

***ANALYTICAL STUDY OF TOXIC PRINCIPLES IN 'KALIHARI KANDA' (Gloriosa superba) BEFORE SHODHANA SANSKARA AND AFTER SHODHANA SANSKARA***

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**ABSTRACT**

Poisonous plants are subjected to *Shodhana* (Purification) prior to their therapeutic use. But in Ayurveda no any process is described to observe the changes taking place after *Shodhana*. Hence to find out the variations observed in parameter like Ash value, Extractive value, TLC, Alkaloidal assay etc. before and after *Shodhana sanskara* of 'Kalihari Kanda' the study was carried out. Well ripened *Kanda* of 'Kalihari' was selected. *Shodhana sanskara* of *Kalihari Kanda* was done by two methods as *Gomutra sthapitam* and *Takra sthapitam* described by *Yogratnakar* and *Bhavprakash* respectively. Later on *shodhana* was carried out with distil water *sthapitam* as control group. Analytical study of *Ashuddha* (impure) and three *Shodhit* (purified) samples of *Kalihari Kanda* were done with the help of parameters such as loss or gain of weight, Alcohol extractive value, Aqueous extractive value, pH of aqueous extract of 5% solution, TLC, Alkaloidal assay. On the basis of observed data it was concluded that there was remarkable differences in various parametric values of *Ashuddha* and all three *Shodhit* samples of 'Kalihari Kanda'. So this may be differentiating test for *Ashuddha* and *Shuddha* samples and can be applied for *shodhit Kalihari Kanda*.

**Keywords:** *Shodhana Sanskara, Kalihari Kanda, Poisonous plants, Upavisha.*

**INTRODUCTION**

The intention of *Ayurveda* is to maintain the health of the healthy person and also to cure the disease<sup>1</sup>. The principles of *Ayurveda* are immortal, extensive research work is necessary to prove it. According to *Acharya Charaka* if we used poisonous drugs after *Shodhana* they act as

a good medicine. Even medicine may act as a poison if do not use in proper dose<sup>2</sup>. *Shodhana sanskara* is mentioned for purification of drugs (*Dravya*)<sup>3</sup>. The poisonous plants are attributed with certain properties like *Rasayana, Yogawahi, Tridoshaghna, Brihan, Viryavardhak*

and *Pranadayi* when *Shodhana Sanskara* is carried out<sup>4</sup>. *Shodhana sanskara* should be done on poisonous plants before its therapeutic use. This process reduces toxicity of poisonous plants considerably and keeps it to required optimum level<sup>5</sup>. So it is interesting to study practically the changes in the toxic principles of poisonous drugs (*Dravya*) before and after *Shodhana sanskara*. As the '*Kalihari Kanda*' is *Sthavar vanaspatic upvisha*<sup>6</sup>, it should be purified before using in medicinal preparation. For the experimental study *Shodhana* is done by two methods mentioned in *Ayurvedic* texts and by distilled water as a control group. So we select here to study changes taking place in '*Kalihari Kanda*' before and after *Shodhana sanskara* according to the modern parameters and the *Ayurved pharmacopoeia I.S.M. Govt. of India*<sup>7</sup>.

#### AIMS AND OBJECTIVES

1. To study the changes taking place in the effect of toxic principles on the basis of chemical analysis before and after *Shodhana sanskara* of *Vanaspatic Upavisha 'Kalihari Kanda'* (*Gloriosa superba*).
2. To develop a laboratory test such as Ash values, Extractive values, Alkaloidal assay, Thin layer chromatography (TLC) etc. to differentiate *Shuddha* and *Ashuddha Kalihari Kanda*.

#### REVIEW OF LITERATURE

*Kalihari (Gloriosa superba)* is a semi woody herbaceous branching climber reaching approximately five meters in height. Plant is common in Bengal and in low jungles throughout India, Burma and Ceylon. Part used is tubers. The entire plant is toxic especially the tubers are extremely poisonous. The toxic properties of plant are essentially due to the highly active alkaloid

'*Cholchicine*'. Another important alkaloid called *Gloriosine*<sup>8</sup>. In *Bruhatrayi* many therapeutic uses of *Kalihari Kanda* are mentioned. It is an abortifacient used for the expulsion of foetus, *kanda lepa* applied on palm and sole it gets labourless delivery<sup>9</sup>. It used in the treatment of *Vatrakta*<sup>10</sup>, *Kushtha*<sup>11</sup>, *Arsha*<sup>12</sup>, *Bhagandar*, *Asadhya Gandmala* can be treated by *Pana*, *Abhyanga* and *Nasya of Gunjyadya taila*<sup>13</sup>. *Kalihari* can also be used as antidote for snake bite described by *Achyarya Vagbhata*. Before using *Kalihari Kanda* as medicine or to prepare *kalpa*, *Shodhana* is advised in *Samhita*. Methods of *Shodhana sanskara of Kalihari kanda* in different *Ayurvedic* texts are as follows.

