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Pharmacognostic standardization and DNA fingerprinting of leaves of *Datura stramonium*, growing naturally in Asir region of Saudi Arabia

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Abstract: Evaluation of the various pharmacognostic quality parameters and DNA fingerprint of Saudi Arabian medicinal plant namely *Datura stramonium* growing in Asir region was the objective of the study. The pharmacognostical parameters were done in terms of macroscopic characters, microscopic details, physico-chemical evaluations, phytochemical analysis, fluorescence analysis and DNA fingerprint by using standard techniques and random polymorphic DNA primer. The detailed microscopy of the leaf revealed the presence of pre-medullary phloem, xylem, endodermis, parenchymatous pericycle, lower epidermis and calcium oxalate crystals. There are various amounts of foreign material, ash values, moisture content and extractive values, found after estimations. Preliminary phytochemical screening exhibited the presence of alkaloids, flavonoids, glycosides, tannins and sterols in variable amounts. Random amplified polymorphic DNA (RAPD) showed there are a prominent scorbale DNA bands. These evaluations provided referential information for correct authentication and quality standardization of the important medicinal plant material. These information will also be supportive to differentiate *Datura stramonium* from the closely related other species.

Keywords: Medicinal plant, physico-chemical parameter, phytochemical screening, DNA fingerprint, Asir region.

INTRODUCTION

Kingdom of Saudi Arabia (KSA) is characterized with a wide variety of flora, consisting of different species of herbs, shrubs and trees. It contains different types of edible and medicinal plants. Asir province is full of natural and ancient herbal resources that inspire ones senses with beauty and fragrance. Here potential medicinal and aromatic plants present a genetic diversity that must not be ignored (Leipzig, 1996). Asir region, KSA is located at the southwest of Saudi Arabia. The hazy high ground gives an attractive bionetwork for the coniferous trees to grow in plenty. The big cities of the province are Khamis Mushayt, Bishah and Abha. There are total 261 plant species found in this region out of which 165 species have medicinal importance but there is lack of full information covering the active constituents of medicinal plants in this region or documented medicinal uses (Yassin *et al*., 2013).

A meticulous, literature survey on Saudi Medicinal Plants of Asir region as well as on the quality standardization of various other medicinal plants have been carried out from various web portals, national and international journals, herbal database from other published research materials. Through the literature, it has also been clear that a large number of medicinal plants in the flora of Saudi Arabia have been used intensively in indigenous medicine from ancient time (Abbas *et al.,* 1992; Alsherif *et al*., 2012; Al-Sherif *et al*., 2013; Hegazy *et al*., 2014; Mossa *et al*., 2000; Al-Yahya *et al*., 1990; Kuete*, et al*., 2013). Pharmacognostic standardization of medicinal plants is extremely essential and crucial to maintain the identity, quality, purity and safety of crude drugs (Anonymous, 1998). From the documentation, it also becomes evident that despite the extensive traditional use of these plants their standardization and pharmacognostical constants have not been investigated. On the basis of literature survey, *Datura stramonium* was selected for its pharmacognostic quality standardization studies.

D. stramonium is commonly known as Jimsonweed belonging to nightshade family. This plant was originated in Mexico, but has now become naturalized in many parts of the world. It is an annual, erect, branching herb and forms a bush up to 0.6 to 1.5 m tall. The leaves of the plant are about 8 to 25 cm in length, smooth and margin is toothed, and irregularly undulated. The lower surface is a week green while the upper surface of the leaves is dark green in color and. The taste of the leaves is bitter nauseating. The white to violet colored flowers are trumpet-shape, and grow on short stems. The egg-shaped capsule of seed is covered with spines or bald (Stace, 1997; Henkel, 1911; Grieve, 1971).

All parts of *D. stramonium* plants contain tropane alkaloids which includes atropine, dl hyoscyamine and hyoscine, which are known as anticholinergics. Due to ignorance in villages, many people get hospitalized after ingestion of the plant due to its psychoactive effects (Preissel, and Preissel, 2002; Giannini, 1986). *D.*

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stramonium intoxication typically produces hyperthermia, tachycardia, delirium, bizarre behavior, and severe mydriasis with resultant painful photophobia which last many days (Freye, 2009). The symptoms generally start around 30 minutes to 1 hour after ingesting the herb and last from 24 to 48 hours (Pennachio *et al*., 2010). In this intoxication, physostigmine can be injected in as an antidote (Goldfrank and Neil, 2006).

