Published online in http://ijam.co.in
ISSN: 0976-5921

Research article

Role of Castor oil in Processing (*Shodhana)* of *Kupeelu* (*Strychnos nuxvomica* Linn.) Seeds: An Approach of Traditional Ayurveda

Swarnendu Mitra[*]; V J Shukla[1]; Rabinarayan Acharya[2]

1. Head, Pharmaceutical Laboratory, 2. Associate Professor, Dept. of Dravyaguna,
Institute for Post Graduate Teaching and Research in Ayurveda,
Gujarat Ayurved University, Jamnagar, India

Abstract

Seeds of *Strychnos nuxvomica* Linn. (Loganiaceae), a poisonous plant drug, is being used in different Ayurvedic therapeutics after proper processing (*Shodhana)* with some specific media. As per the available references in Ayurvedic classics, media like cow's urine, cow's milk, cow's ghee etc. has been incorporated for processing of Nux-vomica seeds. Apart from the classical methods some other methods are also implemented by the traditional practitioners of Ayurveda using castor oil (*Eranda taila*), ginger juice (*Ardraka swarasa*) etc. for the same purpose. In present study an attempt has been made to process the seeds by executing a traditional method employing *Eranda taila* (castor oil) as the medium. This study revealed that the method studied reduces the toxic Strychnine & Brucine contents by 67.36% and 46.97% respectively in comparison to the raw Nux-vomica seeds as determined by HPTLC.

Key words: Kupeelu, nuxvomica, purification, processing, Strychnine, Brucine.

Introduction:

Kupeelu (*Strychnos nuxvomica* Linn), a well known plant in Indian system of medicine is being used extensively in different classical formulations with great therapeutic significance. Though the plant is described under the '*Upavisa Vargas'* (sub poisonous group) (1), it's seeds have been used successfully in different formulations to combat different diseases after proper *Samaskar* known as *Shodhana* (processing or purification) (2). The seeds are mainly used as aphrodisiac, appetizer, anti-periodic, digestive, purgative, and stimulant. Further the seeds are also used in anemia, asthma, bronchitis, intermittent & malarial fever and in weakness of extremities (3). Nux-vomica was introduced in Europe in the sixteenth century, but was not much used in medicine, being chiefly employed to poison dogs, cats, crows, etc (4). It is claimed in the ancient manuscripts of Ayurveda that the *'Visha'* becomes *'Amrita'* after logical use (5) and the physicians of *Ayurveda* successfully employed this drug in a number of diseases after proper purification in some specific media.

The concept of *Shodhana* (processing or purification) in Ayurveda is not only a process of purification/detoxification but also a process to enhance the potency and efficacy of the drug (6). It is reported that Aconite (*Vatsanabha*) purified by cow's

***Corresponding Author:**
Ph.D. Scholar, Dept. of Dravyaguna,
IPGT &RA,
Gujarat Ayurved University,
Jamnagar, Gujarat
E-mail: dr.swarnendu2008@yahoo.com
Ph.No: 9725876839

urine is converted to cardiac stimulant, whereas raw Aconite is cardiac depressant (7). Purified *Kupeelu* is also claimed to be a potent drug in countering old age problems and specially recommended during senility as *Rasayana* (antioxidant) (8).The plant is also found to have analgesic & anti-inflammatory (9), anti-oxidant (10), anti-tumor (11), anti-snake venom (12), anti-diarrhoeal (13) and hepatoprotective (14) activities when studied in animal models.

