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## DEVELOPMENT OF QUALITY STANDARDS AND PHYTOCHEMICAL ANALYSIS FOR *STRYCHINUS NUXVOMICA* LINN

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### Keywords:

*Strychnus nuxvomica*,  
photochemical screening,  
HPTLC, herbal medicine,  
standardization

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### ABSTRACT

*Strychnus nuxvomica* (SN) is an evergreen and medium sized deciduous tree, usually 15-20 m high and up to 70 cm diameter. The fruits are globose, 2.5-5cm. in diameter, orange red when ripe containing many discoid, compressed coins like seed in fleshy pulp. Seeds are used for therapeutic purposes. The objective of the present investigation was development of the quality standards and the phytochemical analysis of SN. This includes morphological and histological characters. pH of aqueous solution, ash values, extractive values, loss on drying, fluorescence analysis, primary phytochemical screening and HPTLC finger printing profile of different extracts. The finding of this study might be useful as supplement information regarding the identification of SN and laying down pharmacopoeial standards as standardization of the herbal medicine is essential and need of today.

## INTRODUCTION

*Strychno* Linn. is a large genus of scandent shrubs or trees found throughout the tropics and sub tropics. Nearly 20 species crop up in India, of which *Strychnus nuxvomica* is renowned for the drug value of its poisonous alkaloid, strychnine and brucine<sup>1</sup>. Seeds are mostly used for therapeutic purposes. Seeds are discoid, concave on one side and convex on the other side being 20 -25 mm in diameter and 4 mm in thickness; ash grey or greenish grey in colour, covered with fine and silky closely adpressed hair, radiating from centre to the circumference, due to which the seed have satiny sheen. Seeds are very hard when dried and are odour less<sup>2</sup>. Seed is a power full poison in large doses, producing titanic convulsions and eventually cause death and in low doses, it may result in mental derangement. In the indigenous system of medicine, it is used as a tonic, stimulant and febrifuge and it is useful in nervous and joint disorders. Nuxvomica seeds have been reported to have different constituents including strychnine, brucine, vomice, alpha-colubrine, beta-colubrine, pseudostrychnine, glycoside loganin, steroids and tannins<sup>1</sup>. Lack of proper production, supply system and increasing demand are the major factors promoting the practices of adulteration and substitution. Therefore, standardization of herbal drugs is essential for assuring the therapeutic efficacy of the drugs. The present investigation was carried out to develop quality standard and phytochemical analysis of nuxvomica seed.

## MATERIALS AND METHODS

### Collection and authentication of the plant material

The seeds of nuxvomica were purchased from Khari Baoli, local market of Delhi and authenticated by Dr. H B Singh, NISCAIRE, New Delhi- 110012 (Ref. No. of the voucher is NISCAIR/RHMP/F-3/Conlt/06/720/37).

### Microscopic and macroscopic study

Macroscopical and microscopical characters of the drug were studied according to WHO and Indian pharmacopoeia guidelines<sup>3, 4</sup>.

### Physiochemical studies

Different physiochemical values such as extractive value (cold and hot extract), ash values (total ash, acid insoluble ash, and water-soluble ash), loss on drying and pH of 1 % and 10% solution of nuxvomica seed were determined according to the standard methods<sup>3</sup>. The presence of heavy metal was determined to ensure the safety of the drug on in human beings.

### Preliminary phytochemical analysis

The preliminary phytochemical screening was carried out using the extracts of different types of chemical constituents as per method described by Trease and Evans<sup>5</sup>. The extracts were subjected to preliminary photochemical investigation for detection of alkaloids, carbohydrates, glycosides, proteins and amino acids, lipids/fats, phenolic and flavonoid compound.

### Fluorescence analysis

Chemical tests of powder drug with different reagents were performed to observe the colour reactions according to the reported method<sup>6</sup>.

