richest bio-resource of drugs in traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.^{2, 3} Ayurveda is one among the other glorious pride of India. It has an enormous treasure of life science for all.⁴ Ayurveda being the oldest system of medicine used the therapeutic effects of these plant derivatives in various dosage forms in prevention and cure of diseases. The wise sages analysed the efficacy of each formulation and explained them along with the formulation. Raw materials were explained on basis of its rasa, guna, veerya, etc. In the present era, the need of explaining everything in detail with proof became a must. The effect of Ayurvedic drugs and its reasoning on basis of traditional way and language started giving way to modern scientific methods and terminologies. So was the introduction of modern dosage forms in the traditional systems of medicine like tablets, capsules, gel, balm etc. Most of the ancient methods of processing of medicines were meant for immediate use and not for long term storage as in case of kashayams. In the present scenario of bulk manufacturing and sales, this is a shortfall. This paved way to different processes which helps to get more yield and methods to increase shelf life. To carry out the extraction of plant materials, various methods like infusion, decoction, digestion, maceration, percolation, reserved percolation, sonication, continuous hot percolation (soxhlation) and microwave-assisted extraction are used.^{5, 6} Different extraction methods broadly talked as cold and hot processes were

introduced. One of the accepted methods of converting the liquid kashayam is to make it into spray-dried powder after preparing the kashayam. The key benefit is that it enables the avoidance of preservatives to increase the shelf life. It is a challenging necessity that the shift from the traditional form of processing to modern ways must not in any ways affect the efficacy of the formulation. This study is to analyse the actives of a traditional kashayam and its comparison with the spray dried extracts through phytochemical analysis and the efficacy assessment through animal trials.

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoids, alkaloids and phenolic compounds.⁷ The study was done through initial screening and then quantitative analysis followed by the efficacy study on animals.

MATERIALS AND METHODS

The tests were conducted at the Research and Development division of Nagarjuna Herbal Concentrates Ltd., Kerala, India. approved by the Department of Scientific & Industrial Research (DSIR), Govt. of India and the Committee for Control and Supervision on Experiments on Animals (CPCSEA), Govt. of India. The raw materials for preparation and the samples for the test were collected and approved from the Quality Control Department of Nagarjuna Herbal Concentrates Ltd.

Seven of the popular kashayams were selected for the study. The products analysed were liquid kashayam and Spray dried powders of:

1. Raasneirandaadi kashayam
2. Maharaasnaadi kashayam
3. Gulguluthikthakam kashayam
4. Mahaamanjishtaadi kashayam
5. Dhaanwantharam kashayam
6. Punarnnavaadi kashayam
7. Pathyaakshadhaathryaadi kashayam

Identification of phytochemicals

The phytochemical identification was carried out by the method of Trease and Evans⁸ and Harborne⁸. All the samples were diluted with distilled water as 12.5mg/ml. From this solution, 0.5ml each was taken for test.

Quantitative Analysis of Phytochemicals

Six batches samples of seven products of different manufacturing dates were randomly collected. They were quantitatively analysed for Alkaloids,⁹ Flavonoids,⁹ Saponins, Phenols and Tannins¹⁰. The quantification of Alkaloids was also done even though no sample has shown its presence. The spray dried powders were prepared. Averages of all the results are shown below. (Table 1).

From the results, it is seen that even though the presence was not indicated in screening as in case of alkaloids, they are present in the samples. This may be due to the higher specificity of the analytical method used in quantitative analysis. The presence of Phenols and Flavonoids are more in the samples when compared with the other factors. The values are somewhat similar with the predominance seen in spray dried powders. This may be due to the powder samples being fresh and were devoid of any preservatives. Still the variation in the values calls for further studies.

Efficacy Study on Animals

To compare the efficacy of the samples, Rasaneirandaadi kashayam was selected due to its known anti-inflammatory activity the sacrificing of animals can also be avoided through this method. The efficacy was compared through Carrageenan induced paw oedema in Wister albino rats. The spray -dried powder was converted into tablets at 600 g/tablet at a dose of 6

tab/50 kg and kashayam at a dose of 15 ml/50 kg. The disintegration time of tablet is kept below 10 minutes.

The study proposal was submitted and got approved by the Institutional Animal Ethics Committee of Nagarjuna Herbal Concentrates Ltd, approved by CPCSEA, Govt. of India. The animals were bred and selected from the Pharmacology lab of Nagarjuna Herbal Concentrates Ltd.

Anti-inflammatory activity (Carrageenan induced paw oedema)

Albino female rats weighing 130-150 grams were divided into six groups of six animals each. The dosage of the drugs administered to the different groups was as follows. Group I - Control (5% tween 20, 0.5 ml/kg, p.o.), Group II Rasaneirandaadi Kashayam (0.3ml/kg, p.o.), Group II Rasaneirandaadi tablet with extract powder (90 mg/kg, p.o.).