In a previous study, 16 alkaloids have been seperated and identified from the crude nux vomica and 80% of them are Strychnine and Brucine, as well as their derivatives such as isoStrychnine and Brucine N-oxide (15). Strychnine ($C_{21}H_{22}O_2N_2$; m.p. 286 to 288^0 C) and Brucine ($C_{23}H_{26}O_4N_2$; m.p. 178^0 C) have been reported as the most important and strongly toxic alkaloids present in this, besides other minor alkaloidal constituents (16). It is also reported that Nux-vomica in large doses, producing tetanic convulsions and eventually death and in lesser doses it may manifest mental derangement (17). So it is mandatory to purify or properly processed Nux-vomica seeds prior to its administration in therapeutics. There are also few reports of previous research works advocating a variety of methods of purification of Nux-vomica seeds as per Chinese (18), Unani (19) and Ayurveda (20) system of medicine. However, the methods of purification and analytical techniques were different from the present study. The purpose of this study was to evaluate the role of purification on the quantitative reduction of toxic alkaloids of *Kupeelu* seeds by HPTLC technique. Therefore the present study was planned to find out the effect of *Shodhana* (purification) with *Eranda taila* (castor oil) on the quantitative reduction of toxic Strychnine and Brucine contents in nux vomica seeds.

Materials and methods

Collection of drugs

Fully matured *Kupeelu* (*Strychnos nuxvomica* Linn.) fruits were collected from the field of Bankura district, West Bengal in India during the month of December and were botanically authenticated by pharmacognosists and sample specimen were kept in the museum for future reference. Seeds were taken out from the fruit pulp, thoroughly washed in tap water and shade dried.

Selection of seeds (14)

The dried seeds were first dropped in a beaker containing water. The seeds which floated on the surface of water or found broken, black in color were rejected and the seeds which were settled at the bottom of the beaker were selected for purification after drying in air and were considered as raw drug (KR).

Collection of media

Eranda taila (castor oil, Brand name: APPU, Manufactured by Shree Western G & C industries, Ahmedabad) was procured from the local market and used for processing the Nux-vomica seeds.

Equipments for *Shodhana* (Purification)

Frying pan (diameter-20cm), stainless steel spatula (length 30 cm), digital weighing machine and, induction heater.

Equipments for HPTLC

A CAMAG (Switzerland) HPTLC system equipped with a sample applicator Linomat V sample applicator was used for application of samples. CAMAG TLC Scanner 3, Reprostar and Wincats 4.02 were used for scanning the plates. CAMAG twin through glass chamber was used for developing the plates.

Chemicals

Pure Strychnine and Brucine were obtained from Sigma Aldrich, U.S.A and

precoated silica gel 60 F_{254} TLC aluminium plates (10×10 cms, 0.2mm thick), AR grade toluene, ethyl acetate, diethyl amine, methanol and chloroform were obtained from M/S Merck Ltd. Mumbai, India.

Method of purification of Kupeelu (*Strychnos nuxvomica* Linn.)

Purification method was carried out in three batches by frying with *Eranda taila* (castor oil) as per the traditional process (21) mentioned below:

100g. of seeds were fried with 20 ml. castor oil in mild temperature (temperature was set at 60^0C) on an induction heater until the seeds became swollen and reddish yellow in color. Seeds were then taken out from the heater, seed coats were removed as much as possible by rubbing them over the fingers and immediately made into powder form. The powdered materials were kept in an airtight glass container and marked as 'KET powder' for further use.

HPTLC method for estimation of Strychnine and Brucine

Preparation of standard Strychnine and Brucine solution

Strychnine standard (10 mg) and Brucine standard (10 mg) were accurately weighed and dissolved in methanol in two standard flasks and final volumes were adjusted to 10 ml with methanol. (1 µg/µl)

Calibration curve for Strychnine and Brucine

The standard solutions corresponding to 2µg to 6µg of standard Strychnine and Brucine were applied on TLC plates (10cm× 10cm), precoated with silica gel as 6 mm bands by using CAMAG Linomat IV sample applicator. The plate was developed in a solvent system of Toluene: Ethyl acetate: Diethyl amine (7: 2:1, v/v) in a CAMAG twin through chamber up to a distance of 7.5 cm at a temperature of 30 ± 2^0 C. The plates were air dried and scanned at a wavelength of 254 nm using CAMAG TLC scanner and CATS V 4.06 software. The peak area of Strychnine and Brucine were recorded for each concentration. The calibration curves of Strychnine and Brucine were obtained by plotting the graphs of peak areas vs. concentrations of Strychnine and Brucine.