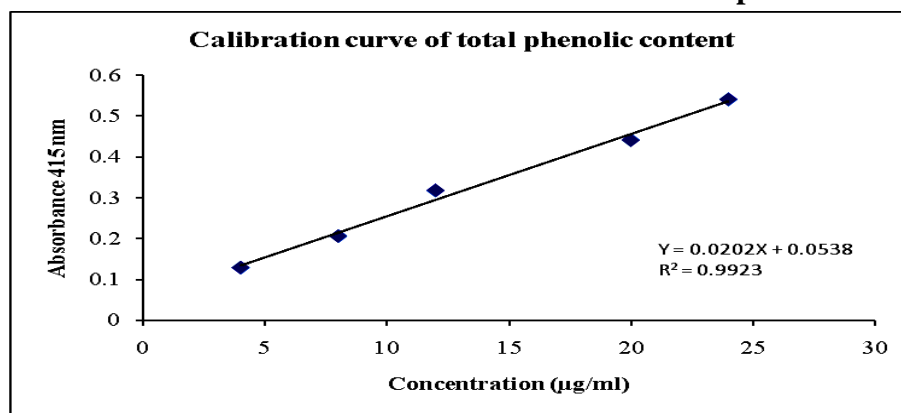
### Determination of total phenolic contents

The phenol was determined in powdered crude drugs, extracts and beverages by Folin Ciocalteu method. Standard stock solution was prepared by dissolving 25 mg of catechin standard in 100 ml distilled water. Different concentrations of the standard solutions were prepared for standard calibration curve starting from 4 to 24 µg/ml in water. The commercial Folin Ciocalteu reagent was diluted (1: 10) with distilled water on the day of use. 1M sodium acetate was prepared by dissolving 82 g of sodium acetate in 1000 ml distilled water.

Sample preparation - 500 mg of the samples were taken in 50 ml volumetric flasks and added around 25 ml of distilled water and solicited for 10 minutes then made up the volume with water.

Procedure - Take 3ml of each standard and sample solution in a 10 ml test tube and to this add 3 ml of FC reagent and 3 ml of sodium carbonate solution. A blank solution was prepared by adding 3 ml each of distilled water, sodium carbonate solution, and FC reagent in test tube. Keep the solution in dark for 30 minutes for colour development. Absorbance was taken at 415 nm against blank solution. After taking absorbance of standard dilutions, calibration curve was plotted (Fig. 1). Phenolic contents in drug were calculated by using standard calibration curve.

**Figure 1: Calibration curve of standard catechin for total phenolic contents**



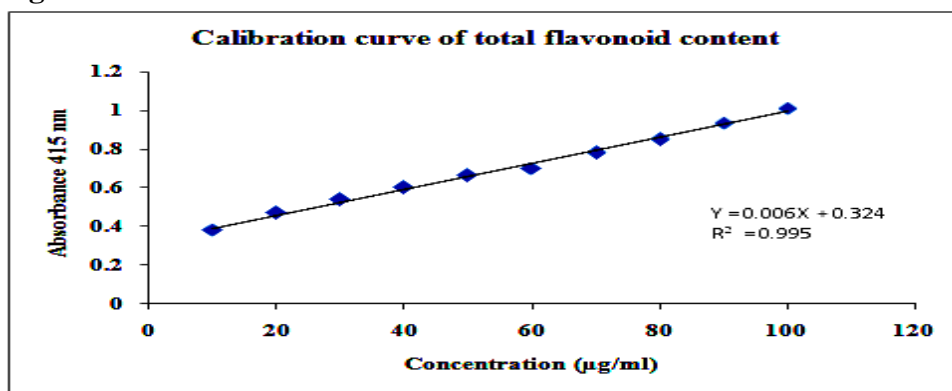
### Determination of total flavonoid contents

The flavonoid content was determined in powdered crude drugs according to method described by Pourmorad et al<sup>7</sup>.  $\text{AlCl}_3$  (0.1 g/ml) and  $\text{CH}_3\text{COONa}$  (1M) were prepared, Prepared dilutions for Rutin (standard) from 10  $\mu\text{g}$ /ml to 100  $\mu\text{g}$ /ml.

Samples Preparation - 500 mg of the samples were taken in 50 ml volumetric flasks and added around 25 ml of methanol water and solicited for 30 minutes then made volume with methanol.

Procedure - 0.5ml of each standard and sample solution was taken in a 10 ml test tube and added 1.5 ml methanol. To this added 0.1ml of  $\text{AlCl}_3$  and 0.1 ml of  $\text{CH}_3\text{COONa}$  reagents and added 2.8 ml Distilled water and kept for 30 minutes. A blank solution was prepared by adding 2 ml of methanol + 0.1ml of  $\text{AlCl}_3$  + 0.1ml of  $\text{CH}_3\text{COONa}$  reagents and then added 2.8 ml Distilled water. Absorbance was taken at 415 nm against blank solution. After taking the absorbance of standard dilutions, calibration curve was plotted (Fig. 2). Flavanoid contents in drug were calculated by using standard calibration curve.

