WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 14, 1372-1382. Research Article ISSN 2277– 7105

COMPARATIVE ANALYTICAL STUDY ON THE EFFECT OF GHRITA BHARJANA ON KUPILU SEEDS OF WILD AND MARKET SOURCES

Shivaram N. G.* and Sathisha M. S.

Article Received on
15 October 2021,

Revised on 05 Nov. 2021,
Accepted on 25 Nov. 2021

DOI: 10.20959/wjpr202114-22465

***Corresponding Author**
Dr. Shivaram N. G.

ABSTRACT

Kupilu (Strychnus nux vomica) is one among the *upavisha dravyas* and its demand as well as utility are increasing daily because of its therapeutic significance in many diseases. Many formulations of *Ayurveda* contain *kupilu as* an important ingredient. Classical references explain about the collection of raw drug in detail. But due to industrialisation, Vaidyas are not collecting all the drugs by themselves. The stage in which the drug has to be collected will also decide the pharmacological action. The samples are collected in the state of raw and fresh in the wild and market (dry). Kupilu contains Strychnine and Brucine as the main chemical constituents which are responsible for its toxic effects. Time of collection and the state (matured / immature) of collection of seed may also impact on the quantification/ quality of these chemical composition. Beeja of matured raw kupilu is a potent spinal poison as well as a potent convulsant, whereas s*hodhita kupilu acts* as nervine tonic, analgesic, aphrodiasic, anthelminthic, cardiac stimulant, purgative etc. *Shodhana (Purification)* is a process by which unwanted and harmful impurities are separated from the substance by various methods like boiling, frying, washing etc. with different media, thereby minimising the unwanted toxicity of the substance. As per *Ayurveda* classics, medias like cow's ghee, cow's milk, cow's urine, *kanji*, castor oil etc have been mentioned for the *shodhana* process of kupilu beeja. Here an attempt has been made to compare the impact of *shodhana* in the form of *bharjana* by cow's ghee on wild and market varieties of *kupilu* seeds by the physico-chemical parameters including HPLC analysis.

KEYWORDS: *Kupilu*, *Upavisha*, *Shodhana*, HPLC, Strychnine, Brucine.

INTRODUCTION

Acharya Charaka clearly opined that "***Yogaadapi visham teekshnam uttamam bheshajam bhavet***[1]," (Even a deadly poison if administered properly and wisely can become a very good medicine).

While highlighting the importance of Visha dravyas, Rasa Vaidyas said that "When diseased conditions & symptoms are **not brought under control by using mercurial & other mineral products** (Which are supposed to be very potent & effective) then in such cases **use of poisons is very effective**. (Ayurveda Prakasha ७/४७,४६).

Standardization of any formulation starts from collection of the raw drug to the end product. Any raw drug or the end product if it is not potent to cure the disease is of no use. Pharmacalogical action of the formulation depends on Samyoga, Vishlesha, Samskara, Kala and Yukti. (Cha.Kal. 12 48). Time of collection and process of detoxification are Kala and Samskara.

Vishachikitsa or *Agada tantra* is one among *Ashtangas* of *Ayurveda* which deals with identification of different *vishadravyas*, their impact on body along with their *chikitsa.* Ancient literatures like *Charaka Samhita, Sushruta Samhita, Ashtanga Hrudaya* etc offer exhaustive information on the concept of *Vishadravyas*. There is a detailed description about *vishadravyas* in *Rasa tarangini* and *Rasaratna samuchaya*[2] which are indicated for the safe usage for human consumption equally to *Kashtoushdhis.*

Broadly *Vishadravyas* are classified as *Sthavara, Jangama* and *Krittrima* on the basis the sources whereas on the basis of its toxicity and potency, it is of two types – *Mahavisha* and *Upavisha.*

Vishadravyas are highly potent and fast acting drugs, they can be used as a catalysts which enhance the properties of other drugs with which they are used. But before administering *vishadravya*, proper *shodhana* should be done to reduce their toxicity and fatality. It is not that we are going to remove all the Visha Guna from the drug completely. The concept of *Shodhana* in *Ayurveda* not only covers the process of detoxification of physical and chemical impurities but also minimises hazardous effects and improve the potency/therapeutic efficacy of the purified drugs. The media used for purification influences the pharmacological action of the product.