In Ayurveda, *D. stramonium* has been used for long in asthma symptoms. In 18th century, the British physician, James Anderson, seek the knowledge of this practice and made it famous in whole Europe (Barceloux, 2008; Pennachio *et al*., 2010). The extract of *D. stramonium*, boiled with specific grease is reported to cure all type of inflammations, burnings and scaldings (Maud, 1971). The native American Pueblo people once used *D. stramonium*as an anesthetic to make patients unconscious while broken bones were fixed (Turner, 2009). The Chinese also utilized it in the form of anesthesia during medical surgery (Nellis, 1997). Thus plant *D. stramonium* was found much of ethno-medicinal importance. Various research and review articles as well as monographs surveyed, give explanation of many parameters like botanical parameters, chemical compositions, ethno medical uses, pharmacological uses, toxicological aspects of different parts of *Datura stramonium* growing in various countries of the world but not explaining about the pharmacognostical as well as genetical quality standardization parameters of leaves in particular of this plant growing in Saudi Arabian geographical region. (Gaire, 2008; Soni, *et al*., 2012; Sayyed and Shah, 2014; Ananth, 2013) Therefore this article aimed to investigate for the first time the pharmacognostic quality parameters and DNA fingerprint of *D. stramonium* plants growing in Asir region, KSA.

MATERIALS AND METHODS

Collection and authentication

The fresh leaves of the plant *Datura stramonium* were collected from the roadside area of Bani-Malik of Abha city, KSA at the altitude of 2200m, in March, 2017. The plant material was identified by Dr. Mahadevan Ninjaian, Senior Professor of Pharmacognosy Department, College of Pharmacy, King Khalid University, Abha, KSA. The fresh leaves were utilized for the study of macroscopic and microscopic characters as well as DNA fingerprinting whereas other part of collected plant material was shade-dried and coarsely powdered by using grinder. This coarse powder was used for physico-chemical parameters and preliminary phytochemical investigation, as per standard methods.

Macroscopic and microscopic studies

The macroscopy of the leaves was done according to standard methods (Brain and Turner, 1975; Trease and Evans, 2002). Hand section of the fresh leaves and dried coarse powder was stained and mounted for micro-techniques (Johansen, 1940). The photographs of microscopy were taken with the help of inverted microscope for photo-documentation.

Physicochemical analysis

Physicochemical parameters includes Moister content analysis, Foreign matter analysis, Total Ash value, Acid-insoluble Ash value, Extractive values, Fluorescent analysis, Powdered drug reaction with reagents and pH value was performed according to standard procedures given in the literature (Harborne, 1998; Ansari, 2008)

Phytochemical analysis

100 g coarse powder of air dried leaves of *D. stramonium* were packed in muslin cloth and subjected to soxhelet extractor for continuous hot extraction with hexane, chloroform, ethanol and distilled water for 8 hours respectively. Then extracts were filtered and filtrate was then evaporated till dryness. Preliminary phytochemical screening was done for the identification of various chemical constituents in the various extracts as described by Harborne and Khandelwal (Harborne, 1998; Khandelwal, 2005)

Fig. 1: Leaf morphology of *D. stramonium*

DNA fingerprint

Forty nine milligram from young leaves of *D. stramonium* was grinded using mortar and pestle. DNA from leaves was isolated by using DN easy plant mini kit (QIAGEN-USA) according to the indication in the Kit. Oligo349, (5'– GGA GCC CCC T–3') wad applied for *D.*

stramonium identification (Zhao *et al*., 1999; Mahmood *et al*., 2016) whereas 49 ng of extracted genomic DNA of the leaves, 200 µM each dNTP, 25 mM $MgCl_2$; 7 pmol of Oligo349 and 0.2 µL of Taq DNA polymerase (3U/µL) were applied in 25 µL of Eppendorf PCR tubes. Thermal cycler Bio-Rad initiated at 94°C for 5 min for denaturation, followed by forty nine cycles at 94°C for 1 minute for denaturation, 30°C for 1 minute for annealing, 72°C for 2 minutes for extension and concluded with 72°C for 7 minutes for final extension. By using 1.4% gel electrophoresis, ethidinium bromide for staining and wide range of molecular size, all amplified bands were analyzed and recorded. No genomic DNA template was examined in PCR amplification reactions as negative control and PCR reaction steps was repeated three times to examine the reproducibility (Aljibouri *et al*., 2013)

