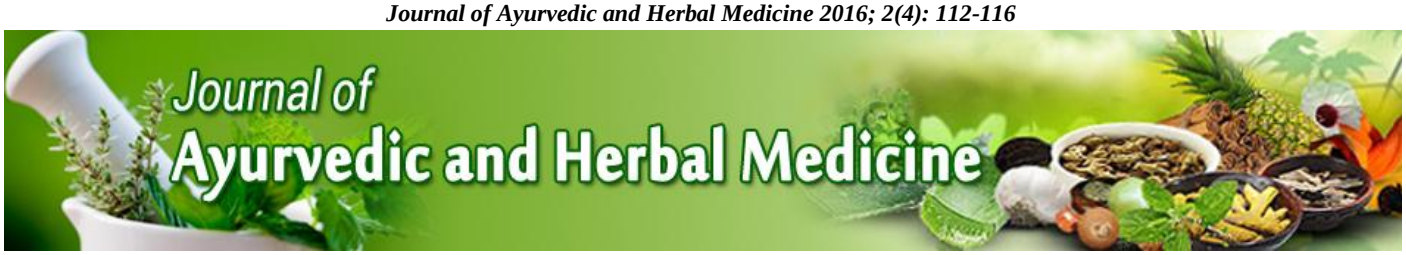

Research Article

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A comparative analytical study of Ashodhitha Kupilu (*Strychnos nux-vomica* Linn.) Shodhitha Kupilu and Vishamushti Vati.

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ABSTRACT

Kupilu (*Strychnos nux- vomica* Linn), is one of the widely used drug in the category of Upavisha. Vishamushti vati is one of the preparation in which Kuiplu is the main ingredient. Kupilu shows toxic symptoms, if it is consumed without proper Shodhana procedures. Strychnine is one of the main chemical constituent available in the Kupilu by which the chemical action of Kupilu is expected. So it is very much essential to study the concentration of Strychnine in raw, Shodhitha kupilu and Vishamushti vati by means of physico-chemical analysis. Physico chemical analysis reveals increased value in loss on drying, total ash, water soluble ash, alcohol soluble extraction, water soluble extraction in Vishamushti Vati and increased value in acid insoluble ash in Ashodhitha kupilu. It is observed that Shodhitha Kupilu was less acidic than other two samples. Strychnine percentage in Ashodhitha Kupilu was 0.44, Shodhitha Kupilu was 0.14 and that of Vishamushti vati was 0.17. Hence it is proved that Shodhitha Kupilu and Vishamushti vati are having less Strychnine percentage compare to Ashodhitha Kupilu.

Keywords: Kupilu, Strychnine, Analytical study, Vishamushti vati, HPTLC.

INTRODUCTION

Kupilu (*Strychnos nux-vomica* Linn) is one of the widely used drug in the category of Upavisha[1]. It is considered as spinal poison of vegetable origin causing serious derangements in the body. Strychnine is the main chemical constituent which is responsible for producing the toxicity & convulsions[2]. It is cited in the treatises of Ayurveda, the Visha (poison) becomes Amritha (nectar) after logical administration[3]. Ancient physicians of Ayurveda successfully used these drugs to combat number of diseases after proper Shodhana in specific media. In classical text books of Ayurveda, actions of Kupilu are explained as Vedanasthapana, Naadibalya and Jwaraghna. Kupilu is indicated in Ajeerna, Grahani, Nadishoola, and Vatavyadhis[4] and is used as an ingredient in preparations of many formulations such as Agnithundi vati, Lakshmi vilasa rasa, Visha garbhathaila. Vishamushti is one among them which is used in condition like Udarashoola, Pakshaghatha, Vibandha and Vishamajwra[5] etc.

After introduction of modern analytical technologies in Ayurveda, it becomes easy to find out the changes in drugs occurred during different Samskaranas. Thus the level of different constituents can be also assessed by using these types of analytical techniques in drug at different stages and by knowing chemical profile of the drugs we can derive the concept behind the mode of action and we can prevent the toxicity. Some previous studies on kupilu revealed the shodhan process reduces Strychnine & Brucine contents[6].

Hence to provide scientific value of different constituents of kupilu before & after shodhana and Visamusti vati, the study A COMPARATIVE ANALYTCLA STUDY OF ASHODHITHA KUPILU (*Strychnos nux-vomica* Linn.) SHODHITHA KUPILU AND VISHAMUSHTI VATI has undertaken.