**Figure 2: Calibration curve of standard rutin for total flavonoid content**



### Development of the HPTLC finger print profile of different extracts

The plant material was coarsely powdered and extracted in Soxhlet apparatus for 6-24 h using solvent petroleum, chloroform and methanol extracts. The extracts were evaporated to dryness in the rota – vapour and the solvents were recovered. Gummy residues so obtained, were stored in the deep freezer at  $-20$  degree until further application. The samples were prepared by dissolving each extract in their respective solvents to get the concentration of 10 mg/ml. These solutions were further passed through syringe filter to remove any impurity and applied on TLC plate for finger printing analysis. The extract was applied on HPTLC aluminium sheets silica gel 60 F 254 (Merck) 10 micro l each with the band length 6 mm using Linomat 5 sample applicator set a speed of 100nl/sec CAMAG, Switzerland.

Different solvent systems were used for the separation of the constituents of the different extracts. The chromatogram was developed in the twin chamber for 20 min up to the distance of 80mm and the spots were visible at 254 and 366 wavelengths.

## RESULTS AND DISCUSSION

The following results have been obtained under different headings

### Macroscopic characters

Seeds are ashy grey or greenish grey in colour, disc shaped about 17.0 to 29.5 mm across and 2.5-7.0 mm thick; usually uneven inside, a little depressed on one side and arched on the other side. At the one end of margin where the microphyle is situated there is distinct prominence from which raised line passes the centre of the seed. The hilum is in the centre of either on raised or the depressed surface. Beneath the seed coat, there is a lining of horny endosperm about 2.0 mm thick. The endosperm is perforated above the microphyle by a cylindrical channel leading into a disc shaped hallow and enclosing a terete radicle of the embryo. Attached to the radical and lying within the hallow are two cordate leafy cotyledons having a distinct palmate venation with 5-7 veins. The endosperm is translucent grey in colour and the embryo is whitish.

### Microscopic

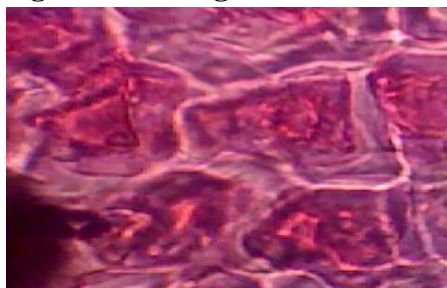
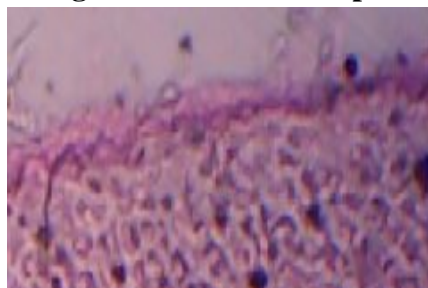
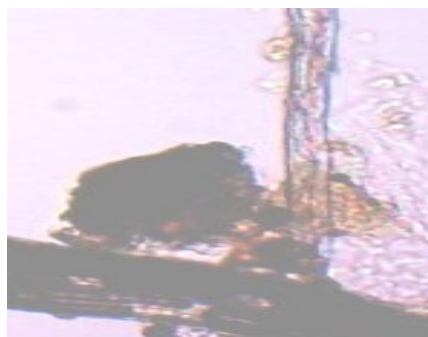
The testa is about 0.1mm thick, the greater part being formed by the epidermis. The testa is an outer trichomatous epidermis and inner layer of ground tissue. Each epidermal cell is extended to form an appressed trichomes. The walls of these cells are highly thickened and lignified, the anticlinal walls being undulated and pierced by tubular pits, which are sometimes more or less twisted. The limb of the trichomes has about 10 longitudinal ribs of lignified thickening upon its wall. The inner region of the testa consists of collapsed cells about 25 mm in thickness. In transverse section, these cells appear as ill-defined polygonal cells. The endosperm is composed of thick walled cellulosic parenchyma and the cells are isodiametric. Fixed aleuronic grains are seen in the protoplasm. See figure 3-9.