After one hour of the administration of the drugs, 0.1 ml of 1% W/V carrageenan solution in normal saline was injected into the sub plantar tissue of the left-hand paw of the rat and the right hand paw was served as the control. The paw volume of the rats was measured in the plethysmograph, at 1hr interval for 4 hrs and compared with control. Percentage inhibition was calculated using the formula, Percentage inhibition = $[(V_c - V_t) / V_c] \times 100$, where, V_t represents the percentage difference in increased paw volume after the administration of test drugs to the rats and V_c represents difference of increased volume in the control groups. (Table 3)

Table 3 shows anti oedema effect of intraperitoneally administered Raasneirandaadi kashayam on carrageenan induced paw oedema in rats. Rasaneirandaadi kashayam different dosage forms showed significant anti-inflammatory effect. However, the activity at Raasneirandaadi kashayam doses 15ml/50kg shows slightly better effect than the tablet with extract powder. The maximum percentage of inhibition was shown from 1st hour itself (52.5%) and this effect was continued till the end of the experiment. In kashayam extract, powder tablet in the dose of 6tab/50kg shows closer action to that of kashayam. When the efficacy was compared to the controls, strong anti-inflammatory activity was observed in Raasneirandaadi kashayam group with a 46 to 52% inhibition of the inflammation and in tablet.

Table 1: Phytochemical screening of kashayams and kashayam powder

SI No	Product	Ak	Fl	Sa	Ca	Ph	Gl	Te	Ta
1.	Raasneirandaadi kashayam	-	-	-	+	++	-	-	+
2.	Raasneirandaadi kashayam ext.	-	-	+	++	+	-	+	+
3.	Maharaasnaadi kashayam	-	-	+	+	++	-	-	+
4.	Maharaasnaadi kashayam ext.	-	-	+	++	+	-	+	+
5.	Gulguluthikthakam kashayam	-	-	-	+	+	-	-	+
6.	Gulguluthikthakam kashayam ext.	-	-	-	+	-	+	-	-
7.	Mahaamanjishtaadi kashayam	-	-	+	+	++	-	++	+
8.	Mahaamanjishtaadi kashayam ext.	-	-	+	++	+	-	+	+
9.	Dhaanwantharam kashayam	-	-	+	+	+	-	+	+
10.	Dhaanwantharam kashayam ext.	-	-	-	+	-	+	-	-
11.	Punarnnavaadi kashayam	-	-	+	+	++	-	++	+
12.	Punarnnavaadi kashayam ext.	-	-	+	++	+	-	+	+
13.	Pathyaakshadhaathryaadi kashayam	-	-	+	+	++	-	+	+
14.	Pathyaakshadhaathryaadi kashayam ext	-	-	+	++	+	-	-	+

Alkaloids – Ak, Flavonoids – Fl, Saponins – Sa, Carbohydrates – Ca, Phenols – Ph, Glycosides – Gl, Terpenoids – Te, Tannins – Ta
Note: Concentration of each sample - 12.5 mg/ml

Table 2: The percentage of phytochemical constituents in kashayams and its kashayam powder

Sl no	Product name	Tannin (%)	Phenols (%)	Flavonoids (%)	Alkaloids (%)	Saponins (%)
1	Raasneirandaadi kashayam	4.93±0.67	9.91±1.67	10.80±1.39	0.61±0.42	8.00±0.96
2	Raasnairandaadi kashayam powder	6.25±1.31	11.11±0.92	14.20±1.96	1.97±0.12	8.50±0.65
3	Maharaasnaadi kashayam	3.18±0.70	6.93±1.29	6.51±0.85	0.36±0.18	3.09±0.44
4	Maharaasnaadi kashayam powder	4.20±0.35	5.63±0.42	13.06±0.23	1.40±0.56	2.97±0.58
5	Gulguluthikthakam kashayam	0.74±0.20	3.69±0.50	3.31±1.69	2.24±0.11	0.92±0.61
6	Gulguluthikthakam kashayam powder	1.28±0.10	1.05±0.22	9.12±0.09	3.45±0.02	1.22±0.13
7	Mahaamanjishtaadi kashayam	3.63±0.54	7.29±0.51	8.41±0.52	0.35±0.16	1.00±0.39
8	Mahaamanjishtaadi kashayam powder	8.52±0.23	6.84±0.21	9.30±0.12	0.41±0.13	6.46±0.42
9	Dhaanwantharam kashayam	2.39±2.36	9.55±8.95	4.10±0.19	2.00±0.14	5.80±1.10
10	Dhaanwantharam kashayam powder	3.46±0.09	11.09±1.23	6.80±0.03	2.20±0.03	4.25±0.04
11	Punarnavaadi kashayam	8.51±0.62	12.23±3.55	10.00±1.22	3.30±0.17	3.35±0.73
12	Punarnavaadi kashayam powder	8.47±1.80	18.79±1.20	12.31±0.84	3.80±0.24	4.20±0.03
13	Pathyaakshadhaathryaadi kashayam	12.52±1.25	17.34±5.02	17.80±1.52	1.27±0.08	2.01±0.31
14	Pathyaakshadhaathryaadi kashayam ext	14.94±2.60	11.66±1.85	22.50±1.85	2.48±0.18	3.10±0.28

Data are presented as mean ± S.D.

