Original article

Physico-chemical and Phyto-chemical Standardization of a Potent Unani Cardiovascular drug *Saad Kufi* (*Cyperus scariosus* R. Br)

Sana Nafees[1], Huda Nafees[2], Sumbul Rehman[3], Syed Ziaur Rahman[4], Kunwar Mohammad Yousuf Amin[5]*

Abstract

Background: Standardization of a single potent Unani drug *Saad Kufi* is a must for maintaining and assessing the quality and safety as to attain the desired therapeutic effect. This will help prepare the ideal monograph which will serve in establishing its authenticity, quality, safety and reproducibility. ***Aim:*** The study aims to prepare the ideal monograph of the *Saad Kufi* (*Cyperus scariosus* R.Br)which is an important cardiovascular drug. ***Methodology*:** Physicochemical and preliminary phytochemical analyses were carried out according to the guidelines given by WHO. ***Result*:** *Saad Kufi* powder was coarse, brown incolor, Agreeable smell, and Tasteless. Total ash, acid insoluble ash, water-soluble ash, loss in weight on drying, pH (1% and 10% solution) were found to be16.3±0.07, 8.616±0.20, 3.1±0.30, 8.5±0.173, 5.76±0.08 and 5.23±0.08 respectively. The crude fibre content was 46.43±0.19 The Phytochemical screening revealed the presence ofalkaloids, carbohydrates, proteins, phenols, sterols, glycosides, Cardiac glycosides, flavonoids, sterols/ terpenes and volatile oil. TLC studies of various extracts of drugs obtained in different solvent system have been conducted and Rf values of various spots in different solvent systems have been noted in day light, UV light and after treatment with iodine vapours. The Rf values in the given solvent are used to characterize the drugs identity and purity. ***Conclusion*:** The above findings could be considered for laying down pharmacopoeial standards. No data exhibits in this regard to compare with, thus our findings may be considered as standard for future references.

Keywords: *SaadKufi;* Physicochemical; Phytochemical; Standardization; Monographs.

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Introduction: Unani System of Medicine (USM) is one of the oldest Traditional system of Medicine (TSM), based on the drugs originated from plants, animals and minerals. The efficacy and safety of these traditional drugs are closely related with their quality assurance. With the tremendous increase of medicinal plants around the globe, several concerns regarding the efficacy and safety of the herbal medicines have also been raised. From cultivation to production and storage of herbal medicine, chances of deterioration of drugs in term of quality is very common, ultimately decline of the efficacy. To overcome

1. Sana Nafees, Department of Ilmul Advia, Aligarh Muslim University, Aligarh, India. Email: dr.sananafees@gmail.com
2. Huda Nafees, Assistant Professor, Department of Saidla, Aligarh Muslim University, Aligarh, India. Email: dr.hudanafees@gmail.com
3. Sumbul Rehman , Assistant Professor, Department of Ilmul Advia, Aligarh Muslim University, Aligarh, India. Email: dr.sumbulrehman@gmail.com
4. Syed Ziaur Rahman, Professor, Department of Pharmacology, Jawaharlal Medical College & Hospital, Faculty of Medicine, Aligarh Muslim University, Aligarh, India.Email: rahmansz@yahoo.com
5. Kunwar Mohammad Yousuf Amin, Professor, Department of Ilmul Advia, Aligarh Muslim University, Aligarh, India. Email: kmya55@yahoo.com

Correspondence: Dr. Sana Nafees, Department of Ilmul Advia, Aligarh Muslim University, Aligarh -202001, India. Email: dr.sananafees@gmail.com

these problems standardization of Unani drugs in each tier is indispensable. For better therapeutic use, availability of genuine and authentic drugs is very important. Since efficacy of drugs mainly depends upon its physical and chemical properties, therefore determination of physicochemical parameters to ascertain the authenticity of drugs is necessary before subjecting it for pharmacological screening. Unani drug namely Saad Kufi *(Cyperus scariosus* R. BR*)* have high therapeutic value but there is less information available about its physicochemical properties to ascertain its identity, quality to check adulteration.

So, the present study was done to create a standardized physico-chemical and phyto-chemical profile for Saad Kufi (*Cyperus scariosus* R. BR.) on the basis of pharmacopeial guidelines for standardization of herbal drugs.