**Figure 3: T.S of seed of nuxvomica**



**Figure 4: T.S of endosperm cells**



**Figure 5: Enlarged view of endosperm cells****Figure 6: L.S. of endosperm****Figure 7: Macerates****Figure 8: Attached trichomes with sclerenchymatus tissue****Powder study of nuxvomica seed**

The powder is a yellowish grey to brownish grey with rancid odour and bitter taste. The powder analysis of the crude drug shows the sclerenchymatous epidermis of the testa, very abundant fragments of lignified rods of the trichomes and abundant fragments of endosperm and large number of aleuronic grains.

**Physicochemical studies**

Different physicochemical parameters such as extractive values (cold and hot extracts), ash values (total ash, acid insoluble ash and water soluble ash), loss on the drying, pH of 1% and 10% solution and the heavy metals analysis of the nux-vomica seed were determined. All the heavy metals (As, Hg, Pd and Cd) were found within the permissible limits as described by the WHO. The results are presented in table 1.

**Table 1: Results of physicochemical analysis**

S. No.	Parameters	Values
1	<b>Extractive values (cold extract)</b>	<b>% Extractable matter</b>
a	Petroleum ether extract	1.6 w/w
b	Chloroform extract	2.0 w/w
c	Methanol extract	7.2 w/w
d	Aqueous extract	26.0 w/w
2	<b>Extractive values (hot extract)</b>	<b>% Extractable matter</b>

a	Petroleum ether extract	8.1 w/w
b	Chloroform extract	2.3 w/w
c	Methanol extract	6.3 w/w
d	Aqueous extract	18.3 w/w
3	<b>Extractive values (successive extract)</b>	<b>% Extractable matter</b>
a	Petroleum ether extract	8.1 w/w
b	Chloroform extract	1.2 w/w
c	Methanol extract	5.3 w/w
d	Aqueous extract	9.2 w/w
4	<b>Ash value</b>	
a	Total ash	1.20 % w/w
b	Acid insoluble ash	1.00 % w/w
c	Water soluble ash	0.29 % w/w
5	<b>Loss on drying of crude drug</b>	7.8 % w/w
6	<b>pH of drug 1%</b>	5.64
7	<b>pH of drug 10 %</b>	5.91
8	<b>Heavy metal analysis</b>	<b>Value in ppm</b>
a	Arsenic (As)	less than 0.1ppm
b	Mercury (Hg)	less than 0.1ppm
c	Lead (Pd)	less than 1.0 ppm
d	Cadmium (Cd)	less than 0.2 ppm

### Preliminary phytochemical analysis

The preliminary phytochemical screening was carried out using the extract for the presence of different types of chemical constituents such as alkaloids, carbohydrates, glycosides, phenolic compounds, flavonoids, proteins and amino acids and fat/lipids. The presence and absence of the different phyto-constituents are presented in table 2.

**Table 2: Results of preliminary phytochemical analysis**

Constituents	Test	Extract			
		Petroleum ether	Chloroform	Methanol	Aqueous
Alkaloids	Hager's reagent	-	+	+	-
	Mayer's reagent	+	+	+	-
	Wagner's reagent	+	+	+	-
Carbohydrates	Fehling's reagent	-	+	+	+
Phenolic compounds	Ferric chloride test	-	+	+	+
	Lead acetate test	-	+	+	-
Flavonoids	Ammonia test	-	+	+	+
	Pew test	+	+	-	-
Proteins and amino- acids	Million's test	-	-	+	+
Glycosides	Borntragger's test	-	-	+	-



### Fluorescence analysis

Chemical tests of the powdered crude drug with different reagents (distilled water, chloroform, NaOH, HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>, I<sub>2</sub>, HCl, toluene, alcohol ammonia, glacial acetic acid and ethyl acetate) were studied in the day light, UV light (254 and 366 nm) and results are presented in table 3.

**Table 3: Effect of different chemical reagents with crude drug powder on the fluorescence behaviour**

S. No.	Treatment	Day light	254nm	366 nm
1.	Powder as such	Chocolate brown	Dark brown	Dark brown
2.	Distilled water	Light brown	Dark green	Dark green
3.	Chloroform	Colourless	White	Milky white
4.	HNO <sub>3</sub>	Green	Black	Greenish yellow
5.	H <sub>2</sub> SO <sub>4</sub>	Black	Black	Dark purple
6.	Iodine	Orange	Black	Brown
7.	Conc. HCL	Dark brown	Black	Dark purple
8.	Toluene	Light brow	Milky white	Transparent
9.	Alcohol	Turbid	Milky white	White
10.	Glacial acetic acid	Light brown	Milky white	White
11.	Ethyl acetate	Turbid	White	Yellow

### Total flavonoid content

The results of total flavonoids content of nuxvomica seed determined by Aluminium chloride colorimetric method found to be 0.09 % w/w.