#### 1. By *Yogratnakar with Gomutra sthapitam*

Quantity sufficient *Gomutra* taken in earthen pot and pieces *Ashuddha Kalihari Kanda* immersed in it. After 24 hrs *Kalihari Kanda* pieces were drown out from the earthen pot and dried.

#### 2. By *Bhavprakash with Takra Sthapitam*

Quantity sufficient *Takra* mix with *Saindhav* taken in earthen pot and *Ashuddha Kalihari Kanda* pieces suspended in it and on next day *Kalihari kanda* were drown out and washed with hot water. After this it again mixed with fresh *Takra* with *Saindhav* and repeated the same procedure for next 6 days. Lastly washed with hot water and dried.

#### EXPERIMENTAL STUDY

As per literature references, *Shodhana sanskara of Kalihari kanda* reduces its toxicity and enhances its therapeutic properties. Physical and chemical changes due to *Shodhana* have not been studied with reference to modern parameters. Hence the study of *Kalihari Kanda* after *Shodhana* was carried out with the help of mod-

ern parameters to see whether it reduces its toxicity or not. Analytical study include Alkaloidal assay as alkaloid is significant ingredient and other parameters like Ash values, Extractive values, Thin Layer Chromatography (TLC) follows testing of drugs which is mentioned in the Ayurvedic Pharmacopoeia of India.

## MATERIAL AND METHODS

*Shodhana of Kalihari Kanda* is done by two methods mentioned in *Ayurvedic* texts and by distilled water as a control group.

A. Well ripened *Kanda* of *Kalihari* were selected

B. *Shodhana sanskara* of *Kalihari kanda* was done by *sthapitam* with *shodhana dravya*'s like

1. *Gomutra*

2. *Takra*

3. Later on *Shodhana* was carried out with distilled water *sthapitam* as control group.

C. *Ashuddha* and three *Shodhit* samples were analyzed for physical and chemical parameters.

*Kalihari kanda* were collected from market of Nagpur city. *Kanda* was cut into many pieces and taken for *shodhana* process. *Gomutra* and *Takra* collected from *Govigyan Anusandhan Kendra* Nagpur and used for *shodhana*. Process of *Shodhana* was carried out according to *Yogratnakar* and *Bhavprakash*.

## SHODHANA PROCEDURE

*Shodhana* of *Kalihari Kanda* is done by three methods

1. **By *Gomutra Sthapitam*** (Ref-*Yogratnakar*<sup>14</sup>)

**Apparatus-** Earthen pot, beaker

**Ingredients-** *Ashuddha Kalihari kanda* -125gm and *Gomutra* q.s.

**Procedure-** Quantity sufficient *Gomutra* taken in earthen pot and 125gm of *Ashuddha Kalihari Kanda* pieces immersed in it and kept for 24hrs and pot covered with plate. After this '*Kalihari kanda*' pieces were drawn out from the earthen pot and dried.

2. **By *Gomutra Sthapitam*** (Ref-*Bhavprakash*<sup>15</sup>)

**Apparatus-** Earthen pot, beaker etc.

**Ingredients-** *Ashuddha Kalihari kanda* -125gm and *Takra* -q.s.

**Procedure-** Quantity sufficient *Takra* mix with *Saindhav* taken in earthen pot and 125gm of *Ashuddha Kalihari kanda* pieces suspended in it and pot covered with plate. On next day *Kalihari kanda* were drawn out and washed with hot water. After this it again mix with fresh *Takra* with *Saindhav* and repeat the same procedure for next six days. Lastly washed with hot water and dried.

3. **By Distilled Water *Sthapitam***

**Apparatus-** Earthen pot, beaker etc.

**Ingredients-** *Ashuddha kalihari kanda* -125gm and distilled water q.s.

**Procedure-** Quantity sufficient distilled water taken in earthen pot pieces of '*Kalihari kanda*' immersed in it for 24hrs. Pot covered with plate after this '*Kalihari kanda*' pieces drawn out and dried.

## ANALYTICAL STUDY

Parameters were taken according to the Ayurved pharmacopoeia I.S.M. Govt. of India with some additional parameters.

- **Loss or gain in weight due to Shodhana:**

For each *Shodhana sanskara* 125gm of *Ashuddha Kalihari Kanda* were taken. After *shodhan sanskara* all three samples were kept for drying at 45° C and 35 % humidity. *Shodhit* sample were weighed. The percentage of loss in weight of *Shodhit* samples are shown in table 1 and figure 1.