Saad Kufi *(Cyperus scariosus* R. BR.) belongs to the family Cyperacae. It is a hardy grass like perennial plant consisting of 600 species distributed in tropical and warm temperate region of the world. Cyperus is a greek word meaning sedge[1]. Medicinally the root of Cyperus scariosus R. Bris used for the same purpose as those of *Cyperus rotundus*[2,3] and this have long been in use in Hindu medicine and perfumery under the Sanskrit name Nagar mustaka[3]. Ibn Sina described Saad as a root of a plant which is nodular, long, slender and plant looks like a wheat plant[4,5,6]. Roots are thick, elongated, slender, and black in colour, aromatic smell with pungent taste[5,6]. Stem is of about one hand long and prostrate surrounded by small leaves and nodes[5]. Leafless or leafy shoots are produced above ground. Infloresence is umbel or head like. Spikelets are one to many flowered[1] and are linear straw coloured[7]. The Unani traditional actions (*Af'al*) or therapeuticuses (*Iste'malateIlaji*) ascribed to it are Exhilarant[6], Cardiotonic[8], anti-hypertensive[9], Nervine Tonic [8, 10, 11, 12] Deobstruent[4, 5, 6] Astringent, Dessicant[13] Carminative [5, 8, 11] Appetizer [12, 14], Anti-emetic [5, 11], Aphrodisiac [6, 14]. It strengthens the urinary bladder [5, 6]. It is use in the Palpitation [6, 10, 14], General weakness [8], Chronic ulcer, Ascites [5].

Material and method:

Collection and Identification: Roots of Saad Kufi (*Cyperus scariosus* R. Br) was procured from the local market of Aligarh (Baradwari) and identified in the Pharmacognosy Section, Department of Ilmul Advia, A. K. Tibbiya College, Aligarh Muslim University. They were also authenticated by National Institute of Science Communication and Information Resources, New Delhi (NISCAIR /RHMD/ Consult/ 2017/3064-13-1). The sample of the test drug was submitted to Mawalid-e-Salasa Museum of the Department after identification, for future reference with the voucher No. SC-0220/17. It was dried at optimum temperature and further crushed and sieved to coarse powder mechanically and stored in air tight container for study (Figure-1a &b).

Figure 1(a) : Plant Image of *Saad Kufi*

Figure 1 (b): Market Sample of *Saad Kufi*

Organoleptic Parameters: Organoleptic characters of test sample such as appearance, colour, smell and taste were observed.

Physico-Chemical Analysis: Physico-chemical analysis include the determination of ash value, moisture content, pH value at 1% and 10% solution, solubility, bulk density, crude fibre content, solubility, extractive values in different organic solvent. These were carried out as per the guidelines of WHO and Govt. of India [15-18].

Phytochemical Analysis Qualitative phytochemical analysis of the chemical constituents present in the drug sample has been done [19, 20]. Fluorescence Analysis of the powdered drugs along with the successive extracts have, alkaloid estimation [21].

Thin Layer Chromatography Thin Layer Chromatography of petroleum ether extract of drug was carried out on aluminium plates precoated with Silica gel-G (Layer thickness 0.20-0.25 mm) for all extract in various phases later sprayed by different spraying reagents. The Rf value of spots was calculated by the following formulae [22].

Rf Value = Distance travelled by the spot / Distance travelled by the solvent

Observations and Results

Organoleptic Characters: The powder of the dried herb of Saad Kufi (*Cyperus scariosus*) was dark brown with characteristic bitter odour and taste. Organoleptic and macroscopic characters were summarized in table-1 and 2.

Tab. 1 Organoleptic Characters of Powdered Drug

S. No.	Parameter	Saad Kufi (*Cyperus scariosus*)
1.	Colour	Brown
2.	Appearance	Coarse
3.	Odour	Agreeable
4.	Taste	Tasteless

Tab. 2 Macroscopic Characters of Saad Kufi (*Cyperus scariosus*)

S. No.	Parameter	Saad Kufi (*Cyperus scariosus*)
1.	Colour	Blackish brown
2.	Shape	Elongated and cylindrical
3.	Size	4-15 x1-2 cm
4.	Surface	Wrinkled and knotted
5.	Fracture	waxy

Physico-Chemical Constants: Different Physico-chemical constants were determined three times and then average values depicted in table-3.