Kupilu beeja,a potent *upavisha dravya* was selected for the present study as it has high demand nowadays in pharmaceuticals for the preparation of *Ayurvedic* formulations. It is highly toxic due to presence of active alkaloids like Strychnine and Brucine.[5] Many classical purificatory methods are explained in *Ayurveda for the shodhana of kupilubeeja.*

In the present study, *goghrita* is selected as the media for purification of *kupilu.* According to *Rasa Tarangini*, it is the quickest method of *shuddhi (twaritam shuddhimayaati).Ghrita* is having similar properties as that of *Ojas* which are quite opposite to *dashagunas* of *visha*. *Vagbhatta* says there is no medicine equal to *ghrita* in all types and all stages of *visha*, especially in *vatapradhanata*. *Bharjana(frying) with ghrita* is one of the purificatory methods mentioned in classics.

Samples from wild and market are selected for the present study as the market samples have chances of adulteration or contamination. To assess the purity, genuinity as well as the effect of *shodhana (ghrita bharjana)* both the samples are selected.

Here an attempt has been made to compare the impact of traditional method of *shodhana* by goghrita bharjana on wild and market sources of *kupilu beeja* and to confirm it by physiochemical parameters and HPLC analysis.

MATERIALS AND METHODS

Source of data

Samples of Kupilu beeja was collected from Bengaluru market, the wild variety of Kupilu was collected from Sonda and Nilkod of North Canara district in the month of December, both were authenticated through analysis.

Cow's ghee was collected from "Gramarajya Trust", Bengaluru and was authenticated.

Raw drug standardization

Both market and wild samples of kupilu were subjected for certain basic tests for their genuinity.

Powder microscopy

2 grams of sample was taken, added to 20 ml of distilled water and mixed. These mixture was then placed on glass slides and a thin layer was made, over that cover slide was placed such

that no air bubbles was formed. Later the slide was placed on microscope for observation of cell arrangements. Same procedure was carried out for other three samples.

***Bharjana* process**

Approximately half the quantity of both the samples were subjected to *Bharjana* with *go ghritha* on low flame till *samyak bharjita Lakshanas* were seen as per the classical procedure explained in *Rasatarangini*. Standard marker compounds strychnine and *brucine* were purchased one gram each by Yucca laboratory Mumbai. *Kupilu beeja* (market and wild sources) and *goghrita* were collected and authenticated for analysis. Seeds of all the samples were subjected to '*Bharjana'* with a small quantity of authenticated *goghrita* on a low flame till *samyak bharjita lakshanas* were seen and later were powdered. Half of the quantity of both market and wild samples were powdered. Both wild and market samples powder were subjected to *Pharmacognostic* (macroscopic, microscopic), Physico-chemical, Preliminary, Phytochemical and HPLC studies in order to confirm the *genuinity* and purity of the samples.

HPLC analysis

Preparation of extracts

25 grams of dried powdered seeds were defatted by using Pet.ether for about 4 hours, extract the defatted material by refluxing in methanol for 8 hours. The extracts were filtered and concentrated, and these were used for the phytochemical screening and HPLC profiling. The methanolic extract of the seeds of Strychnus nux vomica was subjected to preliminary phytochemical screening for the detection of major class of compounds.

Analytical/HPLC method

High-performance liquid chromatography is used for the estimation of to be superscribed as number 4 in the given sample.

Sample preparation

Standard solution: 10mg of standard Strychnine and Brucine were weighed separately and dissolved in 10 ml methanol, filtered through 0.45µm membrane.

Standard solution: 0.5ml of solution A and 0.5ml of solution B measured and mixed.

Sample solution: 10mg methanolic extract of seeds of Strychnus nux vomica were accurately weighed, diluted to 10ml of methanol (HPLC grade) and was filtered through0.45µm membrane filter.

Chromatographic conditions

Name of the system: Waters TM e2695

Column used: Shim-Pack GIST C18

Dimension of column: 250mm*4.6mm, 5µm

Detector: PDA

Wavelength: Ch1: 254nm, Ch2:264nm

Mobile phase: Water: Acetonitrile

Flow rate: 1ml/min

Injection volume: 20 µl

Run time: 40 Min

Sample preparation: Methanol (HPLC)

Procedure

Solvents of HPLC grade were procured from Merck. The solvents were sonicated about 30 min. 20µlSTD sample injected to Waters T Malliance 2695 system with PDA detector (Shim-Pack GIST C18 (250mm*4.6mm, 5µm) column, with the flow rate 87.5 A% & 12.5.)The retention time of the standard solution was recorded.

20 µl of suitably diluted solution of both wild and market seed sample were injected and the retention time was recorded. Same concentrated solutions of both processed wild and market seed sample were injected with the same parameters and compared.