RESULTS

Macroscopic characteristics

The macroscopic study showed that the leaves of D. *stramonium* are simple, glabrous, triangular ovate with acuminate apex and asymmetric wedged base. These are grayish green in color with slight faint characteristic odor and generally 5-20 cm long and 4-15 cm wide. Margin is unequal dentate lobed and irregular serrate type. Petiole is grooved on lower side and slightly bent. Midrib is prominent on both upper and lower surfaces. Reticulate is pinnate venation type and secondary veins generally 5-7, arise on each side, leaving midrib at the angle of about 45^o (fig. 1).

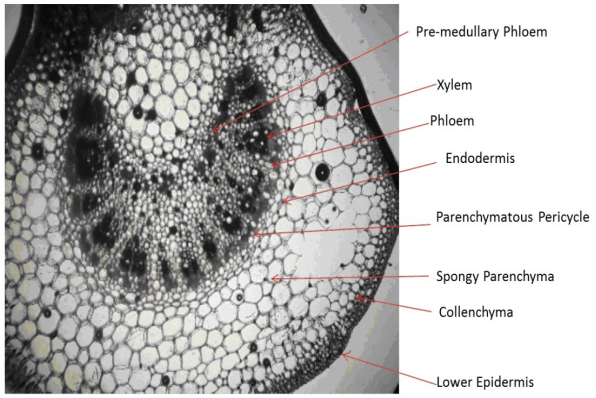

Fig. 2: Transverse section of *D. stramonium* fresh leaf in midrib region

Microscopic characteristics

The leaf sample was studied microscopically by transverse section through midrib. Fig. 2 shows epidermis, spongy parenchyma, collenchyma, central non-lignified phloem, lignified xylem and parenchymatous pericycle in midrib region.

Microscopy of Powdered leaf

The crude powder of leaf was dark green in color with characteristic odor and astringent taste. The powdered leaf of *D. stramonium* under microscopic investigation showed epidermal cells with anisocytic and anomocytic stomata, longitudinal upper and lower palisade cells, matrix showing Ca-oxalate crystals of cluster type, xylem vessels pitted annular and reticulate type (fig. 3).

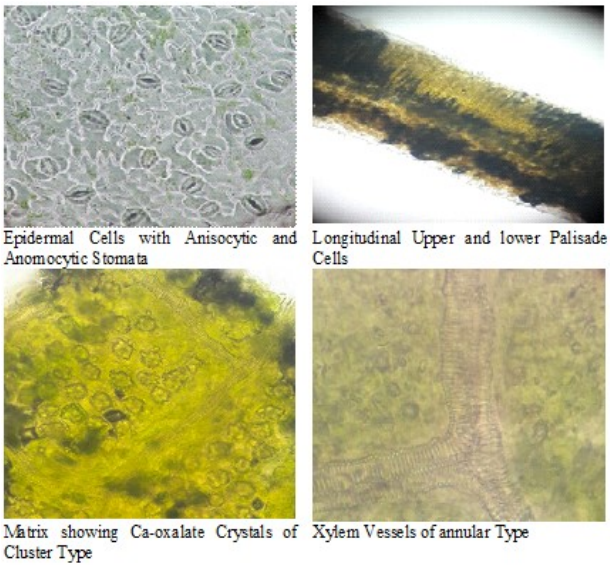

Fig. 3: Microscopical characters of *D. stramonium* leaf powder.

Phytochemical analysis

The result of qualitative phytochemical analysis of various extracts of the crude powder of *D. stramonium* leaf is shown in table 1. The leaf had maximum amount of alkaloid and sterols followed by tannin. The other phyto-constituents like glycosides and flavonoids were present in trace amounts in the leaf while saponins were absent.

Physicochemical analysis

The physiochemical characterizations of leaf are shown in Table 2. The moisture content of leaf was 9.8%. The ash values were determined by two different ways *viz.,* total ash and acid insoluble ash. The total ash in leaf was 15.5%, while acid insoluble ash was 3.1%. The maximum extractive value was found in aqueous solution and minimum was in hexane.