- **Loss on drying:**

For the study of loss on drying 5gm of each sample of *Kalihari Kanda* were taken separately in tare china disc. *Takra shodhit kalihari kanda* was taken 5.0058gm. They were kept for heating in oven at 105<sup>0</sup>C for 2 hrs and cooled in desiccator. Again kept in the oven, cooled and weighed. Procedure was repeated until constant weight was obtained. Loss on drying of *Ashuddha* and various *Shodhit* samples are shown in table 2 and figure 2.

- **Total Ash of Ashuddha and Shodhit Kalihari kanda:**

Total Ash gives information regarding inorganic content of the sample. Accurately weighed about 2.5gm of each sample were incinerated to constant weight in tare silica crucible and ignite it, when black particles of carbon are no more visible, cool it in desiccator and weighed it. Further heat, cool and weight. The procedure was repeated till constant weight obtained. Total Ash value of *Ashuddha* and *Shodhit Kalihari kanda* are shown in table 3 and figure 3A.

- **Water Soluble Ash of Ashuddha and Shodhit Kalihari Kanda:**

Water soluble Ash gives information about alkalis, metals. Water soluble Ash of various sam-

ples of '*Kalihari kanda*' were determined by boiling the Ash for 20 minutes with 50ml water of each sample separately. The residue in sintered glass crucible was weighed and percentage of water soluble ash was calculated. Water soluble Ash of *Ashuddha* and *Shodhit Kalihari kanda* are shown in table 3 and figure 3B.

- **Acid Insoluble Ash of Ashuddha and Shodhit Kalihari kanda:**

Acid insoluble Ash gives information about silicate or sand present in the samples. Acid insoluble Ash of various samples of *Kalihari kanda* was determined by boiling the ash for 20 minutes with 50ml of HCl. Then the residue in sintered glass crucible was weighed and percentage of acid insoluble ash was calculated. Acid insoluble ash of *Ashuddha* and *Shodhit Kalihari kanda* are shown in table 3 and figure 3C.

- **Alcohol Extractive Value of Ashuddha and Shodhit Kalihari Kanda:**

Alcohol extractive value shows the alcohol soluble portion of the sample. 5gm of powdered '*Kalihari kanda*' of each sample was suspended separately in 100ml of rectified spirit in conical flask with their mouth closed. The flasks were shaken for 6 hrs and kept overnight. The extract was filtered the next day. The 25ml of filtrate was evaporated in tare china dish on water bath. After complete evaporation it was dried in oven and weighed. Alcohol extractive values of *Ashuddha* and *Shodhit* samples are shown in table 4 and figure 4.

- **Aqueous Extractive values of Ashuddha and Shodhit Kalihari kanda:**

An aqueous extractive value shows the water soluble portion of samples. 5gm of powdered '*Kalihari kanda*' of each sample was suspended

separately in 100ml of distilled water in conical flask with their mouth closed. The flasks were shaken for 6 hrs and kept overnight. The extract was filtered next day 25ml of filtrate was evaporated in tare china dish on water bath. After complete evaporation it was dried in oven and weighed. Aqueous extractive values of *Ashuddha* and *Shodhit* samples are shown in table 5 and figure 5.

- **pH of Aqueous extract of 5% solution.**

pH is the measure of acidity or alkalinity. It is the logarithm of the reciprocal of the hydrogen ion concentration or is equal to the logarithm of the hydrogen ion concentration with negative sign. This method has the advantage that all scales of acidity or alkalinity between there of solution molar (or normal) with respect to hydrogen or hydroxyl ions can be expressed by series of positive numbers between 0 and 14. The plant material, in general contains many alkaloids, which are basic compounds and also have acidic compounds such as fatty acids (myristic, palmitic). Hence to check pH of the crude and *sanskrit* (treated) samples of *kalihari kanda*, following method was adopted.

5gm of powder of each sample was suspended separately in 100ml of Chloroform water in conical flask with their mouth closed. The flasks were shaken for 6 hrs and then left for 18 hrs and filtered. pH of each filtrate (5% aqueous extract) were measured by pH meter after calibration of electrode at 25° C (Toschon-Toshniwal instrument). Observations were noted for all samples and pH of aqueous extract of various samples is shown in Table 6 and figure 6.

- **Thin Layer Chromatography:**

Thin layer chromatography gives information regarding chemical components present in sample. Thus here change in the chemical composi-

tion due to *Shodhana* with respect to *Ashuddha* sample can be ascertained by TLC by comparing various bands present in their TLC pattern. The alcoholic extract was spotted on Silica Gel 60 F (Merk) plates and were run using solvent system, Ethyl Acetate: Methanol: Water (1.6: 0.225: 0.16). RF values of various samples are shown in table 7.