OBSERVATION AND RESULTS

1. Organoleptic studies

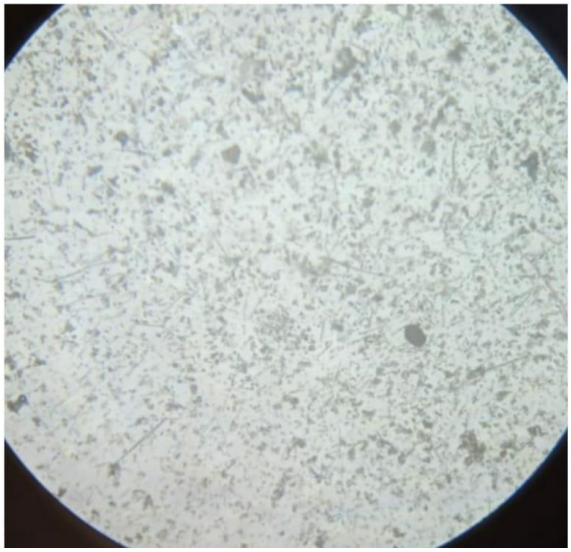
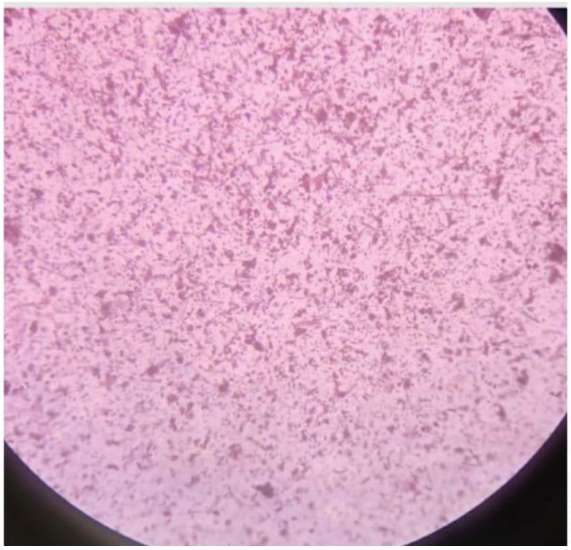
Quality	Wild	Market
1. Colour	Brownish	Light brown
2. Odour	Odourless	Odourless
3. Taste	Bitter	Bitter
4. Touch	Hairy and hard	Hard, hairy

2. Powder microscopy

a) Wild sample

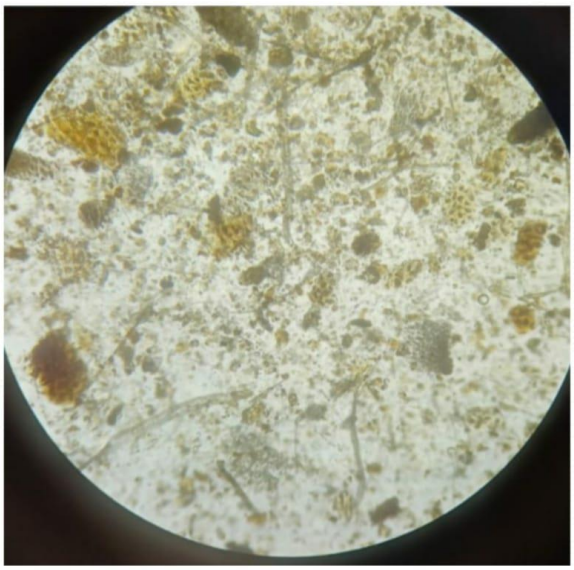
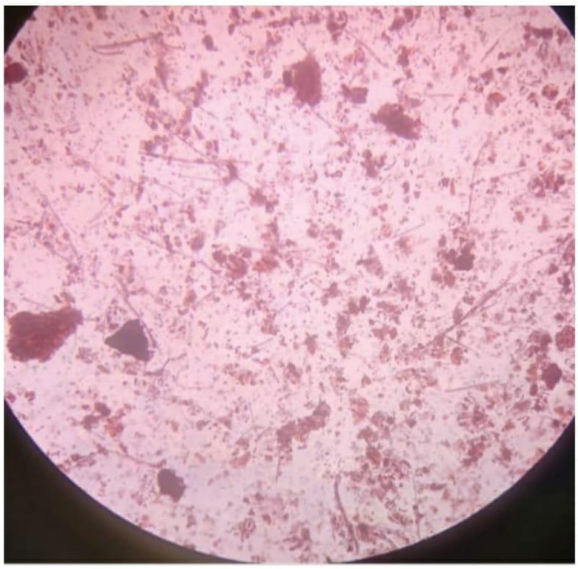
1. Unprocessed