Preparation of sample solutions for estimation of Strychnine and Brucine

The raw & the purified samples (2g. each) both were defatted individually with petroleum ether. Defatted samples were then mixed with 10% ammonia and finally extracted with 25 ml methanol for 1 hr. under reflux. The methanol extracts were filtered and concentrated to 5 ml and used as test solutions. 5µl of each test solution was spotted along with 2 to 6 µl standard solutions of Strychnine and Brucine. The plates were developed in mobile phase of Toluene: Ethyl acetate: Diethyl amine (7:2:1, v/v) and scanned at 254 nm for Strychnine and Brucine. Peak areas were noted and quantity of Strychnine and Brucine were calculated by comparing the areas of standard solutions from calibration curve.

Results and Discussion

It was observed in the organoleptic study that the greyish powder of the raw seeds turned into reddish brown in colour after frying in *Eranda* (castor) oil and pungent smell was coming out from the purified seeds. The organoleptic characters of raw and purified samples were tabulated in table no.1. While carrying out the preliminary phytochemical investigations the presence of alkaloids, tannins, carbohydrates, proteins, fixed oils were detected in methanolic extracts of raw and purified seeds (table no.2). The presence of Strychnine and Brucine was confirmed by comparing the Rf values with that of standard markers by HPTLC. Both the samples were evaluated for physicochemical parameters like loss on

drying, total ash, Ph value, water soluble extractive, alcohol soluble extractive etc. and the results were put into table no.3.

In HPTLC chromatogram, UV spectrum at 254 nm of standard Strychnine (R_f 0.54) and standard Brucine (R_f 0.34) were shown in Figure 1-2 and peak areas of Strychnine and Brucine in both the samples were exposed in Figure 3-4. Calibration curves of Strychnine and Brucine were prepared by plotting concentrations of Strychnine and Brucine in the range of 2-6 µg/spot versus average area of the peak. The responses for concentrations of standard Strychnine and Brucine were found to be linear (Figure-5 & Figure-6). The amount of Strychnine and Brucine in raw & purified samples were computed from the calibration curves which suggests the reduction of Strychnine and Brucine content by 67.36% and 46.97% respectively in the castor oil purified sample (table no.4). It might be due to the fact that some amount of Strychnine and Brucine might had been converted into less toxic derivatives like isostrychnine, isobrucine, Strychnine N-oxide, Brucine N-oxide etc. during the frying process in *Eranda taila*. Although further more study is required to explore the exact reason for decreasing the Strychnine and Brucine content.

Conclusion

From this study it may be concluded that castor oil (*Eranda taila*) is an effective media for purification of *Kupeelu* seeds as far as toxic alkaloids are concerned. The findings strongly confirm the claims of the traditional practitioners of Ayurveda that *Shodhana* (proper processing) of *Kupeelu* by *Eranda taila* successfully reduces the toxic elements of the drug. The method was found to be very simple, less time consuming and cost effective also.

Acknowledgement

The authors are very much thankful to Director of I.P.G.T & R.A, G.A.U, Jamnagar, India for providing all necessary facilities in carrying out this work.

References:

1. Sharma SN, Shastri KN. Rasa Tarangini. 11th edition. New Delhi; Motilal Banarasidas Publication; 2000, 163-164p.
2. Gogte VM. Ayurvedic Pharmacology & Therapeutic Uses of Medicinal Plants. 1st edition. Mumbai; Bharatiya Vidya Bhavan; 2000, 345-347p.
3. Sabnis Mukund. Chemistry & Pharmacology of Ayurvedic Medicinal Plants, 1st edition. Varanasi; Chaukhamba Amarabharati Prakashan; 2006, 455-456p.
4. Evans CWilliams. Pharmacognosy. 16th edition. United Kingdom; Elsevier Limited; 2009, 399p.
5. Sharma P.V. Charaka Samhita, Text with English translation. 9th edition,Vol.I. Varanasi; Chowkhamba Orientalia; 2005, 13p.
6. Shastri JLN. Dravyaguna Vijnana. 1stedition, Vol.I. Varanasi; Choukhamba Orientalia; 2009, 320p.
7. Singh LB, Singh RS, Bose R, Sen SP. Studies on the pharmacological action of Aconite in the form used in Indian Medicine. Bull Med Ethnobot Res. 1985;6:115-123.
8. Pandey G. Anti-Aging Herbal Drugs of India. 1stedition. Delhi; Sri Satguru Publication; 2002, 248p.
9. Yin W, Wang TS, Yin FZ. Analgesic and anti-inflammatory properties of Brucine and Brucine N-oxide extracted from seeds of *Strychnos nux-vomica.* J Ethnopharmacol 2003;88(2-3):205-214.
10. Tripathi YB, Chaurasia S. Effect of Strychnos nux vomica alcohol extract on lipid peroxidation in rat liver.

Pharmaceutical Biology 1996;34(4):295-299.
11. Deng XK, Yin W, Li WD, Yin FZ, Lu XY, Zhang XC, Hua ZC, Cai BC. The anti-tumor effects of alkaloids from the seeds of Strychnos nux-vomica on HepG2 cells and its possible mechanism. J Ethnopharmacol [Internet]. 2006 June [cited 2010 April 21];106(2):179-186. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16442763
12. Chatterjee I, Chakravarty AK, Gomes A. Antisnake venom activity of ethanolic seed extract of Strychnos nux vomica Linn. Indian J Exp Biol. 2004;42(5):468-475.
13. Shoba F. Gricilda, Thomas Molly. Study of antidiarrhoeal activity of four medicinal plants in castor-oil induced diarrhea. Journal of Ethnopharmacology [Internet]. 2001[cited 2010 July 2];76(1):73-76. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11378284
14. Gopalkrishna SV, Lakshmi narasu M, Ramachandra Setty S. Hepatoprotective activity of detoxified seeds of nux vomica against CCl_4 induced hepatic injury in albino rats. Pharmacologyonline 2010;1:803-815.
15. Cai BC, Hattori M, Namba T. Processing of nux vomica. II. Changes in alkaloid composition of the seeds of Strychnos nux-vomica on traditional drug-processing. Chem Pharm Bull [Internet].1990[cited2010June3];38(5): 1295-1298. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2393954
16. Anonymous. The Wealth of India, Raw Materials. Vol.X. New Delhi; National Institute of Science Communication, Council of Scientific & Industrial Research; 1998, 62-66p.
17. Anonymous. Database of Indian Medicinal Plants. Vol.V. New Delhi; Documentation and Publication Division CCRAS; 2005, 139p.
18. Cai BC, Hattori M, Namba T. Processing of nux vomica. II. Changes in alkaloid composition of the seeds of Strychnos nux-vomica on traditional drug-processing. Chem Pharm Bull [Internet].1990[cited2010June3];38(5): 1295-1298. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2393954
19. Akbar S, Khan SA, Akbar M, Iqbal M. Use of strychnos nuxvomica (Azraqi) seeds in Unani System Of Medicine: Role of detoxification. Afr J Tradit Complement Altern Med., 2010;7(4):286-290.
20. Mehta N, Prajapati PK, Chadhuary AK. Role of milk in Shodhana(detoxification) with special reference to Nux-vomica. Aryavaidyan. 2007;20(2):100-104.
21. Trivedi Krishnaprasad. Dhanwantari Banausadhi Visesanka. Vijaygara, Dhanwantari Karyalaya, 1963;2:270.