- **Total Alkaloidal Assay (Estimation of Alkaloids)**

5 gm of the each sample was taken in 50ml of 70% ethanol and shaken well for 6 hrs and left overnight. The Solution was filtered and 35ml filtrate of each sample was evaporated on water bath. The residue was distilled with 40ml of water and filtered in the separating funnel. All four samples were collected in separating funnel and saturated by sodium chloride and successive portion of chloroform 20ml, 15ml, 15ml, 10 ml. Then chloroform layers were collected and combine chloroform extract was evaporated. The residue of each sample dissolved in 30ml of hot water and filtered in beaker. In each cold filtrate 3ml of the phosphotungstic acid solution (10gm sodium tungsten +6 gm sodium phosphate +50ml water acidified with nitric acid) added. Further 7 ml of diluted sulphuric acid added and it shake and centrifuged. After centrifugation the supernatant contain rejected and remaining precipitates were transferred in to the separator funnel by transferring in water. 30ml of chloroform was added, shaken and chloroform layer was collected. Again 10ml of chloroform added and shaken it and chloroform layer collected. This combine chloroform solution of each sample evaporated to dryness. 5ml of ethanol transferred in residue and again evaporated. Finally all sample were dried at 100° C till constant weight obtained. The total Alkaloidal as-

says of *Ashuddha* and *shodhit* samples are shown in table 8 and figure 7.

## DISCUSSION AND CONCLUSION

This study shows remarkable difference in various parametric values of *Ashuddha* and three *Shodhit* samples of *Kalihari Kanda* conclusion can be drawn.

The percentage of loss in weight due to *Shodhana* with *Gomutra sthapitam* is highest i.e. 12.8% and lowest *Shodhana* with distilled water *sthapitam*. *Takra Sthapitam shodhit* sample shows lowest percentage of loss on drying (9.065%) as compared to *Ashuddha* sample which is 12.92%. Total Ash value is highest in *Gomutra Sthapitam Shodhit* 3.656% and *Ashuddha* sample 2.824%. Water soluble ash value is highest in *Gomutra Sthapitam Shodhit* sample 3.376% where as acid insoluble ash is highest in distilled water *Sthapitam Shodhit* sample i.e. 0.064. Alcohol Extractive value is highest in *Takra Sthapitam Shodhit* which is 1.552%. Aqueous Extractive value is highest in *Takra Sthapitam Shodhit* sample i.e. 25.904% as compared to *Ashuddha* 13.536%. pH of aqueous extract of *Gomutra Sthapitam Shodhit* is 8.64% while *Takra Sthapitam Shodhit* is 3.97% as compare to *Ashuddha* which is 5.04%. Thin layer chromatography with iodine shows *Takra Shodhit* sample shows highest four spot while Distil water *sthapitam shodhit* shows lowest number spot and they very light in colour. It

means large number of chemical constituent washed out due to *Shodhana* by Distil water *sthapitam* and one chemical constituent is added in *Takra Sthapitam Shodhit* which is very dark brown in colour. Highest percentage of alkaloids are in *Ashuddha* sample (0.292%) while lowest are in distilled water *sthapitam shodhit* which is 0.016% *Gomutra Sthapitam Shodhit* and *Takra Sthapitam Shodhit* shows 0.214%, 0.71% respectively. The result shows remarkable differences in various parametric values of *Ashuddha* and *Shuddha* samples which can be considered differentiating tests for *ashuddha* and *shuddha* samples and can be applied for *Shodhit Kalihari Kanda*. So for any formulations (*kalpa*) of *Kalihari kanda* available in market, further test can be developed to find out whether properly *Shodhit Kalihari Kanda* has been used in it. Also this can be applied for doubtful (toxic) formulations in market. To study toxicity of plant further animal experimentation in this regard will add a useful parameter.

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## TABLES AND FIGURES

**Table 1:** Table showing the percentage of loss in weight due to *Shodhana* of various samples

Samples of <i>Kalihari Kanda</i>	Wt. before <i>Shodhana</i>	Wt. after <i>Shodhana</i>	% of loss in wt.
<i>Gomutra Sthapitam Shodhit</i>	125 Gm	109	12.8%
<i>Takra Sthapitam Shodhit</i>	125 Gm	110	12.0%
<i>Distilled water Sthapitam Shodhit</i>	125 Gm	113	09.6%