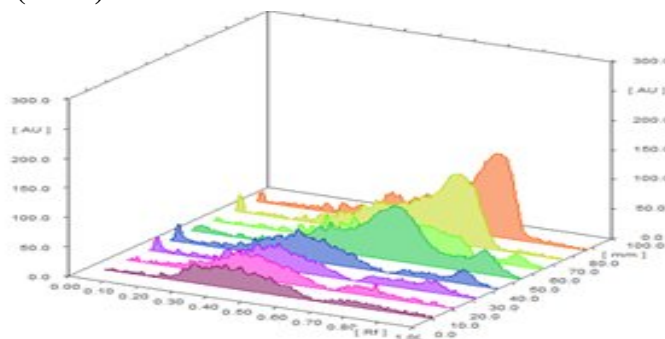
### Total phenolic content

The amount of phenolic content determined by Folin Ciocalteu method using the standard calibration curve was found to be 0.38 % w/w.

### HPTLC chromatographic finger print profile of different extract of nuxvomica

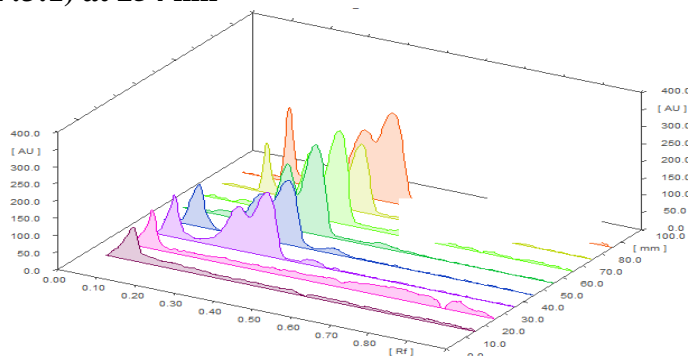
HPTLC analysis was performed for different extracts by using different solvent systems and the chromatograph obtained were scanned different wavelengths at 254 and 366nm. The results were summarized in table 4. Moreover, 3D views of different extracts of nuxvomica given in Fig. 9-12.

**Figure 9: 3 D view of different extracts of nuxvomica with solvent system Benzene: Ethyl acetate: Formic acid (8:1:1) at 366 nm**