Unstained stained

2. Processed

Unstained stained

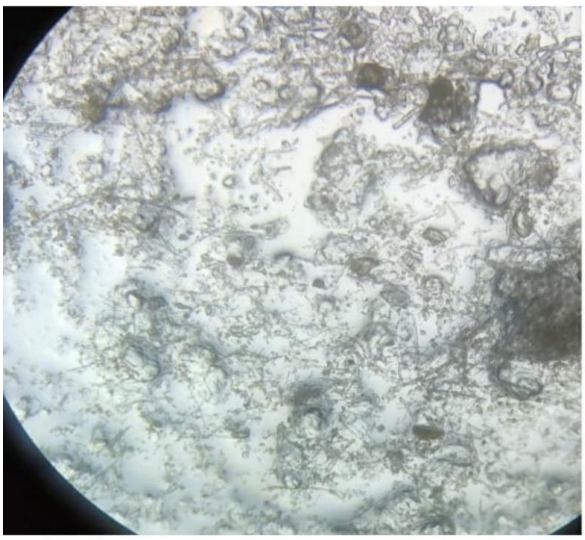
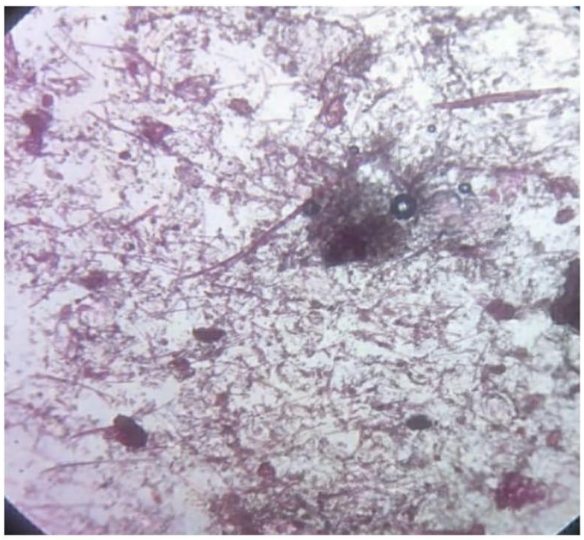
Observed for presence of

1) **Aleurone grains**
2) **Oil globules**

Market sample

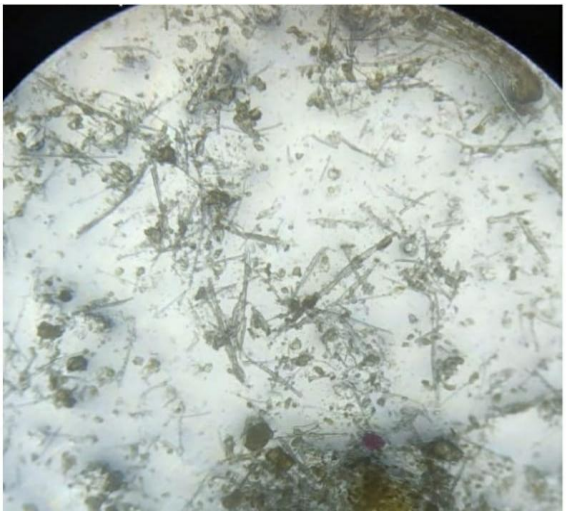
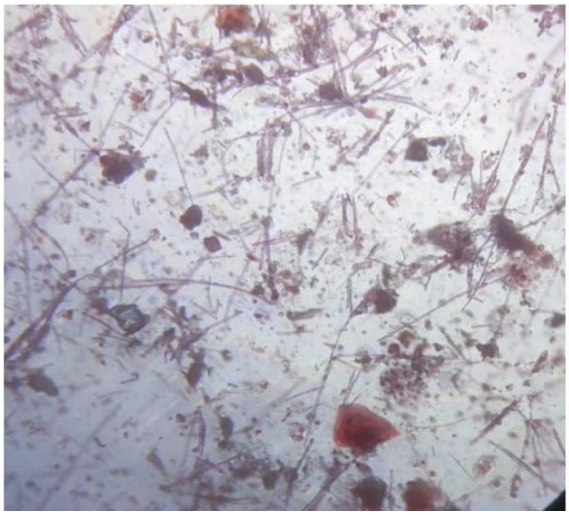
Unprocessed