Published online in http://ijam.co.in
ISSN: 0976-5921
International Journal of Ayurvedic Medicine, 2011, 2(2), 62-71

Table No.1: Organoleptic characters of raw & purified *Kupelu* seeds powder

Parameters	**Organoleptic characters of raw *Kupelu* seeds powder (KR)**	**Organoleptic characters of *Kupelu* seeds powder purified by *Eranda taila* (KET) in three batches**		
		Batch 1	**Batch 2**	**Batch 3**
Texture	Smooth	Smooth	Smooth	Smooth
Colour	Greyish white	Reddish brown	Reddish brown	Reddish brown
Odour	Slightly acidic	Pungent	Pungent	Pungent
Taste	Intense bitter	Bitter	Bitter	Bitter

Table No.2: Qualitative tests for various functional groups

Functional group	**Test**	**Raw drug (KR)**	**Purified drug (KET)**
Carbohydrate	Molish's test	+ ve	+ ve
Protein	Precipitation test with 5% lead acetate , 5% $CuSO_4$ and 5%ammonium sulphate solution	+ ve (white colloidal ppt. obtained)	+ ve (white colloidal ppt. obtained)
Oil	Filter paper test	+ ve (Filter paper gets permanently stained with oils)	+ ve (Filter paper gets permanently stained with oils)
Tanin	5% $Fecl_3$ test	+ ve	+ ve
Steroid	Libermann-Buchard test	- ve	- ve
Alkaloid	Dragendorff's test	+ ve	+ ve
	Wagner's test	+ ve	+ ve
Flavonoids	Lead acetate test	- ve	- ve

Table No.3: Physicochemical parameters of raw and purified seeds

Parameters	Samples	
	Raw *Kupeelu*	Purified by *Eranda taila* (Castor oil)
Loss on drying	3.39 % w/w	4.03 % w/w
Ash value	1.11% w/w	1.07 % w/w
Water soluble extractive	37.83 % w/w	32.78 % w/w
Methanol soluble extractive	3.89 % w/w	7.72 % w/w
pH	5.75	4.51

Table No.4: **Results of estimation of Strychnine and Brucine in raw and purified samples of *Kupeelu* by HPTLC**

Samples	Amount of Strychnine found (% w/w)	Amount of Brucine found (% w/w)
Raw *Kupeelu* (KR)	1.44	0.66
Kupeelu purified by *Eranda taila* (KET)	0.47	0.35

Published online in http://ijam.co.in
ISSN: 0976-5921
International Journal of Ayurvedic Medicine, 2011, 2(2), 62-71

