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RESEARCH ARTICLE

ANALYSIS AND QUANTIFICATION OF ECCENTRIC DRUGS FROM HERBAL MEDICINES USING HPTLC

Akanksha[1] and Dr. Jyoti Singh[2]

Amity Institute of Forensic Science, Amity University, Noida sec-125, U.P., India-201303

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***Corresponding author:**
Dr. Vijender Singh

ABSTRACT

The exploitation of the drug is a conglomerate chronic disease that affects the functioning of the brain thus resulting in compulsive drug seeking and use, despite harmful consequences. The array of psychoactive substances often includes alcohol, opioids, cannabinoids, sedative hypnotics, cocaine, stimulants, hallucinogens, tobacco, and use of multiple drugs. Eccentric drugs such as aspirin, diclofenac, cough syrups etc. can also be abused of non-dependency exists under an additional code (Singh & Gupta, 2017). The unprecedented demand for herbal drugs leading to the adulteration and substitution for genuine drugs. The herbal drugs used in the current study are Sarpagandha, Shankhpushpi, Jatamansi. This study focuses on the examination and quantification of sedative contents in herbal drugs/medicines by using HPTLC. The steroidal components are analysed by using various analytical techniques and this signifies that herbal drug have also tendency to be abused. Long-term use of herbal medications developed dependency on people.

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INTRODUCTION

By the modernisation in human antiquity, people have susceptible approach towards the vulnerability of the substances validates pantomiming such as herbs, alcohol and drugs. Exploitation of the drugs disturb the normal function of the brain thus results compulsive drug seeking and use, despite harmful consequences (Chauhan , Gandhi, & Shukla, 2018) . Classification of substance use disorder by ICD-10 under "Mental and behavioural disorders due to psychoactive substance use (F10–F19)" defines a definitive pattern of substance use such as acute intoxication, destructive use, dependency syndrome, and withdrawal state. The array of psychoactive substances often includes alcohol, opioids, cannabinoids, sedative hypnotics, cocaine, stimulants, hallucinogens, tobacco, and use of multiple drugs. Eccentric drugs such as aspirin, diclofenac, cough syrups etc. can also be abused of non-dependency exists under an additional code (Singh & Gupta, 2017). Drugs can be classified according to their biological effects produced by them as Depressants (further classified as alcohol, benzodiazepines, barbiturates), Stimulants and Hallucinogens. In the contemporary times, the juveniles are oblivious about the consequences of certain acts done under the influence of drugs. In our country, consumption of drugs is increasing day by day.

Moreover, the slums are one of the most affected areas by the consumption of modalities in form of eccentric drugs (Chauhan , Gandhi, & Shukla, 2018). According to the reports of National drug dependence treatment centre New Delhi, the slums start to intake of modalities by the age group of 9-10 years of age while the consumption of other drugs (Cannabis, heroin etc.) start by the age group of 12- 13 years (Bunaciu & Aboul- Enein, 2020) . Around 7.21 crore population consumes several drugs which is dependent on availability and expenses of these drugs. According to the statistics of 2016, about 396 million slums are located in India, in which approx. 82,400 are located in the capital and national capital region of India. Approximately 42,000 of these residents/ slums from the age group of 7 to the commonly used modalities. In 2016, During the statistical survey of Delhi aids control society, found that substance abuse in slums of Delhi is about 30,000 while tobacco 9,450; alcohol 27,910 and inhalants 15,600. Most commonly drugs which are being abused in slums because of their easy availability are cannabis 840, heroin and some pharmaceutical drugs such as sedatives & injections 210. In India, according to the reports of NCRB (2019) 38.5% drug cases were reported in Punjab. After Punjab, the second maximum rate of crime in the category was in Andaman and Nicobar Islands (33.4 per cent) and 133 cases, and Kerala (26.3 per cent) where 9,245 cases were registered, which is also fourth highest in the country.