Tab. 3 Physicochemical Parameters

S. No.	Physicochemical Parameter	Results Mean±S.E.M.
1.	Moisture Content (%)	
	Loss of Weight on Drying	8.5±0.173
	Toulene Distillation Method	6.66±0.33
2.	Ash Value (%)	
	Total Ash	16.3±0.07
	Acid Insoluble Ash	8.616±0.20
	Water Soluble Ash	3.1±0.30
3.	pH Values (%)	
	pH at 1%	5.76±0.08
	pH at 10%	5.23±0.08
4.	Bulk Density (gm/ml)	
	Poured Density	0.38±0.01
	Tapped Density	0.60±0.01
5.	Crude Fibre Content	46.43±0.19
6.	Solubility (%)	
	Alcohol Soluble extractive	4.2±0.11
	Water Soluble extractive	6.46±0.17
7.	Extractive values in different organic solvent	
	Petroleum ether	1.14±0.01
	Diethyl Ether	0.19±0.01
	Chloroform	0.24±0.01
	Acetone	1.03±0.08
	Alcohol	1.77±0.033
	Aqueous	3.92±0.01

Phyto-Chemical Analysis: Qualitative analysis of the Phyto-Chemicals reveals the presence of alkaloids, carbohydrates, proteins, phenols, sterols, glycosides, Cardiac glycosides, flavonoids, sterols/ terpenes and volatile oil presented in table-4.

Table 4. Qualitative Analysis of the Phytochemicals present in test drugs

S.No.	Chemical Constituent	Test/Reagent	Saad Kufi
1.	**Alkaloid**	Dragendroff's Test	+
		Hager's Test	_
		Wagner's Test	_
2.	**Carbohydrate**	Molisch's Test	_
		Fehling's Solution Test	
3.	**Flavonoids**	Mg. Ribbon Test	+
4.	**Cardiac Glycosides**	Baker's yeast Test	+
5.	**Tannins**	Ferric Chloride Test	_

S.No.	Chemical Constituent	Test/Reagent	Saad Kufi
6.	Proteins	Millon's Reaction	+
		Biurette's Test	_
		Xanthoproteic Test	
7.	Starch	Iodine Test	_
8.	Phenols	Lead Acetate Test	+
9.	Sterols/ Terpenes	Hosse's Reaction	+
		Liebermann-Burchard's Test	
10.	Amino Acid	Ninhydrin Solution Test	_
11.	Resin	Acetic Anhydride	_

Indications: ' -ve 'Absence and '+ve' Presence of constituents

Florescence Analysis Florescence analysis under UV light is sometime very characteristic for a drug. As many drugs and the constituents present in the drug emit specific colour when they are exposed to ultraviolet radiations because the radiant energy excites the solution which emits that particular colour known as fluorescence.

The fluorescence analysis of the powdered and successive extracts treated with different chemical reagents was done and change in the colour so appeared was observed and mentioned in the table 5(a, b).

Table 5 (a). Fluorescence Analysis of Powdered Drug Saad Kufi*(Cyperus scariosus)* in different chemical reagents

S. No.	Powdered drug	Day light	UV short	UV long
1.	P. drug + Conc. HNO_3	Brown	Greenish black	Black
2.	P. drug + Conc. HCl	Brown	Greenish black	Black
3.	P. drug + Conc. H_2SO_4	Brownish black	Greenish black	Black
4.	P. drug + 2% Iodine solution	Brownish black	Greenish black	Black
5.	P. drug + Glacial Acetic acid + HNO_3	Dark Brown	Greenish black	Black
6.	P. drug + Glacial acetic acid	Brown	Greenish black	Dark Brown
7.	P. drug + NaOH(10%)	Brown	Brownish black	Black
8.	P. drug + Dil. HNO_3	Light Brown	Greenish black	Black
9.	P. drug + Dil. H_2SO_4	Dark Brown	Brownish black	Black
10.	P. drug + Dil. HCl	Brown	Greenish black	Black
11.	P. drug + Dragendorff's reagent	Dark Brown	Brownish black	Black
12.	P. drug + Wagner's reagent	Brown	Brownish	Black
13.	P. drug +Benedict's reagent	Dark Brown	Greenish black	Black
14.	P. drug + Fehling reagent	Light Brown	Green	Black
15.	P. drug + KOH (10%) Methanolic	Brown	Brownish black	Black
16.	P. drug + $CuSO_4$ (5%)	Brown	Greenish black	Black
17.	P. drug + Ninhydrin(2%) in Acetone	Brown	Black	Brownish black
18.	P. + Picric Acid	Brown	Black	Black
19.	P. drug +Lead Acetate (5%)	Brownish black	Black	Black