Figure 1: HPTLC profile of standard Strychnine

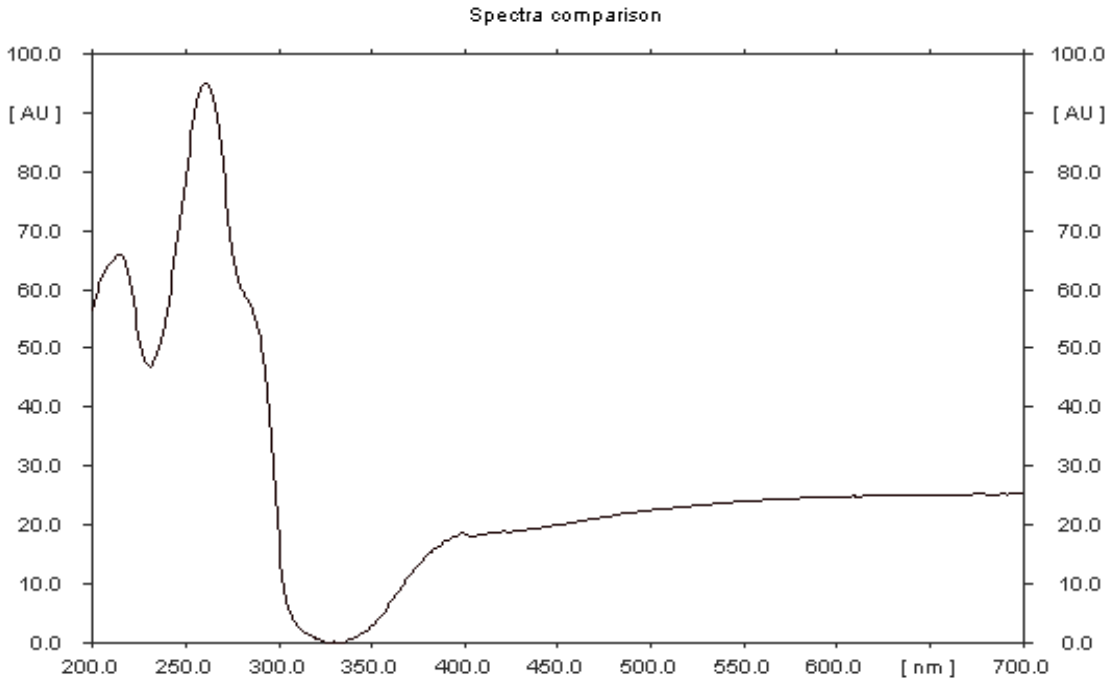


Figure 2: HPTLC profile of standard Brucine

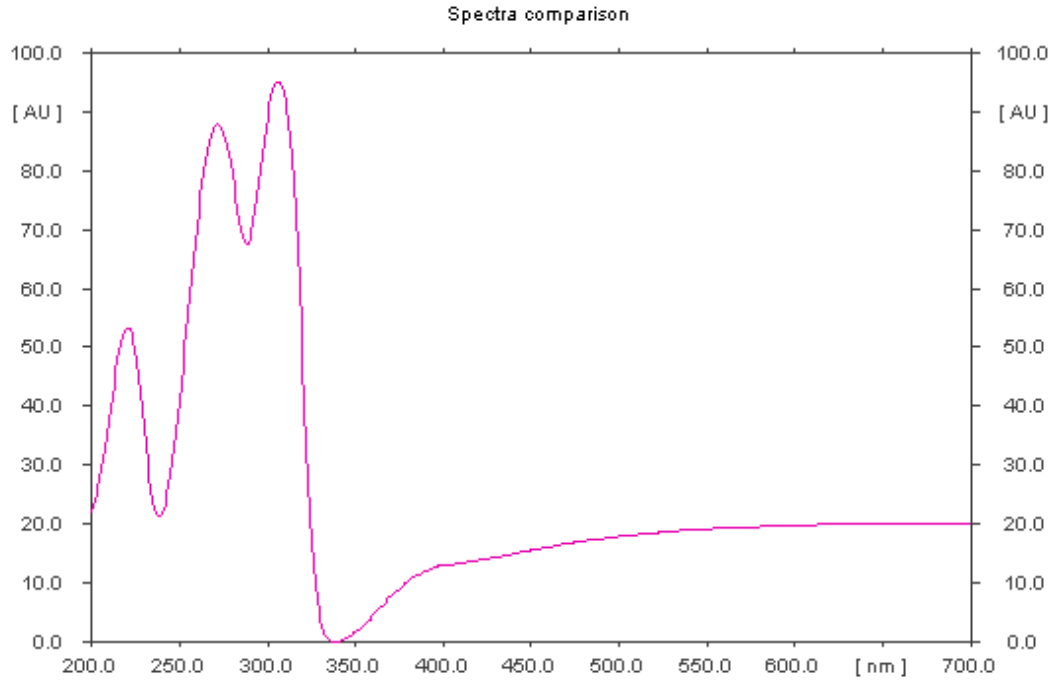


Figure 3: HPTLC of raw *Kupeelu* showing