Kilambi Pundarikakshudu *et al*. in 2019 conducted a study on "Development and Validation of a High-Performance Thin Layer Chromatographic (HPTLC) Method for Simultaneous Quantification of Reserpine, Atropine, and Piperine in *Sarpagandha Ghanvati,* a Classical Ayurvedic Preparation" and concluded that HPTLC is an easy, sensitive, and genuine technique established to determine reserpine, atropine, and piperine from a classical ayurvedic formulation, Sarpagandha Ghanvati (Pundarikakshudu, Sharma , Bhatt, & Kanaki, 2019). Saba Irshad *et al*. in 2020 conducted a study on "Quantification of Shankhpushpi using Phytochemical and Molecular Markers" and concluded that the phytochemical quantification and molecular examination found that the market sample of Delhi, Hisar, and Jaipur were pure C. pluricaulis. Though, other market samples of Shankhpushpi are the mixture of two species. These methods play a vital role in the quality control and determination of adulterant/substituent of herbal drug by providing a substantial identification marker at phytochemical and DNA level to maintain batch to batch consistency of herbal drug and ascertain the drug quality (Irshad, Singh, Khatoon, & Rana, 2021).

MATERIALS AND METHODS

Herbal medicines are obtained by using numerous parts of medicinal plants that have beneficial effects thus, these medicines are often known as phytomedicines. The drugs/medicines sold in the markets contains an exact amount of active ingredients, preferred by a set of standards. Set a standards or consistent parameters assures the quality, efficacy, safety, and repeatability for herbal medicines. The drugs used in the current study are Sarpagandha (Baidyanath Ayurved Bhavan Pvt. Ltd, Kolkata), Shankhpushpi (Patanjali), Jatamansi (Birla Ayurveda, Satpur, Nashik) are herbal drugs. Sarpagandha is attained from the local market of Delhi. Shankhpushpi, & Jatamansi are obtained from the ayurvedic hospital of Delhi (Parihar, Hooda, Kakkar, & Bhan , 2022) . These drugs are taken randomly to check the level of toxicity in these drugs. In the current study, HPTLC (High Performance Thin Layer Chromatography) technique is used in the given study.

Sarpagandha: The quality of the medicine taken was determined using HPTLC. Before the analysis using HPTLC there are certain parameters that should be assessed:

Determination of the total ash- 1 g of the test medicine is taken in a crucible at 450^0C in a muffle furnace and cooled. This sample was weighed and the total percentage of ash was calculated. Determination of alcohol soluble extracts- **T**he powdered test medicine was mixed with 100ml of alcohol in a closed flask for 24 hours. It was then leave for 18 hours and then filtered. 25 ml of each filtrate was evaporated and dried in a porcelain dish at 105^0C to constant weight and the alcohol soluble extracts are calculated.

Determination of water-soluble extracts- The powdered test medicine was mixed with 100ml of water in closed flask for 1hour then it is boiled gently for another hour cooled and weighed, 25 ml of this sample is evaporated in a porcelain dish at 104^0C to constant weight, the percentage of water-soluble extract was calculated.