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MATERIAL AND METHODS

MATERIALS

Kupilu seeds were collected from Hebri surrounding areas and were authenticated in SDM centre for research in Ayurveda and allied sciences udupi. All the materials required for the Shodhana of Kupilu and preparation of Vishamushti Vati were collected from SDM Ayurveda pharmacy Udupi and SDM herbal garden. Pharmaceutical study was conducted at Rasashastra & Bhaishajya kalpana practical hall, SDM college of Ayurveda udupi. Organoleptic, Physico chemical analysis & HPTLC of Ashodhitha Kupilu, Shodhitha Kupilu and Vishamushti Vati were conducted at SDM centre for research in Ayurveda and allied sciences, Udupi.

METHODS

Shodhana of Kupilu & preparation of Vishamushti vati were carried out as per the reference of Siddhabhaishajya mani mala[5]. The references required for analytical studies were referred from protocols for testing of ASU drugs[7].

Shodhana of Kupilu

Shodhna of Kupilu was carried out as per the reference of Siddhabhaishajya manimala[5]. Kupilu seeds were collected and washed properly with water then dried in sunshade. Kupilu seeds were kept in mud for 7 days, after 7 days seeds were taken out and Ankura's were removed. 400 g of dried Kupilu seeds were subjected for bharjana in 50 ml of Go gritha. This process continued until brown colour was seen. Kupilu seeds were powdered and stored in a tight container.

Extraction of Indravaruni swarasa

Extraction of Indravaruni swarasa was carried out as per the reference of bhaishajya ratnavali[8]. 48 indravaruni fruits were taken, washed, cut into pieces and juice was collected using juice extractor.

Preaparation of Vishamushti Vati

Preparation of vishamushti vati was carried out as per the reference of Siddhabaishajya mani mala[5]. 250 g of Shodhitha Kupilu, 250 g of maricha powder was taken in khalwa yantra and triturated with indravaruni swarasa (500 ml, gravimetrically equal to the Shodhitha kupilu & Maricha quantity to get samyak pluta state) and Bhavana was given for 7 hours continuously. Vati's were prepared in 2 Sarshapa pramana and dried in sun shade.

ANALYTICAL STUDY

Samples of Ashodhitha Kupilu, Shodhitha Kupilu and Vishamushti vati were subjected to organoleptic parameters like colour, odour, taste, touch, appearance and physic chemical parameters like loss on drying[7], total ash value[7], acid insoluble ash[7], water soluble ash[7], extractive values[7] and pH[7]. Concentration of Strychnine in the 3 samples were analysed by using High performance thin layer chromatography[7].

OBSERVATIONS AND RESULTS

The Kupilu seeds turned to brown colour after Shodhana process. There was 90 g of weight loss was noted during Shodhana process (initial weight of Kupilu was 400 g and weight of Kupilu after Shodhna was 310 g). Vishamushti vati were grey in colour and Tiktha rasa. There was 25 g of loss was observed during the preparation of Vishamushti vati

The Ashodita kupilu, Shodhita kupilu and Vishamusti vati were subjected for assessment of organoleptic characters and the results were tabulated in Table 1.

The Physico chemical analysis values of Ashodhitha Kupilu, shodhitha Kupilu and Vishamushti vati were analysed and tabulated in Table 2.

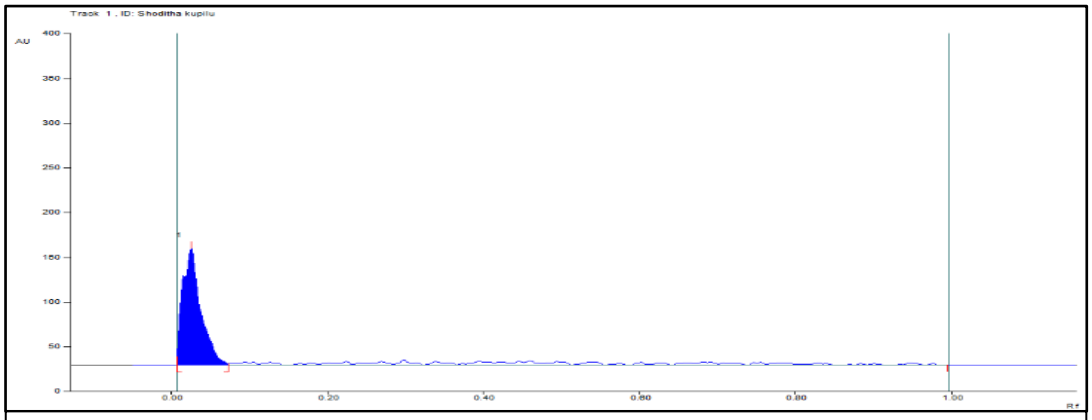
Table 1: Organoleptic characters of Samples