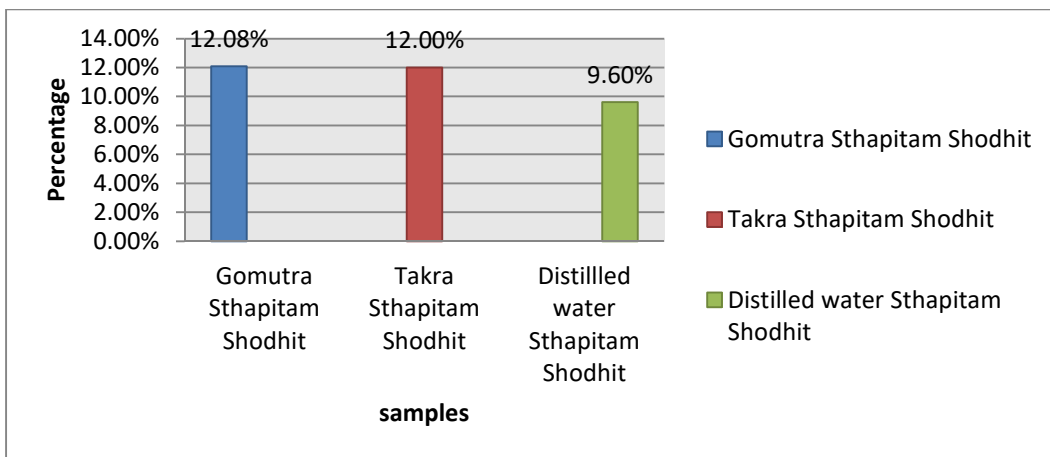


Figure 1: Graph showing the percentage of loss in weight due to Shodhana of various samples

Table 2: Table showing loss on Drying at 105° C of various samples of "Kalihari Kanda"

Samples of Kalihari Kanda	Wt. of samples in gms.	Total loss in wt. after drying	% of L.O.D.
Ashuddha	5	0.6462	12.92%
Gomutra Sthapitam Shodhit	5	0.5846	11.692%
Takra Sthapitam Shodhit	5.0058	0.4538	9.065%
Distilled water Sthapitam Shodhit	5	0.5188	10.376%

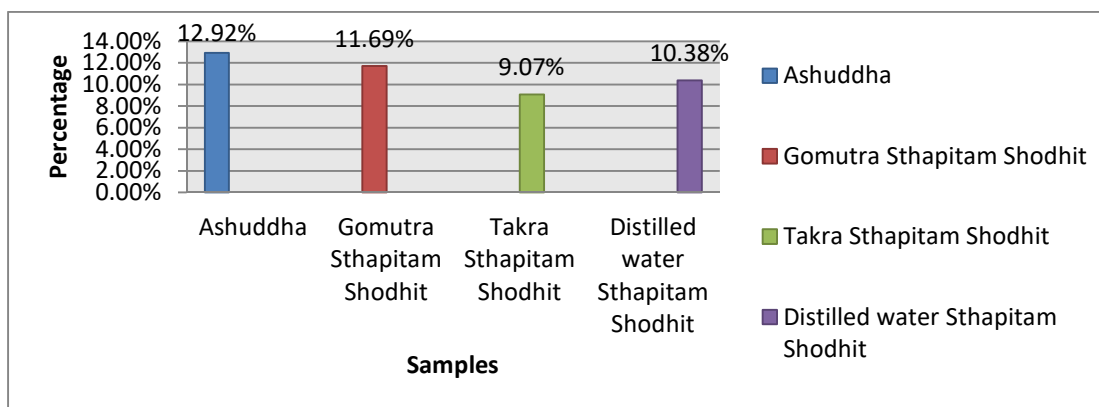


Figure 2: Graph showing loss on Drying at 105° C of various samples of "Kalihari Kanda"

Table 3: Table showing Total Ash, Water Soluble Ash and Acid Insoluble ash of various samples of "Kalihari Kanda"

Samples of Kalihari Kanda	Total Ash	Water Soluble Ash	Acid Insoluble Ash
Ashuddha	2.824%	2.144%	0.064%
Gomutra Sthapitam Shodhit	3.656%	3.376%	0%
Takra Sthapitam Shodhit	1.736%	1.573%	0%
Distilled water Sthapitam Shodhit	1.304%	1.048%	0.016%