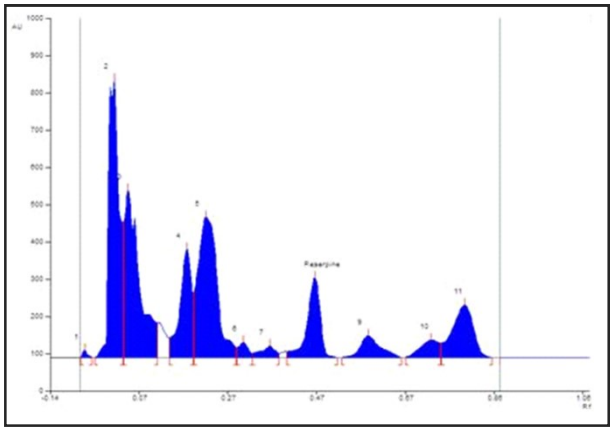

Figure a. HPTLC spectra of standards

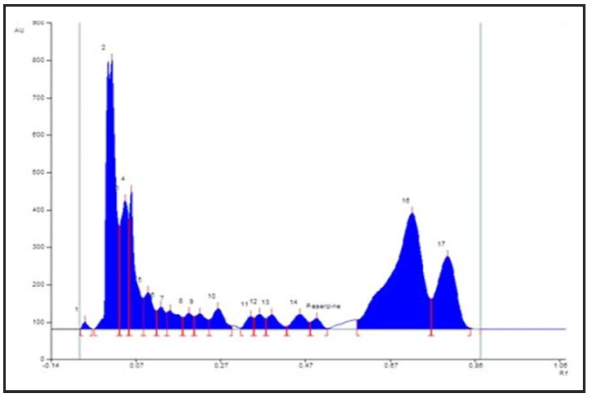

Figure b. HPTLC of Sarpagandha churna

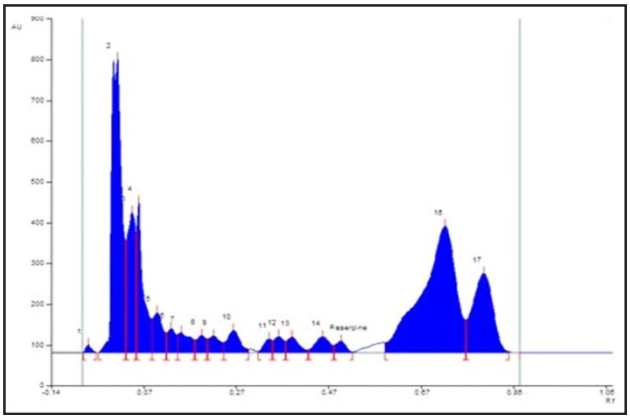

Figure c. HPTLC of Sarpagandha Ghana Vati

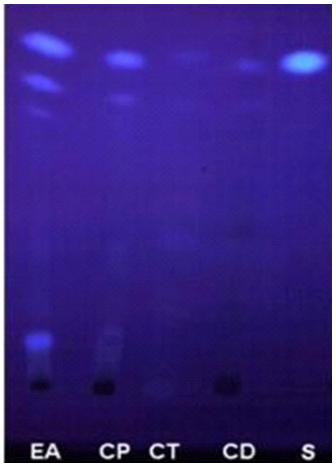

Figure d: Comparatives TLC of ethanolic extracts of CD, CT, EA and CP with scopoletin (S) (Rf− 0.81 in UV 366 nm.)

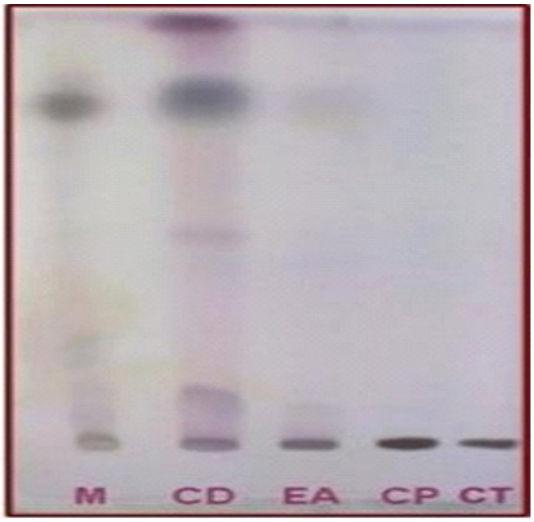

Figure e. Comparatives TLC of ethanolic extracts of CD, CT, EA and CP with mangiferin (M) (Rf: 0.72 with 1% FeCl3 sol)

Table I. Description of test drug formulations

Test/ Drug Formulation	Ingredients	Quantity (%)
Sarpagandha Churna	Sarpagandha root powder	100
Sarpagandha Ghana vati	Sarpagandha ghansatwa	50
	Khursaniajawain ghansatwa	10
	Jatamansi ghansatwa	5
	Bijay ghansatwa	5
	Pippalimool churna	25
	Excepients	5
M-Sarpagandha Mishran	Sarpagandha root churna	15.6
	Jatamansi root churna	15.6
	Vacha leaf churna	15.6
	Punarnava whole plant churna	15.6
	Brahmi whole plant churna	15.6