Fluorescence analysis

The fluorescent analysis under visible light and UV light by treatment of different chemical reagents showed different color. The fluorescence characteristics of leaf are summarized in table 3.

Powdered drug reaction with different reagent

The dried powder of *D. stramonium* was reacted with different organic and inorganic reagents and color appeared in the test-tube was noted as given in table 4.

Determination of pH drug

The pH of 1% and 10% aqueous solution of *D. stramonium* dried leaf powder was observed using pH meter and the results found are given in table t.

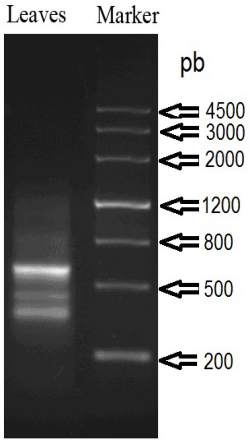

Fig.4: Assessment of DNA finger print of *D. stramonium* leaf fragments obtained with primer sequence (5'– GGA GCC CCC T–3'); Marker: Wide Range DNA Marker

DNA fingerprinting of D. stramonium leaf

Applying the Oligo349, (5'– GGA GCC CCC T–3') primer on *D. stramonium* plant, generated 3 scorable bands, ranged from 730 pb to 370 pb (fig. 4). The most prominent bands was 730 pb followed by 370 pb while the 470 pb was found to be the least one (fig. 4).

DISCUSSION

Pharmacognostic standardization is extremely essential and crucial to maintain the identity, quality, purity and safety of crude drugs. According to World Health Organization (Anonymous, 1998), the macroscopic and microscopic information of a crude drug is the first step towards fixing its identity and purity. Macroscopical and microscopic studies are reliable, simple and cheapest in establishing the identity of source materials (Anonymous, 2000). Macroscopical evaluation is a method of qualitative evaluation based on morphological characters of plant parts or sensory profiles of drugs (Thomas, 2008). The macroscopic and microscopic characters of *D. stramonium* noted in this study, serve as analytical tool for the analysis of a particular plant species which are helpful in recognizing the plant at species level and also in prevention of adulteration or substitution. Though, an additional difficulty takes places when the drug is in dried powder form. Even if the plant is identified correctly, it can be misused or rather substitution can occur in powder form (Deswal and Saini, 2015). Hence it is essential to have some microscopic characteristics of the particular plant parts in powder form also. Therefore, the coarse powder of the leaf of *D. stramonium* was analyzed for some diagnostic characters like type of stomata, crystals, xylem vessels, etc.

The powder was also examined for various phytochemical constituents and this analysis revealed more amount of

Table 1: Phytochemicals in *D. stramonium* leaf extracts

Test	Test type	Hexane Extract	Chloroform Extract	Ethanol extract	Aqueous extract
Sterols	1-Salkowaski test	-	+	+	+
	2- Libermann-burchards test	+	+	-	-
Alkaloids	1-Dragandorff	-	-	+	+
	2-Wagners	-	+	+	+
	3-Mayers	-	+	+	+
Glycosides	1-Bontrager's test	-	-	-	+
Tannins test	1-Ferric chloride test	-	-	+	+
Flavonoids	1-Shinoda test	-	-	+	+
Carbohydrate	1-Fehling test	-	-	+	+
	2-Molish test	-	-	+	+
	3-Barfoed's test	-	-	+	+

Table 2: Physicochemical parameters of *D. stramonium* leaves

S. No.	Parameters	% Value (w/w*) leaf
1	Moisture Content Analysis	9.80±0.25
2	Foreign Matter Analysis	4.17±0.0
3	Total Ash Value	15.5±0.31
4	Acid Insoluble Ash Value	3.1±0.0
5	Hexane Soluble	2.28±0.01
6	Chloroform Soluble	2.30±0.02
7	Ethanol Soluble	11.37±0.11
8	Aqueous Soluble	15.30±0.19

Table 3: Fluorescence analysis of leaves of *D. stramonium*

Solvent used	Day Light	UV light (254nm)	UV light (366nm)
1N HCl	Greenish	Light green	Blackish green
50 % HCl	Greenish yellow	Blackish green	Grayish green
50 % HNO_3	Orange brown	Dark green	Greenish black
50 % H_2SO_4	Brown	Light green	Dark brownish green
1N NaOH	Reddish brown	Dark brown	Brown
Alcoholic NaOH	Blackish brown	Green	Greenish brown
Methanol	Blackish green	Light orange	Dark brown
1% KOH	Blackish orange	Light green	Blackish green
Lead acetate	Yellowish brown	Black	Greenish black