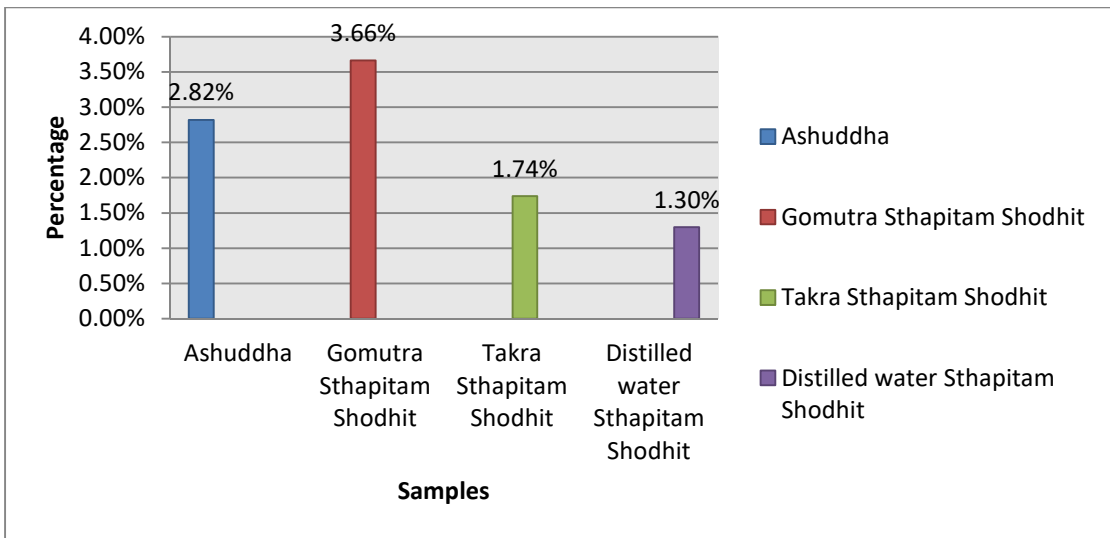


Figure 3A: Graph showing Total Ash % of various samples of "Kalihari Kanda"

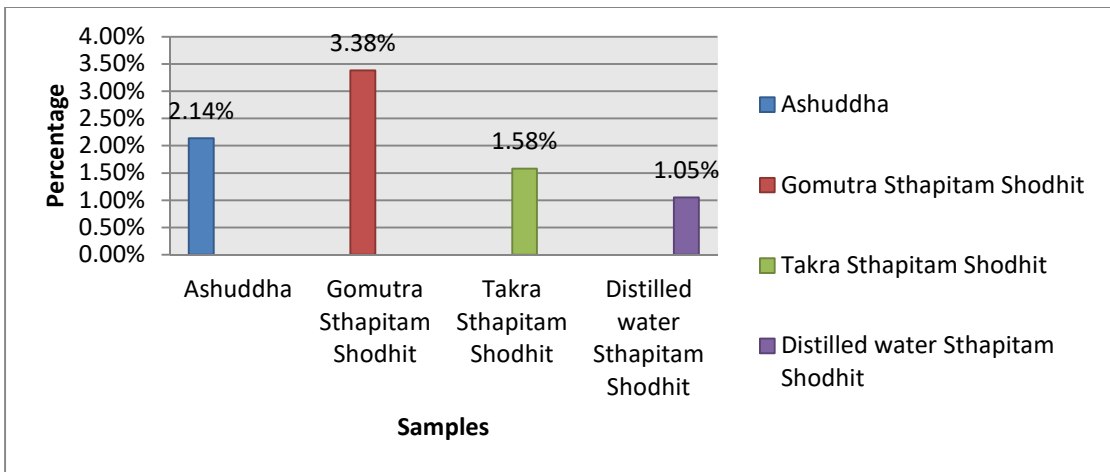


Figure 3B: Graph showing Water Soluble Ash % of various samples of "Kalihari Kanda"