Extraction of alkaloids- 1g of each test powdered drug and 0.1g of Sarpagandha root powder were taken. Each material was refluxed with 10 ml methanol containing 0.1M HCl in water bath for an hour. Then the sample solution was cooled, filtered and liquid-liquid separation was performed using n-hexane. The residual matter after hexane extraction was concentrated in reduced pressure and the residue was then dissolved in methanol-chloroform (98:2, v/v) in a 10 ml volumetric flask. Each sample solution was filtered through 0.22µm filter force using HTLC analysis. Phytochemical analysis: The phytochemical screening of the prepared samples was carried out in order to test the presence of tannins, saponins, flavanoids, carbohydrates, phenol, and steroids. Others drugs are also processed by employing above given procedure and then these digested drugs are accessed for the analysis

Shankhpushpi: Preparation of leaves extract: The aerial parts of the herbs were shade dried and crushed into a coarse powder at room temperature. In a Soxhlet device, the coarsely powdered shade-dried plant material was extracted with petroleum ether. The defatted marc of the drugs was extracted with 95% ethanol. To obtain the ethyl acetate soluble fraction and the aqueous fraction, the ethanol extract was suspended in distilled water and separated with ethyl acetate. Materials: Scopoletin was used as a reference standard, Mangiferin standard, Methanol and water, Glacial acetic acid, petroleum ether and ethanol. Mobile phase: Methanol-water-glacial acetic acid (26:55:0.5 v/v) found out to be the best mobile phase for analysis. The flow rate was at 1.0 ml/min.

Preparation of scopoletin and mangiferin standard solution: A total of 10 mg of mangiferin/scopoletin was accurately weighed and transferred to a 10 ml volumetric flask, where it was dissolved in methanol. 1 mL was taken and diluted with methanol to make 10 mL. The stock solution had a concentration of 100 µg/ml. This stock solution was used to prepare the required dilutions, which contained 5–100 g/ml of mangiferin/scopoletin solution. Sample preparation from extracts: 10 mg of each of the four extracts (CD, CT, CP, and EA) were accurately weighed and transferred to 10 ml volumetric flasks, where they were dissolved in methanol, 1 ml was taken and diluted to 10 ml with methanol. The stock solution had a concentration of 100 g/mL. Methanol was used to dilute 1 ml of each of these solutions to 10 ml. As a result, 10 g/ml solutions of all extracts were prepared.

Jatamansi: HPTLC analysis was carried out on a liquid chromatography system consisting of Waters, 515 pumps and equipped with an online degasser, a Waters PCM (Pump Control Module), a Rheodyne 7725 injection valve furnished with a 20 μL loop, a Waters 2996 photodiode array detector (PDA), and Waters Empower software. Each analysis has to be done 3 times, and average retention times obtained. HPLC conditions: Purospherstar RP-8 column (5 μm, 4.6 × 250 mm; Merck), guard column (4.6 × 40 mm) packed with the same material; solvent system: solvent A-water: phosphoric acid (99.7 : 0.3 v/v), solvent B-acetonitrile : water : phosphoric acid (79.7 : 20 : 0.3 v/v); gradient 0–5 min with 88–85% A, 5-6 min with 85–82% A, 6–9.5 min with 82–75% A, 9.5–10.5 min with 75–74% A, 10.5–12 min with 74–73% A and 12–20 min with 73–70% A, 20– 30 min with 70–30% A, and isocratic from 30 to 35 min with 30% A; flow rate: 0.8 mL/min; column temperature: 30°C; injection volume: 10 μL; standard concentration: 0.1 mg/mL; sample concentration: 50 mg/mL; PDA detection: 280 nm, spectra 200–600 nm.