Table 4: Powdered drug reaction with different reagent

Treatment	Observation (Color)
Conc. HCl	Dark green
Conc. HNO_3	Orange Brown
Conc. H_2SO_4	Blackish brown
Glacial Acetic acid	Light greenish yellow
Iodine solution	Dark brown
NaOH in Methanol	Brownish green

Table 5: pH

Sample	pH
pH of 1 % solution	6.2
pH of 10 % solution	5.8

alkaloids in leaf in comparison to other phytoconstituents. The phytochemical screening of various extracts of leaf powder reveals the presence of various classes of phytochemical which already have shown medicinal potential in other plants (Jasmeet *et al.,* 2017). The phytochemical analysis exhibited comparative more amount of alkaloid in leaf in comparison to other phytoconstituents. The physicochemical parameters like moister content, ash values and extractive values were also evaluated and may assist in preparing quality standard parameters for monograph of the plant. In leaf, the extractive value was highest in water followed by methanol. However, it indicates that polar compounds are more. The extractive values are helpful to identify the type of phytoconstituents present in the crude drug based on polarity of solvents (Patel and Dhanabal, 2013). Thus, preliminary analysis of phytochemical along with physicochemical analysis ensures purity, identity and quality of the drug and also gives an idea about the phytoconstituents present in *D. stramonium* especially for the future studies of pharmacological type at molecular level (Mukherjee, 2002).

The fluorescent analysis helps in the qualitative evaluation of crude drugs which can be used as a reference data for the identification of adulterants. The fluorescence study of the drugs under UV light and visible light by treatment of different analytical grade chemical reagents showed different color. This is attributed to the ultra violet light which produces fluorescence in many phytoconstituents present in the drug that do not fluoresce in daylight (Nagani, 2011). Thus fluorescence is used for qualitative assessment of *D. stramonium*. The microscopic characters, the physiochemical studies and fluorescence analysis collectively can be helpful in the quality control of the crude drug and are prime stem for evaluation (Chanda, 2014).

DNA fingerprint is an important tool in authenticating and characterizing a particular plant for its correct identification (Showkat *et al*., 2015). The earlier reporters recommended that RAPD-PCR fingerprints should be applied to evaluate the molecular variability among individuals with specific fingerprints (Chowda-Reddy *et al*., 2012). It was also reported that the numbers of amplified bands by using specific primer could identify the plants species precisely (Aljibouri*et al.*, 2013). The obtained results were in agreement with the outcome of the number of RAPD fragments of *Acer negundo* plant whereas 3 scorable bands identified male and female plants (Zhao *et al*., 1999). By using the sequence (5'CAACAATGGCTACCACCC3') of RAPD primer for the endangered species due to habitat loss of *Pittosporum meriocarpum* plants characterized also by three amplified bands (Thakur, 2016). Investigating the plant species

using molecular techniques play an important role in exploring their taxonomic status Therefore, *D. stramonium* a medicinally important taxon growing in Abha area, KSA could easily be identified by the random amplified polymorphic DNA (RAPD) primer for the plant conservation and to meet the demand of herbal plants formulations at the molecular level.

Pharmaognostic studies on different plants and their parts have already been reported (Bhide *et al*., 2011; Sandhya *et al*., 2011; Baravalia *et al*., 2011). *D. stramonium* is reported as the traditional plant of Saudi Arabian flora (Alfarhan, 1999). Therefore this study is an addition to the wealth of knowledge of Saudi Arabian flora of medicinal type, especially concerning its quality standardization. As we know, fixing standards is an integral part of fixing the quality and correct identity of a crude drug and *D. stramonium* is traditionally used to treat many important ailments and illness as mentioned above hence it was important to standardize it for its inclusion as an official drug in the national list of medicinal plants.

CONCLUSION

It can be stated that the Pharmacognostic studies resulted in quality standards of the plant which could be useful for checking the authenticity of *D. stramonium* of Saudi Arabian geographical origin. These standards can ensure in preserving the quality of the crude drug and can also be helpful for the preparation of a monograph as well as contribution in the medicinal plant literature of national wealth.

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