peak area of Strychnine and Brucine

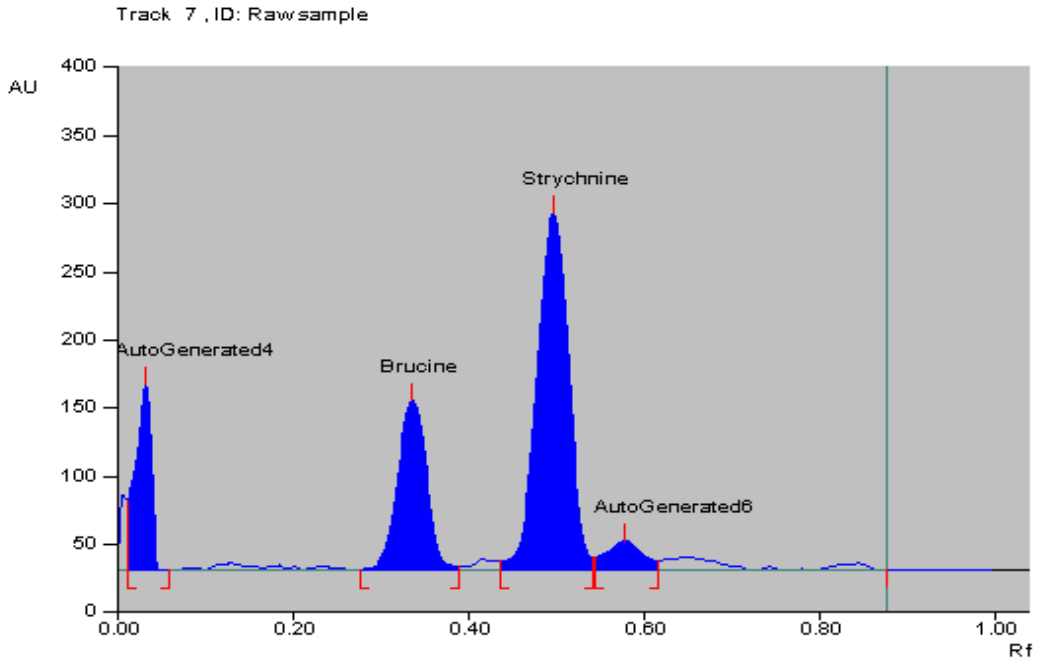
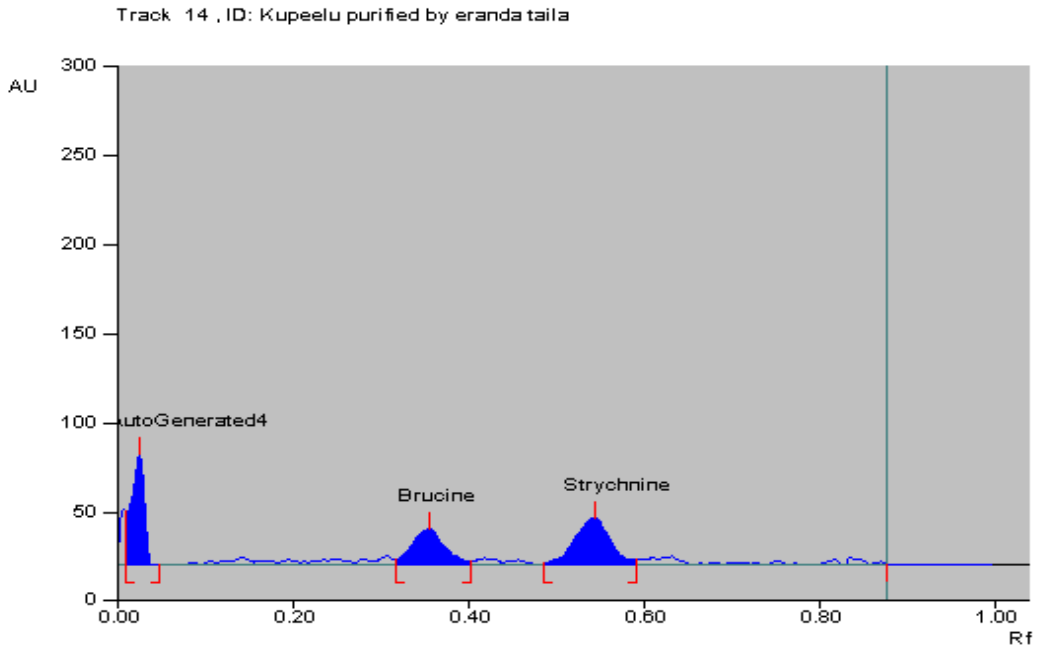


Figure 4: HPTLC of *Kupeelu* purified by *castor oil* showing peak area of Strychnine and Brucine