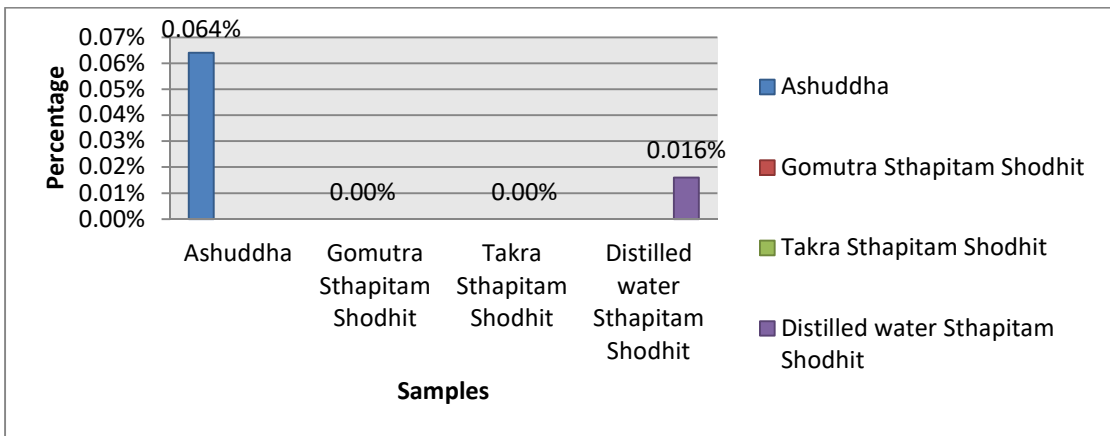
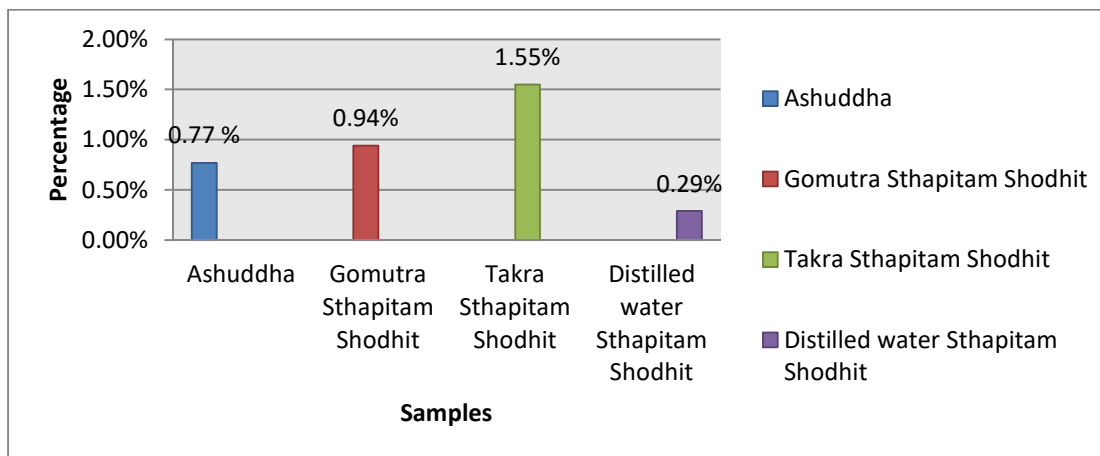


Figure 3C: Graph showing Acid Insoluble Ash % of various samples of "Kalihari Kanda"



**Table 4:** Table showing Alcohol Extractive values of various samples of "Kalihari Kanda"

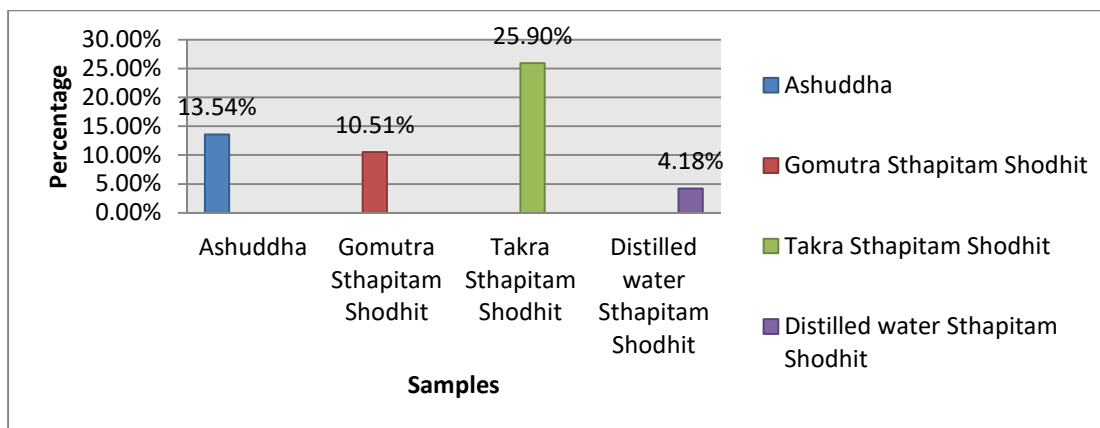
Samples of Kalihari Kanda	Alcohol Extractive values
Ashuddha	0.768%
Gomutra Sthapitam Shodhit	0.944%
Takra Sthapitam Shodhit	1.552%
Distilled water Sthapitam Shodhit	0.288%