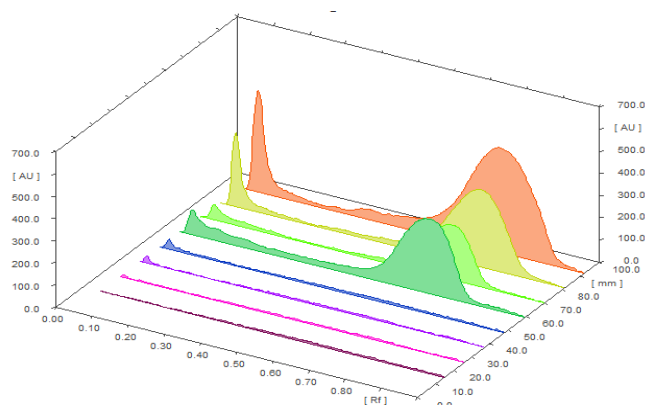


**Table 4: HPTLC fingerprint of various extracts**

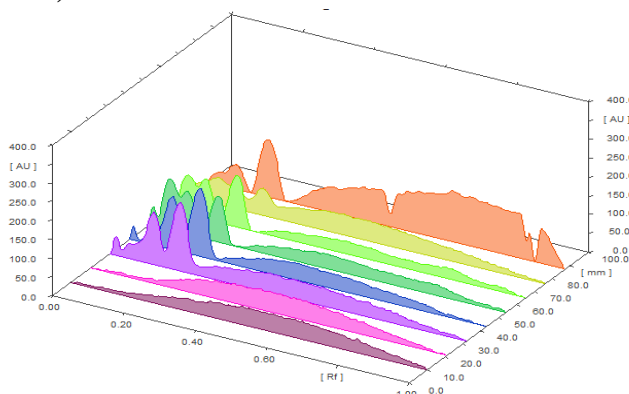
Extract	Solvent system & Rf Value							
	Toluene: Ethyl acetate: Formic acid (5:4:1)		Benzene: Ethyl acetate : Formic acid (8:1:1)		Chloroform : Methanol (7:3)		Methanol: chloroform: water (7:3:1).	
	254nm	366nm	254nm	366nm	254nm	366nm	254nm	366nm
<b>Petroleum ether</b>	0.23		0.37	0.06	0.49		0.12	0.12
	0.33		0.72	0.37	0.97		0.83	0.86
	0.42		0.77	0.42			0.91	
	0.81		0.88	0.51				
				0.73				
<b>Chloroform</b>				0.77				
	0.02	0.02	0.07	0.07	0.64	0.07	0.14	4.1
	0.12	0.06	0.35	0.28	0.07		0.30	
	0.20	0.81	0.42	0.36			0.37	
	0.34		0.75	0.42			0.48	
<b>Methanol</b>	0.73		0.90	0.70				
				0.86				
	0.06	0.01	0.09	0.37	0.08	0.08	0.15	0.15
	0.11	0.11	0.30	0.42	0.32	0.15	0.20	0.33
	0.19	0.81	0.65	0.46	0.42	0.23	0.32	
<b>Water</b>	0.33		0.90	0.53	0.70	0.74	0.40	
	0.78			0.65		0.93	0.57	
	0.92			0.76				
				0.92				
	0.08	0.07	0.42	0.30	0.10	0.09	0.12	0.13
<b>Water</b>	0.17	0.31	0.46	0.44	0.18	0.26	0.17	0.17
	0.35	0.43	0.74	0.51	0.43	0.34	0.36	0.94
	0.44	0.69		0.54	0.46	0.50	0.44	
	0.48	0.93		0.75	0.73	0.77	0.80	
	0.59						0.94	
	0.69							
	0.86							

**Figure 10: 3 D view of different extracts of nuxvomica with solvent system Methanol: Chloroform: Water (7:3:1) at 254 nm**

**Figure 11: 3D View of different extracts of nuxvomica with solvent system Chloroform: Methanol (7:3) at 366 nm**



**Figure 12: 3D View of different extract of nuxvomica with solvent system Toluene: Ethyl acetate: Formic acid (5:4:1) at 254 nm**



The study on the development of the quality standards and the phytochemical analysis of nuxvomica seed revealed a set of parameters, which may enable those who handle this plant to maintain its quality control. Adulteration and substitution has become a major problem due to absence of standard relating to genuineness of drug. The quality of the natural medicines must be as high as that of other synthetic medicinal plants. Quality refers to intrinsic value of the drug, the amount of medicinal principles or active constituents present. The pharmacognostical parameters include HPTLC are help full for further identification and authentication of this plant in the herbal industry. The physical parameters, such as loss on drying, ash values, and the extractive values will be help full to identify the authenticity of the drug even from the crushed or powdered plant materiel. It will serve as standard data for the quality control of the preparations containing this plant in future. The information obtained from the ash value and the extractive values are useful during the time of collection and the extractive process. Using these

standards the plant can be differentiated from the related species. The plant may be considered as the biosynthetic laboratory for the variety of compounds (secondary metabolites) like alkaloids, glycosides, flavonoids, volatile oils and saponins that exert physiological effect. The curative properties of the medicinal plants are due to the presence of various secondary metabolites. Thus, the preliminary screening tests may be useful in the detection of bioactive principles. HPTLC results indicate the number of constituents and further facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds.

## CONCLUSION

Standardization of herbal drugs is a topic of great concern. They are subject to variability as derived from heterogeneous sources. The variability can have both merits and demerits. The main demerits are that the activity of material may vary and inferior material may be produced. *Nuxvomica* is an important plant and has found to have various biological properties, so efforts have been made to provide scientific data to standardize the plant material for further studies. Microscopic and macroscopic data and other physical values including HPTLC will be help full to identify the correct species of the plant. The research out comings of the standardization parameters can also be used for the evaluating the quality and purity of the drug and its formation. However, more phytochemical research work is required for isolation, purification and characterization of biologically active compounds.

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