Published online in http://ijam.co.in
ISSN: 0976-5921
International Journal of Ayurvedic Medicine, 2011, 2(2), 62-71

Figure 5: Calibration curve of Strychnine

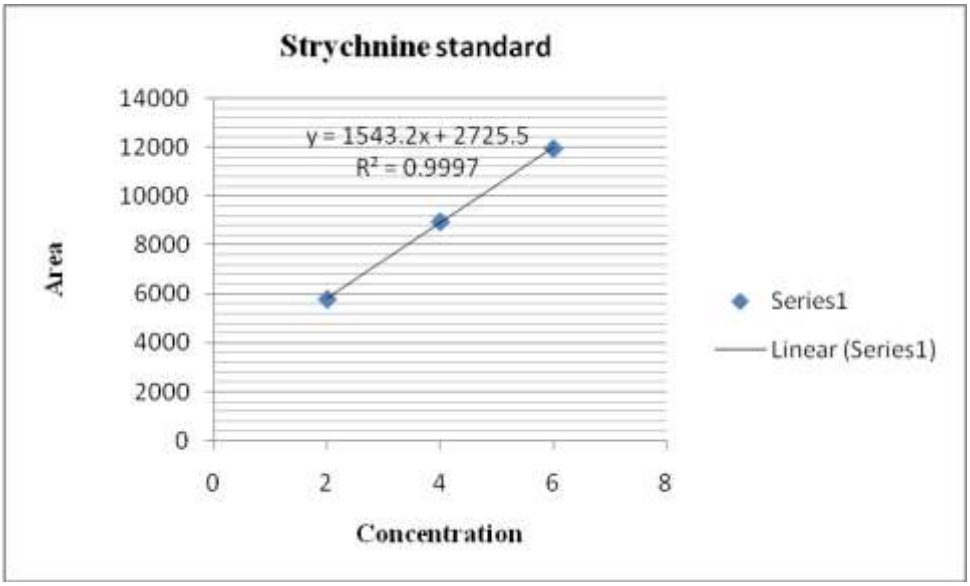
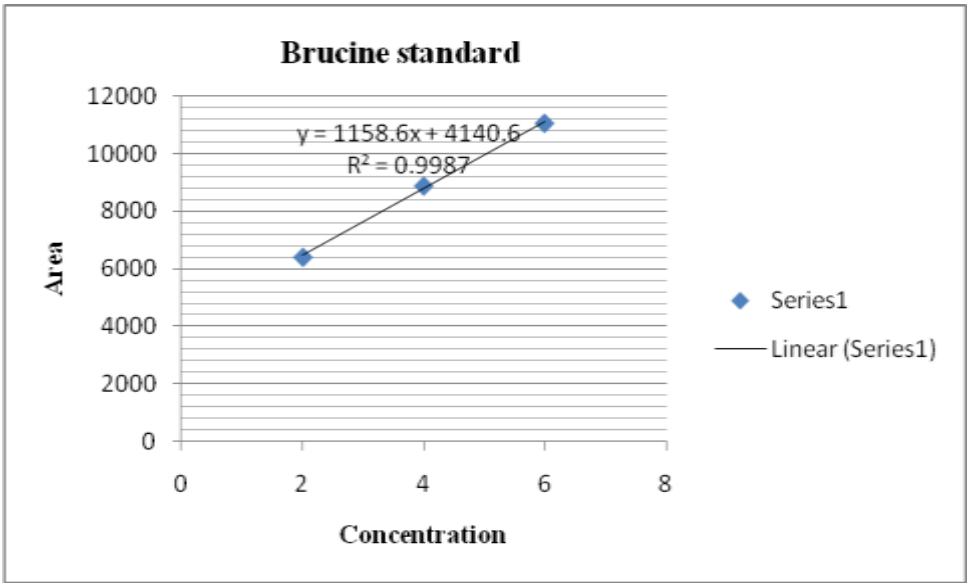


Figure 6: Calibration curve of Brucine



*****