Table 3: Anti-inflammatory effects of normal control, Raasneirandaadi kashayam (0.3ml/kg), and Raasneirandaadi kashayam tablet (90mg/kg b.wt.) on carrageenan induced paw oedema in rats

Treatment	Dose	1st hour	2nd hour	3rd hour	4th hour
Control	Tween 20	0.2±0.02	0.215±0.016	0.23±0.02	0.25±0.19
Raasneirandaadi Kashayam	15ml/50kg	0.095±0.01 [#]	0.115±0.010 [#]	0.115±0.01 [#]	0.133±0.01 [*]
Raasneirandaadi kashayam tablet	6 tab/50kg	0.115±0.01 [*]	0.145±0.011 [*]	0.130±0.01 [*]	0.130±0.01 [*]

Data are presented as mean ± S.D. * indicates statically significant (p<0.05), compared to respective control. # indicates statically higher activity (p<0.05), compared to another treated group.

Statistical analysis

The experimental results were expressed as mean ± standard deviation (SD) of three replicates. Data were analyzed using statistical software Graph Pad Prism version 7. Two-way repeated measure ANOVA test used to assay the differences in volume of paw oedema in rats and increased latency response in pain was measured in mice. Data are shown as mean ± S.D. All data were considered significant at P < 0.05

RESULT AND DISCUSSION

Phytochemical screening

From the results (Table 1) it is seen that there is absence of Alkaloids in all samples. This may be due to the low quantity presence or their ready decomposition on heating and the lesser presence in many plants. The presence of Carbohydrates, Phenols and Tannins are there in all samples. Saponins and Terpenoids were absent in Gulguluthikthakam kashayam both samples. Terpenoids were also absent in Pathyaakshadhaathryaadi kashayam powder. The presence of Glycosides was seen only in Gulguluthikthakam and Dhanwantharam kashayam samples.

The phytochemical compounds detected are known to have medicinal importance. For example, alkaloids have been reported as powerful poison and many alkaloids derived from medicinal plants show biological activities like, anti-inflammatory¹¹ antimalarial¹², antimicrobial¹³, cytotoxicity, antispasmodic and pharmacological effects^{14,15}. Similarly, steroids derived from plants are known to have cardiotoxic effect and possess antibacterial and insecticidal properties¹⁶. Tannins, according to research, are known to have antibacterial¹⁷, antitumor and antiviral activities¹⁸. They work by precipitating microbial protein thus making nutritional protein unavailable for them. These phytochemical compounds identified in the kashayams are responsible for the biological

activities. To get a clear view on all of them, the quantitative analysis was done.

Quantification of phytochemicals

The percentage of phytochemical constituents in the Kashayams was shown in table I. From the results of the phytochemical analysis, it is seen that even though the presence is not shown in the screening, they can be assessed quantitatively in very low quantity. It also shows that the phytochemicals present in each formulation vary in presence and percentage. The medicinal value of plants is connected to the chemical substances that have a definite physiological action on the human body⁶. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases¹⁹. For example, saponins possess both beneficial (cholesterol-lowering) and deleterious (cytotoxic permeabilization of the intestine) properties and also exhibit structure dependent biological activities²⁰. Plants with tannins are used for healing of wounds, varicose ulcers, hemorrhoids, frost-bite and burns^{21,22}. It is a possibility to further analyse the phytochemicals into their sub divisions and correlate with the actions of each of them with the action of the drug. There are many chemical changes occurring when multiple herbs are processed together in water using heat. The phytochemical presence also depends on nature of extraction, time, temperature, concentration, polarity, etc., the standardization may help in the sustaining the product and process standards and retaining its efficacy without variation. This will help in better explanation of the traditional Ayurvedic drug to the modern scientific fraternity improving the acceptance of traditional medicines, but will call upon for expanded research in the area.

Anti-inflammatory property of Raasneirandaadi kashayam

The results of efficacy study on animals shows that the modern dosage form of the tablet prepared from the spray dried powder is showing closer results to that of liquid. The slight variation in

the effect may also be due to the difference in the time duration for absorbance into the system with that of traditional liquid form. The maximum percentage of inhibition shown from the 1st hour itself (52.5%) is indicative of the immediate bioavailability of the drug. The sustained effect continued till the end shows the longer duration of the effect which also supplements to the dosage of the product.

CONCLUSION

The result of the present study shows that the kashayams contain many phytochemical components. These compounds have potentially significant applications in curing disease. However, these studies can only be taken as a preliminary step towards the betterment of traditional medicine. These drugs are to be further individually analysed to ascertain the major active compounds and its efficacy. This may also be related and researched for the ideal pH value of the stomach for each of the drugs and the ideal site of actions to further improve the efficacy of new dosage forms.

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