S. No.	Features	Shodhitha Kupilu	Ashodhitha Kupilu	Vishamushti vati
1	Colour	Brown	Light brown	Greyish
2	Odour	Smell of ghee	Odourless	Smell of all ingredients
3	Touch	Slightly soft	Rough	Soft
4	Taste	Bitter	Bitter	Bitter
5	Appearance	Powder	Powder	Tablet

Table 2: Physico Chemical Analysis Results

Parameter	Results n = 3 %w/w		
	Shoditha kupilu	Ashoditha kupilu	Vishamushti vati
Loss on drying	12.53	11.60	15.86
Total Ash	2.09	2.64	5.62
Acid Insoluble Ash	0.10	1.0	0.69
Water soluble Ash	1.39	0.90	2.69
Alcohol soluble extractive value	4.96	4.88	6.29
Water soluble extractive value	13.85	17.83	23.16
pH	5.18	3.80	4.22

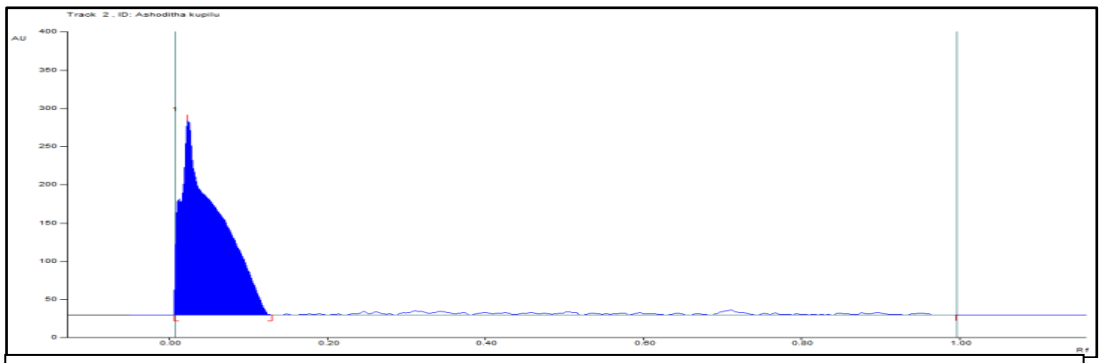
HPTLC: Densitometric Scan at 366nm of Ashodhitha Kupilu, Shodhitha Kupilu, Vishamushti Vati



Track 1, ID: Shoditha kupilu

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.01 Rf	9.1 AU	0.03 Rf	130.5 AU	100.00 %	0.07 Rf	1.3 AU	2172.5 AU	100.00 %	unknown *

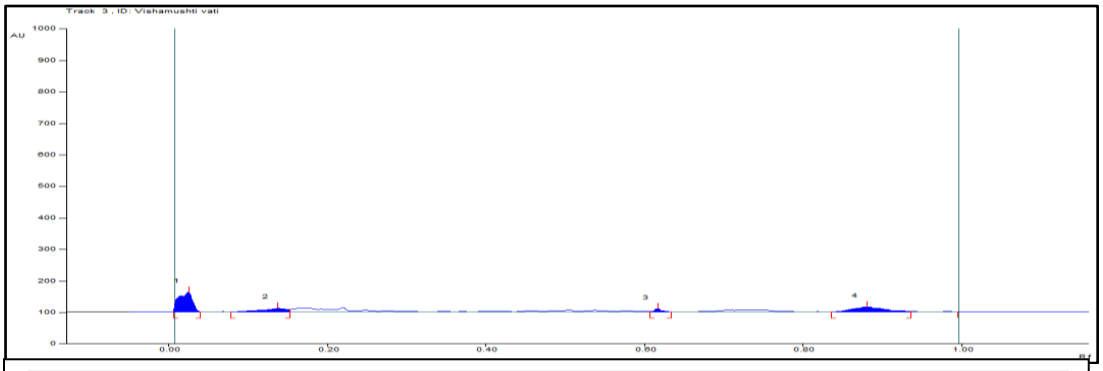
Fig 1: Shodhitha Kupilu. Densitometric scan at 366 nm the sample Ashodhitha Kupilu (0.03)