Table 5 (b) Fluorescence Analysis of Successive extracts of Saad Kufi (*Cyperus scariosus*)

S.No.	Extracts	Day Light	UV Short	UV Long
1.	Petroleum Ether	Brown	Dark green	Black
2.	Diethyl Ether	Brown	Darkgreen	Black
3.	Chloroform	Brown	Light green	Black
4.	Acetone	Brown	Light green	Light black
5.	Alcohol	Brown	Green	Black
6.	Distilled Water	Brown	Dark green	Dark black

TLC studies of various extracts of drugs obtained in different solvent system have been conducted and Rf values of various spots in different solvent systems have been noted in day light, UV light and after treatment with iodine vapours. The Rf values in the given solvent are used to characterize the drugs identity and purity. Results were depicted in Table 6 and figure 2.

Table 6. Thin Layer Chromatography of Saad Kufi(*Cyperus scariosus*)

Extract	Solvent System	Treatment	No. of spots	R_f Value & colour of spots
Petroleum ether Extract	**Petroleum ether : ethylacetate (24:1)**	UV short	5	0.033,0.316,0.383,0.466, 0.633(Purple)
		UV Long	4	0.216(Translucent White), 0.533(White), 0.716,0.816 (Purple)
		Iodine Vapour	3	0.676(Yellow), 0.8, 0.907(Light Yellow)
Chloroform Extract	**Toulene:ethylacetate (8:2)**	UV Long	5	0.631(White),0.684(Purple), 0.736(Pink),0.842, 0.947 (Blue)
Acetone Extract	**Chloroform: Methanol (9:1)**	UV Long	1	0.076(White)
		Iodine Vapour	2	0.153(Brown), 0.246(light brown)
Alcoholic Extract	**n- Butanol : Acetic acid: Water (5:1:4)**	UV Long	3	0.285,0.730,0,769 (White)
		Iodine Vapour	1	0.285 (Brown)
Aqueous Extract	**n-Butanol: Acetic acid: Water (5:1:4)**	UV Long	2	0.177(Brown), 0.903 (White)
		Iodine Vapour	1	0.177(Brown)

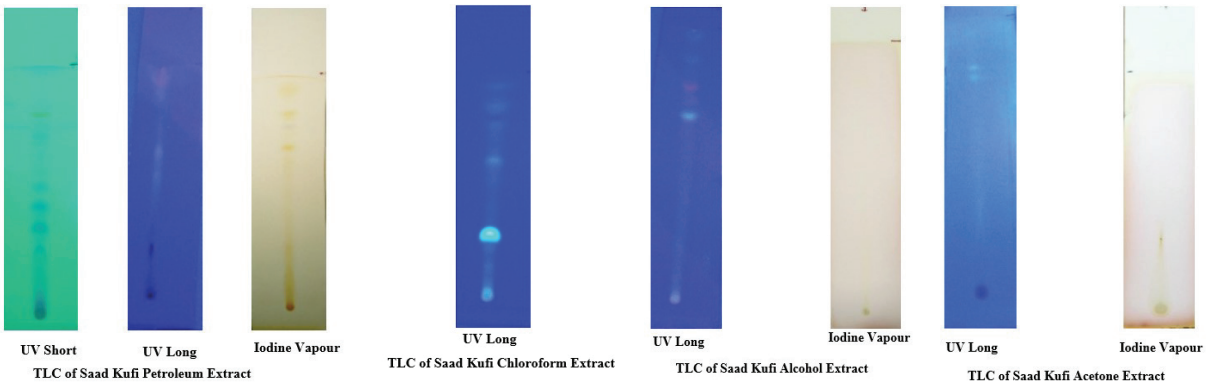

Fig. 2 Thin Layer Chromatography

Discussion

These days, it is obligatory by all medicine regulatory bodies to enforce strategies to improve and protect the health and safety of patients and the concepts like Pharmacovigilance, Cosmetovigilance, Herbovigilance, Haemvigilance and Materiovigilance are being taken up and incorporated as National Health Program in most of the countries[23].Identification of the actual plant is very important in quality control. It is not an easy task. Sometimes two or more plants are known by the same local name, whereas the same plant by two local names. While collecting the medicinal plants from the fields it is very possible that another plant is being collected and mixed with the genuine plant. To establish identity, the gross morphology will give definite information about drugs. Histology and microscopy is valuable both for powders and for unpowdered drugs. The identity

of many adulterants of drugs can be established or confirmed by an examination of calcium oxalate crystals, details of trichomes, anomalous character visible in cross sections, stomatal characters and quantitative microscopy.