RESULTS AND DISCUSSION

Sarpagandha: Chemical constitution: Ajmalicidine, Ajmalicine, Rouhimbine, Indobinine, Reserpiline, Reserpine, Sarpagine, Serpentine, Serpentinine, Yohimbine, Ajmalimine, Ajmaline, Rauwolfinine (Perakenine), Sandwicolidine, Serpinine etc. The HPTLC fingerprinting profile is a major quality control aspect in which the phytochemical components of the formulations can be revealed and the efficacy, quality and safety can be reassured. The HP-TLC technique is used for both comparison of reference and sample and can work as a quality control tool other than the identification, the peak profiles and their intensities and the HPTLC images will give both quantitative and qualitative result in comparison with reference standards, the percentage of purity and minimum content information can also be obtained by this technique. The HPTLC analysis of the sarpagandha medicine shown that the sarpagandha churna exhibits 11 peaks and the sarpagandha Ghana vati has 17 peaks that indicate the fingerprint of test drugs. The individual peaks in fingerprint chromatogram are usually depends on chemically distinct components. The concentration of this chemically active components are not only responsible for its therapeutic properties but also different symptomatic and non- symptomatic side effects therefore it is important to determine the concentration of test drug dosage form and dosage regimen before prescribing it.

Table II: Comparison of chromatographic techniques

Parameters	HPLC (High performance liquid chromatography)	HP-TLC (High performance thin layer chromatography)	GC (Gas chromatography)
Stationary phase	Column	paper/glass	liquid /solid
Mobile phase	Solvent mixture	Solvent mixtures	Pure inert gas
Sample	One at one run	Many at a single run	One at one run
Pressure	High	normal	closed
Results	System peaks	System peaks visual by bands	System peaks
Resolution	High to very high	Moderate to high	High to very high
Time	2-60 min	1-30min	2-60 min
Temperature	Constant	Constant	Increasing

Shankhpushpi: When exposed to UV at 366 nm, the presence of Scopoletin was detected as a blue fluorescence spot. Mangiferin is a xanthone that was identified in Butanol: Acetic acid: water (4:1:2), after spraying with 1% ferric chloride reagent to produce apricot yellow green spot. HPTLC analysis: The results were as follows: Drowning where 2% foreign matter was determined. Loss on drying 1.6%, total ash obtained was 9%, acid insoluble ash was 1% and water-soluble extractive was 12% and Alcohol soluble extractive was 13%. The phytochemical investigation revealed the presence of various phytochemical constituents such as alkaloids, flavonoids, carbohydrates, Steroids and Saponin Glycoside. HPTLC chromatograms of methanol extracts obtained from root of Withania somnifera revealed that higher quality of with a nolides was present. Hence the root of Withania somnifera was considered to mostly prefer for commercial preparation of drugs.

Chemical constitute present in Jatamansi: Alpha-patchoulenese, angelicin, beta- eudesemol, beta-patchoulenese, beta-sitosterol, calarene, calarenol, elemol, jatamansin, jatamansinol, jatamansone, n-hexacosane, n-hexacosanol, n-hexacosanyl arachidate, n- hexacosanyl isolverate, nardol, nardostechone, norsechelanone, oroselol, patchouli alcohol, seychelane, seychellene, valeranal, valeranone. Volatile essential oil, resins, sugar, starch, bitter extractive matter, gum, ketone, sesqueterpin ketone, spirojatamol etc.

CONCLUSION

The herbal products have been a part of traditional medicine system new formulations should be required for the standardization, safety, efficiency and potency of the medicines that are produced, it is required that various quality control measures should be required for the production of herbal medicines and this help the humankind to use safer and effective treatment. The present study focuses on the qualitative analysis of herbal drugs. The quantity of chemically active compounds is in higher or lower amount in the marketed product in comparison to plant-based products. The steroidal components are analysed by using various analytical techniques and this signifies that herbal drug have also tendency to be abused. Long-term use of herbal medications developed dependency on people. HPTLC, FTIR, HPLC are the most advantageous techniques which can be used for the analysis of such herbal drugs.

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