Track 2, ID: Ashoditha kupilu

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.01 Rf	0.0 AU	0.02 Rf	254.4 AU	100.00 %	0.13 Rf	0.1 AU	8479.2 AU	100.00 %	unknown *

Fig 2: Ashoditha Kupilu. Densitometric scan at 366 nm the sample Shodhitha Kupilu (0.02)



Track 3, ID: Vishamushti vati

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.0 AU	0.03 Rf	62.3 AU	62.73 %	0.04 Rf	0.7 AU	774.6 AU	49.78 %
2	0.08 Rf	0.0 AU	0.14 Rf	11.0 AU	11.11 %	0.15 Rf	7.8 AU	257.2 AU	16.53 %
3	0.61 Rf	1.6 AU	0.62 Rf	10.2 AU	10.26 %	0.64 Rf	0.1 AU	67.9 AU	4.36 %
4	0.84 Rf	0.1 AU	0.88 Rf	15.8 AU	15.90 %	0.94 Rf	1.7 AU	456.3 AU	29.33 %

Fig 3: Vishamusti Vati. Densitometric scan at 366 nm the sample Vishmusht Vati (0.03), (0.14), (0.62), (0.88)

Table 3: Showing Rf values of Shoditha kupilu, Ashoditha kupilu and Vishamushti vati Densitometric scan at 254 nm

S. No.	Samples	Rf value
1	Ashodhitha Kupilu	0.66
2	Shodhitha Kupilu	0.69
3	Vishamushti Vati	0.67
4	Strychnine (1000 ng)	0.67
5	Strychnine (2000 ng)	0.68
6	Strychnine (5000 ng)	0.69

Table 4: Showing Rf values of Shoditha kupilu, Ashoditha kupilu and Vishamushti vati in Densitometric scan at 366 nm

S. No.	Samples	Rf value
1	Ashodhitha Kupilu	0.03
2	Shodhitha Kupilu	0.02
3	Vishamushti Vati	0.03 0.14 0.62 0.88

Percentage of Strychnine

All the three samples were subjected for extraction and Strychnine percentage was obtained by using HPTLC, results were tabulated in table 5

Table 5: Percentage of Strychnine in test extracts

Sample name	X (calculated) via Height	X (calculated) via Area	Percentage (%)
Shoditha Kupilu	1.423µg	1.326 µg	0.14
Ashoditha Kupilu	4.645 µg	4.181 µg	0.44
Vishmushti vati	1.890 µg	1.771 µg	0.17

DISCUSSION

Shodhana of Kupilu was carried out as per the reference of Siddha bhaishajya mani mala[5]. 400 g, of Ashodhitha Kupilu seeds were kept in wet mud for 7 days and Ankura was removed, then fried in Gritha, quantity of Gritha was not mentioned in the reference, so 50 ml of Gritha was taken for bharjana with the advice from experienced teachers. Mandagni was maintained throughout the process of heating. This might be to avoid the excessive burning of Kupilu seeds during the Bharjana. Brown colour kupilu seeds were seen after the Shodhana and the seeds became brittle, hence it was easy to make into powder form. There was a loss of 90 g at the end of powdering may be due to physical impurities like (sand), chemical impurities and due to adherence to the micro pulveriser which was used to powder the seeds. Extraction of Indravaruni swarasa was carried out as per reference of Bhaishajya ratnavali[8]. Pakwa indravaruni fruits were collected, washed, cut into pieces and juice was collected using juice extractor. Fruits were made into pieces so that it helps in easy extraction of juice. Seeds were discarded, 48 fruits were taken and 750 ml of Swarasa was obtained and consistency of Swarasa was thick. Preparation of Vishamushti vati was carried out as per the reference of Siddha bhiashjya manimala[5]. 250 g of Shodhitha Kupilu seeds powder and 250 g of Maricha powder were taken and triturated with Indravaruni swarasa for 7 hours. When the paste became thick in consistency, Vati's were prepared. Vati's were grey in colour, Tikta in taste. The total weights of Vati's were 475 g.