**Figure 4:** Graph showing Alcohol Extractive values of various samples of "Kalihari Kanda"

**Table 5:** Table showing Aqueous Extractive Values of Various samples of "Kalihari Kanda"

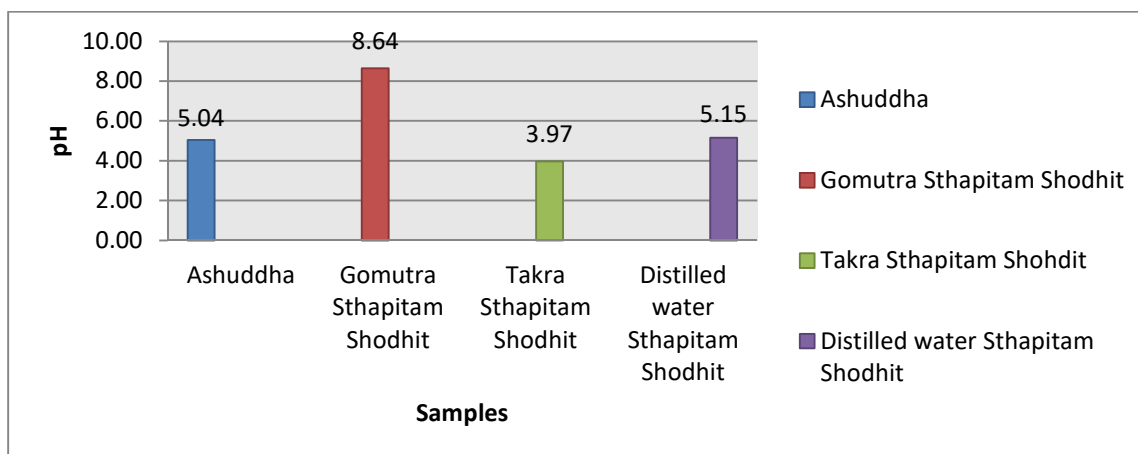
Samples of Kalihari Kanda	Aqueous Extractive Values
Ashuddha	13.536%
Gomutra Sthapitam Shodhit	10.512%
Takra Sthapitam Shodhit	25.904%
Distilled water Sthapitam Shodhit	4.176%



**Figure 5:** Graph showing Aqueous Extractive Values of Various samples of "Kalihari Kanda"

**Table 6:** Table showing pH of Aqueous Extract of various samples

Samples of Kalihari Kanda	pH Aqueous Extractive Values
Ashuddha	5.04
Gomutra Sthapitam Shodhit	8.64
Takra Sthapitam Shodhit	3.97
Distilled water Sthapitam Shodhit	5.15



**Figure 6:** Graph showing pH of Aqueous Extract of various samples

**Table 7:** Table showing Rf. Values of various samples in Thin Layer chromatography with Iodine

Samples of Kalihari Kanda	R.F. Values with Iodine			
	0.11	0.37	0.78	0.89
Ashuddha	-	DB <sup>1</sup>	LB <sup>2</sup>	-
Gomutra Sthapitam	-	LB	DB	-
Takra Sthapitam	LB	LB	DB	VDB <sup>3</sup>
Distilled water Sthapitam	-	VLB <sup>4</sup>	VLB	-

<sup>1</sup>DB- Dark Brown, <sup>2</sup>LB- Light Brown, <sup>3</sup>VDB- Very Dark Brown, <sup>4</sup>VLB- Very Light Brown

**Table 8:** Showing Alkaloid Assay of Various Samples

Samples of Kalihari Kanda	Alkaloid Assay (mg/100gm)
Ashuddha	0.292
Gomutra Sthapitam Shodhit	0.214
Takra Sthapitam Shodhit	0.071
Distilled water Sthapitam Shodhit	0.016

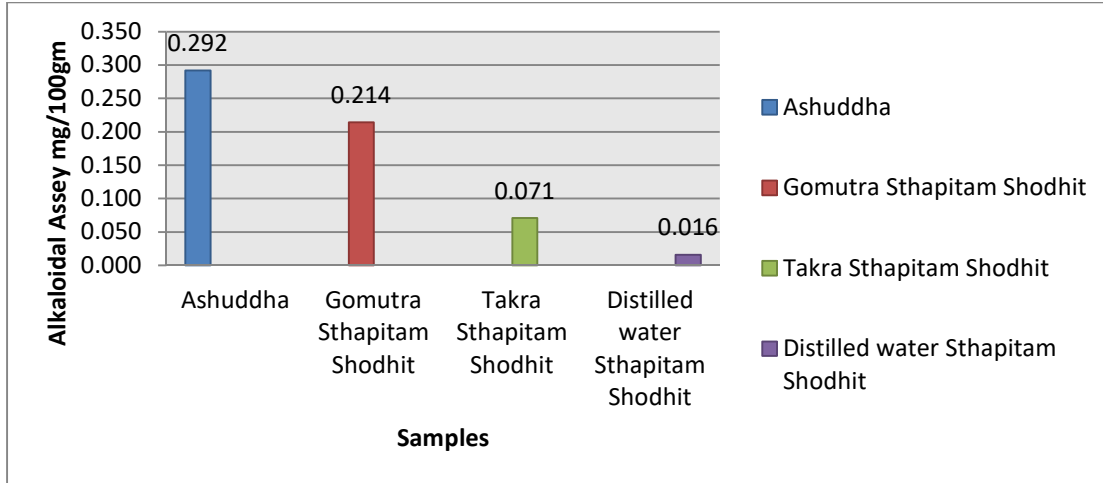


Figure 7: Graph Showing Alkaloid Assay of Various Samples

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