Unstained stained

Processed

Unstained stained

Observed for presence of

1) **Aleurone grains**
2) **Oil globules**

3. Physicochemical analysis

Test name	Wild sample		Market sample	
	raw	Processed	Raw	Processed
Ash value	15.4%	15.2%	12.4%	14.4%
Ph	6.21	5.80	7.59	7.55
Loss On drying	1.993%	2.393%	3.103%	2.068%

4. Phytochemical screening of strychnus nux vomica seeds

Si. No.	Secondary metabolites	Test	Wild Unprocessed	Wild processed	Market unprocssed	Market processed
1	Alkaloids	Dragodroffs test	+	+	+	+
2	Steroids	Salkowski test	+	+	+	+
3	Carbhohydrates	Fehlings test	+	+	+	+
4	Protein	Biuret test	-	-	-	-
5	Tannic And phenolic	Ferric chloride test	-	-	-	-
6	Saponins	Foam test	-	-	-	-
7	Glycosides	Extract + h_2o_4	-	-	-	-

Presence of Alkaloids confirmed the strychnine and brucine contents, Steroids, Carbohydrates confirmed in all the 4 samples of Kupilubeeja.

5. HPLC ANALYSIS

1. Strychnine concentration decreased from 55.19% to 38.71% in the processed wild sample **(net decrease of 16.48%)**
2. Brucine content decreased from 17.33% to 14.87% in the processed wild sample **(net decrease of 2.46%)**
3. Strychnine concentration decreased from 30.84% to 23.42% in the processed market sample **(net decrease of 7.42 %)**
4. Brucine concentration decreased from 31.31% to 26.24% in the processed market sample **(net decrease of 5.07%)**

DISCUSSION

"Anena nidarshanena nanoushadhibhootam jagati kinchit dravyamasti". *Ayurveda* says each and every *dravya* in the universe can be a medicine depending on purpose and utility. Even a highly toxic *dravya can* also become a life saver and can combat disease if administered judiciously.[1] Inspite of their toxic effect or fatal effect, many visha *dravya* are still being used as medicine after proper *shodhana* in ample number of formulations for various diseases. Different *shodhana* procedures are mentioned in classics, in order to detoxify the toxic principles and to incorporate other desirable *gunas* to it. Apart from detoxification, *shodhana enhances* the potency and efficacy of the drug. The Dasha *gunas* attributed to *visha* may get reduced due to *shodhana* procedure, thereby allowing it to utilise internally without toxic effects.

Procedures like *swedana, mardana, prakshalana, dhalana, nirvapana, bharjana, bhavana , nimmajjana etc.* are used for purification of *vishadravyas*.

Kupilu (Strychnosnux -vomica Linn) is one among the *upavisha dravyas and* it's *beeja* is widely used after *shodhana* in various *Ayurvedic* formulations since long time. *Kupilu* is a potent *visha dravya with* seed as its *visha adhisthana* which has *tikta-katu rasa, laghu,ruksha, teekshnaguna, katuvipaka* and *ushnaveerya.[6]* It is having actions like *kaphanashanam, grahi, rochana, agnikrit, madakrit, medohara, krumihara, swasahara, gulmahara, arshohara, mushikavisha hara, kushtahara, pramehahara*[7] etc and used in many formulations like *Lakshmi vilas ras, Agnitundirasa, Vishamushtivati, Vishatindukataila, Krimimudgara rasa, Navajeevan rasa, Ekangaveera rasa*[3] etc. According to modern toxicology, seeds of *Strychnos nuxvomica* is a spinal poison containing active alkaloids like Strychnine and Brucine, which are responsible for its toxic effects.

But it is always advised to utilise these Vishwa dravyas in formulations after proper purificatory procedures as mentioned in the *Ayurvedic* texts. Different medias like *goghrita, gomutra, gomaya, goksheera, kanji* etc have been explained in *Ayurveda* for the *shodhana of kupilu.[8]* Toxicity reduction depends on the media selected for *shodhana* procedure also.

Among the different purificatory methods explained, *Shobhana of kupilubeeja by ghritabharjana was* selected for the current research work. It is the easiest and less time consuming method for purification of *kupilubeeja*, so that one can make use of less toxic *kupilubeeja* without any tedious process.