It was noted that Shodhitha Kupilu sample shown brown colour, odour of ghee and tikta in taste and Ashodhita Kupilu shown light brown colour, odourless, tikta in taste & Vishamushti vati was grey in colour and Tikta in taste. Physico chemical parameters of 3 samles were shown in table 1. The loss on drying of Ashodhitha kupilu was -11.60, Shodhitha kupilu was 12.53 and Vishamushti vati was 15.86. Loss on drying indicates stability of the sample. Increased value in loss on drying in Shodhitha Kupilu and Vishamushti vati samples when compared with Ashodhitha kupilu sample indicates the presence of more moisture, it may be because of seeds which were kept in wet mud for seven days during the process of Shodhana, and in Vishamushti Vati may be because of Bhavana process followed while preparing vati. The total ash value of Ashodhitha Kupilu was 2.09, Shodhitha Kupilu was 2.64 and Vishamushti vati was 5.62. The ash value helps us to determine the amount of inorganic substance present in the sample. Among the three samples Vishamushti vati showed increased ash values, it may be due to the other ingredient and procedures followed while preparing vati. Acid insoluble ash value of Ashodhitha kupilu was 1.0, Shodhitha kuplu was 0.1 & Vishamushti vati was 0.69. Acid insoluble ash value indicates the purity of the sample and almost all inorganic material present is soluble in acid and digestible in human G.I tract. Water soluble ash value of Ashodhitha kupilu was 0.90, Shodhitha Kupilu was 1.39 and Vishamushti Vati was 2.69. Vishamushti vati sample shown increased ash value compare to other samples which indicates maximum quantity of ash was soluble in water media. Alcohol soluble extractive value of Ashodhitha Kupilu was 4.88, Shodhitha Kupilu was 4.96 & Vishamushti vati was 6.29. Increased Alcohol soluble extractive value of Vishamushti vati indicates more number of phytochemical constituents are soluble in alcohol media. Water soluble extractive value of Ashodhitha kupilu was 17.83, Shodhitha kupilu was 13.85 & Vishamushti vati was 23.16. Increased water soluble extractive value of Vishamushti vati was noted when compared to other two samples. pH value of Ashodhitha kupilu was 3.80, Shodhitha kupilu was 5.18 & Vishamushti vati was 4.22. pH values of Shodhitha Kupilu & Vishamusti vati shown alkaline changes when compared to Ashodhita sample may due to the procedures followed on them during processing. Previous studies on kupilu shodhana also indicated the change of pH towards alkaline media after shodhana process[9].

In Densitometric scan at 254 nm the samples Ashodhitha Kupilu (0.66), Shodhitha Kupilu (0.69) and Vishmusht Vati (0.67) shown the peaks at Rf values as shown in table 3 and they compared with Rf values of the marker compound (Strychnine) in different concentrations. In Densitometric scan at 366 nm the samples Ashodhitha Kupilu (0.03), Shodhitha Kupilu (0.02) and Vishmusht Vati (0.03), (0.14), (0.62), (0.88) shown the peaks at Rf value as shown in table 4 and they compared with Rf values of the marker copmpound (Strychnine) in different concentrations. Decrease in Strychnine content found in Shodhitha sample and Vishmushti vati sample when compared to raw sample. In previous studies no any change in Strychnine percentage was noted after shodhana procedure by following bharjana in cow's ghee[9]. But in the present study remarkable reduction of Strychnine percentage was observed in shodhita kupilu when compared with ashodhita kupilu sample. Probably the additional procedure followed (Kupilu seeds were kept in mud for 7 days) may contributed in the reduction of Strychnine percentage. During entire shodhana procedure Strychnine percentage reduced may be due to the diffusion process when it comes in contact with mud for 7 days and ghrita bharjana might have been converted Strychnine in to their N-oxidative derivatives with lesser toxicity. There is slight increase in the Strychnine percentage in Vishamusti vati when

compared with shodhita kupilu sample, may be due to synergetic action of bhavana dravya Indravaruni swarasa which was used while preparing Vishamusti vati.

CONCLUSION

Comparative Analytical study of Ashodhita kupilu, Shodhita kupilu & Vishamusti vati reveals that; Loss on drying value is more in Vishamusti vati & Shodhita kupilu than Ashodhita kupilu. The pH value of kupilu turned towards alkaline media after shodhan process. The HPTLC report indicates the reduction of Strychnine percentage after shodhana process in kupilu from 0.44 to 0.14.

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