Physicochemical study is of prime importance in quality control of Unani drugs. As the efficacy of many drugs correlates with their physical and chemical properties. Therefore, the determination of physicochemical characters for the authenticity of a drug is necessary before studying its medicinal properties. It is also more important because it helps in characterization of constituents or group of constituents that frequently establish the structure activity relationship and the likely molecular mechanism of action of the drugs. For these purposes the following physicochemical parameters were performed. The extractive values are a parameter for detecting the adulteration in any drug. The amount of the extracts that a drug yields in a solvent is often an approximate measure of the amount of certain constituents that the drug contains. It serves as index of purity of the drugs. Percentage of solubility is considered as an index of purity. Ash determination furnishes the basis of judging the identity and purity of drug and gives information relating to its adulteration with inorganic matter [17].pH value is also important parameters of standardization. It affects the stability, therapeutic activity and pharmaceutical elegance of medicinal agents in aqueous or hydrochloric preparation. The plants may be considered as biosynthetic laboratory for secondary metabolites which contribute to the therapeutic effects. Phytochemical constituents present in the drugs may vary, not only from plant to plant but also among different samples of same species. Some phytochemicals like alkaloids, quinones, terpenoids, flavonoids and tannins have property of precipitating proteins [24]. Their presence may help in identifying the phytochemicals responsible for therapeutic effect which will further establish scientific revalidation of drug being used in specific diseases.Many biological activities such asbactericidal, antiviral, cytotoxic, analgesic, anti-inflammatory have been attributed to the presenceof saponins[25].

Thin layer chromatography is one of the important parameters used for detecting the identity, adulteration or for judging the quality of the drugs. The various compounds present in the drugs got separated, depending on the affinity of mobile and stationary phases. If the drug is adulterated with other compounds it may increase the number of spots. On the other hand the exhausted or deteriorated drugs may lose the component or change its chemical character, resulting in the less number of spots or appearance of any new spot with different Rf value [26].

Conclusion: In spite of the efficacy of Unaniand other traditional systems of medicine, they have been widely criticized due to lack of standardization and poor-quality presentation. Many of the medicinal plants available in the market have ambiguous identification along with adulteration and contamination. Therefore, standardization of Unani drugs is must for maintaining and assessing the quality and safety. The standard parameters used for Standardization of Saad Kufi (*Cyperus scariosus)* include organoleptic tests (colour, taste, odour, and appearance), preliminary phytochemical analysis, percentage of loss in weight on drying, ash value, acid insoluble ash, water soluble ash, pH value, moisture content, fluorescence analysis of powder drug and different extracts, TLC, which will help to prepare ideal monograph. These parameters will serve through in setting up its safety, authenticity, reliability, quality, and reproducibility and also these parameters will wide open its research areas like in vitro, in vivo and in silico for further reference and analysis in searching out for new drug targets as an ailment for human diseases. Endorsing the results of this study will stand in for setting up standards of Saad Kufi (*Cyperus scariosus)*.

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Authors's contribution:

Data gathering and idea owner of this study: SN, KMYA

Study design: SN, KMYA, SR

Data gathering: SN

Writing and submitting manuscript: SN, SZR

Editing and approval of final draft: SN, HN, SZR, KMYA

References:

1. Bhattacharjee SK. (2004): "Handbook of Aromatic Plants", Pointer publishers, Jaipur, India. pp.166.
2. Dey K. L. (1973): "The Indigenous Drugs of India" 2nd Edi. PamaPrimlane, The ChronicaBotanica, New Delhi.
3. Dymock W., Warden C. J. H., Hooper D. (). "PharmacographiaIndica" The Institute of Health and Tibbi Research, Hamdard National Foundation, Pakistan,Vol. III, pp. 554-55. https://doi.org/10.5962/bhl.title.10717
4. Ibn Sina. Al-Qanoon Fil Tibb (Urdu Translation), *Idarakitab-us- Shifa, Delhi*, 2014;pp. 753
5. Ibn Baitar. "Al-Jameul Mufridat al Adviawa Al-Aghzia" (Urdu Translation), *Central council for Research in Unani Medicine, New Delhi*, 1999;**3**: pp. 46,47
6. Najmul Ghani: "Khazainul Advia", Central council for Research in Unani Medicine, New Delhi, 2010; **4**: pp. 364-66
7. Bhattacharjee S. K. and De L. C.: "Medicinal Herbs and Flowers" *Aavishkar Publishers*, *Distributors Jaipur, India*, 2005; pp. 78
8. Kabiruddin M.(2007):" Makhzanul Mufridatyaani Kitabul Advia", Idara Kitabul Shifa, pp. 400
9. Nafees S., Rahman SZ., Amin KMY (): Evaluation of anti-hypertensive activity of ancient Unani cardiovascular drug "Saad Kufi" (*Cyperus scariosus*) in adrenaline induced hypertensive rats. *Future Journal of Pharmaceutical Sciences. Springer@ nature* 2020;**6**:124; https://doi.org/10.1186/s43094-020-00142-x
10. Noor Kareem. (1879): "Tarjuma Makhzanul Advia-ba-zabanurdu", *Munshi Nawal Kishore*, *Lucknow*, pp. 234-235
11. Yousuf Harwi (2008).Ainul Hayat. Urdu Translation Ibn Sina Academy Aligarh. pp. 198
12. Azam Khan, (2014). "Muheet-e-Azam", CCRUM, New Delhi, Vol.III, pp.95-7
13. Anonymous "Standardization of Single Drugs of Unani Medicine", Central Council for Research inUnani Medicine,3, pp. 124-30.
14. Ghulam Nabi, (2007)" Makhzanul Mufridatwa Murakkabat Maroofba Khawasul Advia., Central council for Research in Unani Medicine, New Delhi, pp. 237
15. Anonymous (1968). "British Pharmacopoeia" General Medical Council, Bloomsbury Square London.pp.1276-7.
16. Anonymous (2009). The Unani Pharmacopoeia of India", Central council for Research in Unani Medicine, New Delhi, Part I. Vol. VI. pp. xxvi
17. Jenkins G. I., Kenevel A. M. and Digangi F. E. (2008). "Quantitative Pharmaceutical Chemistry", 6th Edition, CBS Publisher and distributers, Pvt. Ltd., New Delhi, pp. 229, 225, 233, 496.
18. Anonymous. "The Unani Pharmacopoeia of India", Central council for Research in Unani Medicine, New Delhi, 2016; **3**: pp. XXiii.
19. Anonymous "Standardization of Single Drugs of Unani Medicine" Central Council for Research in Unani Medicine, New Delhi,1987;**I**: pp. 162-5
20. Afaq, S. H., Tajuddin., Siddiqui, M. M. H. (1994): "Standardization of the Herbal Drugs", AMU press, Aligarh, pp. 33-34, 41-42, 100, 143-6.
21. Anonymous (1992). Standardization of Single Drugs of Unani Medicine, Central Council For Research In Unani Medicine, New Delhi., pp.4.
22. Anonymous "The Unani Pharmacopoeia of India", Central council for Research in Unani Medicine, New Delhi, 2007; 2: pp. Xvii
23. Rahman, S. Z. Concept of Materiovigilance in Unani Medicine. *Bangladesh Journal of Medical Science*, 2018;**18**(1): 5-6. https://doi.org/10.3329/bjms.v18i1.39539
24. Tiwari P., Kumar B., Kau Phytochemical screening and extraction: A Review, International Pharmaceutical sciences,2011; **1**(1): p.101.
25. Sohail T, Ferheen S, Imran H, Yaqeen Z, Rehman A, Khan RA Phytochemical and antibacterial screening of different fractions of root part of Ipomea Turpethum. *Bangladesh Journal of Medical Science* 2018 ;**17** (01): p: 93-97. https://doi.org/10.3329/bjms.v17i1.35288
26. Kaur AD., Ravichandran V., Jain PK.,: High-performance thin layer chromatography method for estimation of conessine in herbal extract and pharmaceutical dosage formulations. *J. Pharm. Biomed. Anal*. 2008;**46**: 391-4. https://doi.org/10.1016/j.jpba.2007.10.001