Ghrita is considered as an unparalleled *bheshaja* or *agada* in all types and stages of *visha*, **" *Sarveshu sarvaavasthasu visheshu na ghritopamam vidyate bheshajam*[9]"** especially in *Vatapradhanata*.Therefore *ghrita* was selected as the media for *shodhana* process (in the form of *Bharjana*) of *kupilubeeja*.

Wild species of *kupilu* is more potent in its *therapetauic* effects maximum therapeutic effects as it is collected according to classical *dravyasangrahana vidhi* with respect to *desha, kala* and safe precautionary measures. There is a chance of adulteration or substitution with substandard drugs or it may be contaminated in case of market samples. It's purity and genuinity are questionable as there are chances of variations/deviations during collection procedure in terms of varied geographical areas, improper time of collection, microbial as well as insect infestation etc before and after. Therefore samples from wild and market were selected for the present study to assess the effect of *shodhana (ghrita bharjana)* on both the samples before and after *ghritabharjana kriya.*

Physico chemical analysis and phytochemical screening were done for wild and market samples of *kupilubeeja* before and after *shodhana.*

As per this study, it was confirmed that shodhana by ghrita bharjana reduced the strychnine and brucine in both *shodhita* wild and market samples as determined by High performance liquid chromatography (HPLC). **Strychnine** decreased from **55.19%** to **38.71%in processed wild sample**with **net decrease of 16.48%**where as in **processed market sample** it decreased from **30.84%** to **23.42% with net decrease of 7.42%**. It might be due to the contact of the seeds with *Ghrita* which helped to diffuse out some quantity of the alkaloids from the seeds and also some amount of strychnine might have been converted into less toxic derivatives like isostrychnine, strychnine N-oxide etc.

After purification, the **Brucine** decreased from **17.33%** to **14.87 %** in **processed wild sample** (**net decrease of 2.46%**) and in **processed market sample** it was reduced from **31.31%** to **26.24% (net decrease of 5.07%)**. Probably the Brucine might also have been converted into less toxic derivatives like isobrucine, brucine N-oxide etc.

Strychnine concentration is more reduced when compared to Brucine concentration of both wild and market samples. Increased affinity of Strychnine towards cow's ghee might be one of the probable reasons for its more reduction than Brucine.

Gunas of ghrita are similar to *ojas and o*pposite to *visha*. *Ghrita* might have counteracted the *laghu, rooksha, teesksha guna* and *ushnaveerya* of *kupilubeeja* by virtue of its *madhura rasa, guru, snigdha guna, madhura vipaka and sheeta veerya*. This might be the probable reason for *Visha Shamana* by *ghrita bharjana* method of purification. Therefore as per this study it is confirmed that bharjana by goghrita reduces the Strychnine and Brucine concentration considerably and can be taken as the easiest procedure for the purification of *Kupilubeeja*.

CONCLUSION

The findings as per this study confirms with the references of *Ayurveda* that is *shodhana* of *kupilu* by *goghrita bharjana* reduces the toxic elements of the drug. Thus it can be concluded that *goghrita* is an effective media for purification of toxic drug like *kupilubeeja*. The method was found to be very simple and less time consuming.

REFERENCES

1. Agnivesha, Charaka Samhita, edited by Vaidya Yadavji Trikamji Acharya, Chaukhambha Surabharti Prakashan, Varanasi, 2008.
2. Shastri Ambikadatta (Suratnojivala Hindi Commentary), Rasaratnasammuchaya, Chaukhambha Amarbharati Prakashan, Varanasi, 1998; 8: 170.
3. Rasatarangini edited by Pandit Kashinath Shastri, Motilal Banarasidas, Delhi, 110007; 11.
4. Singhal, S.K., Toxicology At A Glance, The National Book Depot, Mumbai, Ed, 2009; 7: 114.
5. Dr. Shastry J.L.N., Dravyagunavijnana, Chaukhambha Orientalia, varanasi, Ed, 2008; 2: 353.
6. Prof. Lavekar G.S., Database on Medicinal Plants Used in Ayurveda and Siddha, CCRAS, New Delhi, Reprint, 2008; 5.
7. Sharma P V, Dravyaguna-Vigyana, Chaukhambha Bharati Academy, Varanasi, Reprint 2005; 85.
8. Ilanchezhian R et al. Importance of media in shodhana (Purification/Processing) of poisonous herbal drugs; Ancient science of life, 2010; 30(2): 27-30.
9. Vagbhatta, Ashtanga hrudayam with Arunadatta commentary Sarvangasundara, Varanasi Chaukambha Sanskrit Sansthan Varanasi, 2014.