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PHYSICOCHEMICAL ANALYSIS, QUALITATIVE AND QUANTITATIVE INVESTIGATION OF ATROPINE IN MEDICINAL PLANT ATROPA BELLADONNA

Mahrukh Malik[1]*, Shahzad Hussain[1], Naila Sajjad[2], Sara Nazir[1], Muhammad Usman Gondal[3], Javeria Arshad Malik[4]

[1]Drugs Control and Traditional Medicines Division, National Institute of Health, Islamabad, Pakistan.
[2]University institute of Biochemistry and Biotechnology (UIBB), PMAS-Arid Agricultural University Rawalpindi.
[3]Drug Regulatory of Pakistan, Islamabad, Pakistan.
[4]Holyfamily Hospital Rawalpindi, Pakistan.

***Corresponding Author: Mahrukh Malik**

Drugs Control and Traditional Medicines Division, National Institute of Health, Islamabad, Pakistan.

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ABSTRACT

Atropa belladonna, commonly known as belladonna or deadly nightshade. The plant contains tropane alkaloids, including atropine, scopolamine, and hyoscyamine, which are used as medicinal, herbal and homeopathic remedies. These alkaloids are highly dose dependent. A greater understanding of this fact is critical in order to develop better treatment strategies, therapies, regulations, education of at-risk populations, and a more cohesive paradigm for future research. This research offers an integrated view of the homeopathy of *Atropa belladonna*. Present investigation is conducted to evaluate the physiochemical measures of accessible mother tinctures of Atropa Belladonna. Mother tinctures were collected from five manufacturers in Pakistan and evaluated for physiochemical parameters, % alcohol contents, Weight on dry/ml, % Non-volatile Matter, pH and for identification of atropine. World synchronization to follow WHO specific guidelines for herbal products standardization are of greatest need and to endorse regulation to control herbal market to adapt WHO guideline.

KEYWORDS: Herbal drugs, Standardization, Quality control, Atropa Belladonna, atropine,uv-visible spectrophotometer, FTIR, HPLC, Atomic absorption spectrophotometer.

INTRODUCTION

The plant *Atropa belladonna* belongs to the family Solanaceae is a perennial herb (Southgate et al., 2000). The name Atropa is derived from *"Atropos"* in Greek mythology that refers to one of the three fates which cut the fate of life; and in Italian Belladonna means "beautiful women" (Joshi et al.,2003). In ancient Roman times, the extract of this plant was used by women as eye drops to dilate the pupils of their eyes, seen as an attractive feature at the time (Berdai et al., 2012) and also applied to the cheeks to give a pinkish–red glow to the skin (Cikla et al., 2011). It is commonly known as belladonna, deadly nightshade, devils herb, divale, dwale, devil's cherries, dwayberry, gray morel, naughty man's cherries, and poison black cherry (Lacković et al., 2017). Traditionally in Europe, the plant was used as an herb to treat various illnesses during the middle ages (Moulton et al., 2011). The plant grows in the wild and is native to Asia, Europe, Africa, and sometimes cultivated as an ornamental plant in the United States. It has thick oval dark green foliage with black cherry-like berries making it look identical to blueberries and attractive to eat and It grows about 4 to 5 feet tall. After ingestion of these berries in children's and adults this also happens to be a common reason for intoxication (Berdai et al., 2012). And purple hermaphrodite bell-shaped flowers pollinated by insects. Its large leaves grow in pairs on either side of the plant stem; however, one of each leaf pair located near the flowers is noticeably smaller in size and 0.18 m long leaves, with some similarity to the tobacco plant. (Fatemeh et al., 2011, Lacković et al., 2017) (Figure 1). The intoxication which are present in the berries, leaves and roots is caused by the alkaloids atropine, scopolamine and hyoscyamine. The intoxication causing anti-cholinergic toxidrome. Causes anti-cholinergic effects on the body. The level of the intoxication depends on the dose of alkaloids ingested. Depending on the species the concentration of the alkaloids present in the berries and leaves may also differ. Some species of *Atropa belladonna* are hybrid and

may not produce all the symptoms of toxic anti-cholinergic syndrome. Repetition should also be kept in mind when determining the severity of the symptoms, as central effects are dose and source dependant. (Joshi et al., 2003, Berdai et al., 2012).

The fruits are cherry-l. In Europe, Asia, and Africa where the plant grows in the wild, confusion between black/dark belladonna fruit berries with other edible berries and the sweet and pleasant taste of belladonna berries poses a danger to children and adults (Laffargue et al., 2011). In conditions of stress the berries, leaves, and roots of *Atropa belladonna* contain up to 20 different tropane alkaloid including atropine, scopolamine, and hyoscyamine that function as the plant's chemical defense (Wink et al., 1998). S-(-)-Scopolamine (hyoscine) and S-(-)-hyoscyamine are the main alkaloids of various plants from the solanaceae family (Burrows et al., 2012, Gupta et al., 2012). These alkaloids are biosynthesized from S-(-)-phenylalanine. Upon ingestion of *Atropa belladonna*, hyoscyamine is converted to atropine, a racemic mixture, consisting of 50% l-hyoscyamine and 50 % d-hyoscyamine. In the over ground parts of the plant the total alkaloid content of hyoscyamine is reported to be 0.2% - 2.0%. 0.2% - 1.2% in the roots and 65% in the belladonna berries. Hyoscyamine makes up 68.7% in the roots and 87.6% of the total alkaloid complex in the leaves (Ulbricht et al., 2004). Thus, in *Atropa belladonna* atropine and scopolamine are the two most important alkaloids.

Although the plant was largely confined to the world of witchcraft in the medieval period, herbalists, and apothecaries began to study the plant in the sixteenth and seventeeth century. Eventually, in the ninteenth century the alkaloids in *Atropa belladonna* was incorporated into non-FDA approved over the counter drugs for human use as anticholinergic in cough-cold drug products, sedative to stop bronchial spasms in asthma and whooping cough, analgesic in motion sickness, colic, Parkinson's disease (PD), neuralgia, and rheumatism, as well as in plasters for treating psychiatric disorders associated with hyperkinesis, excessive sweating and brochial asthma (Lee et al., 2007).

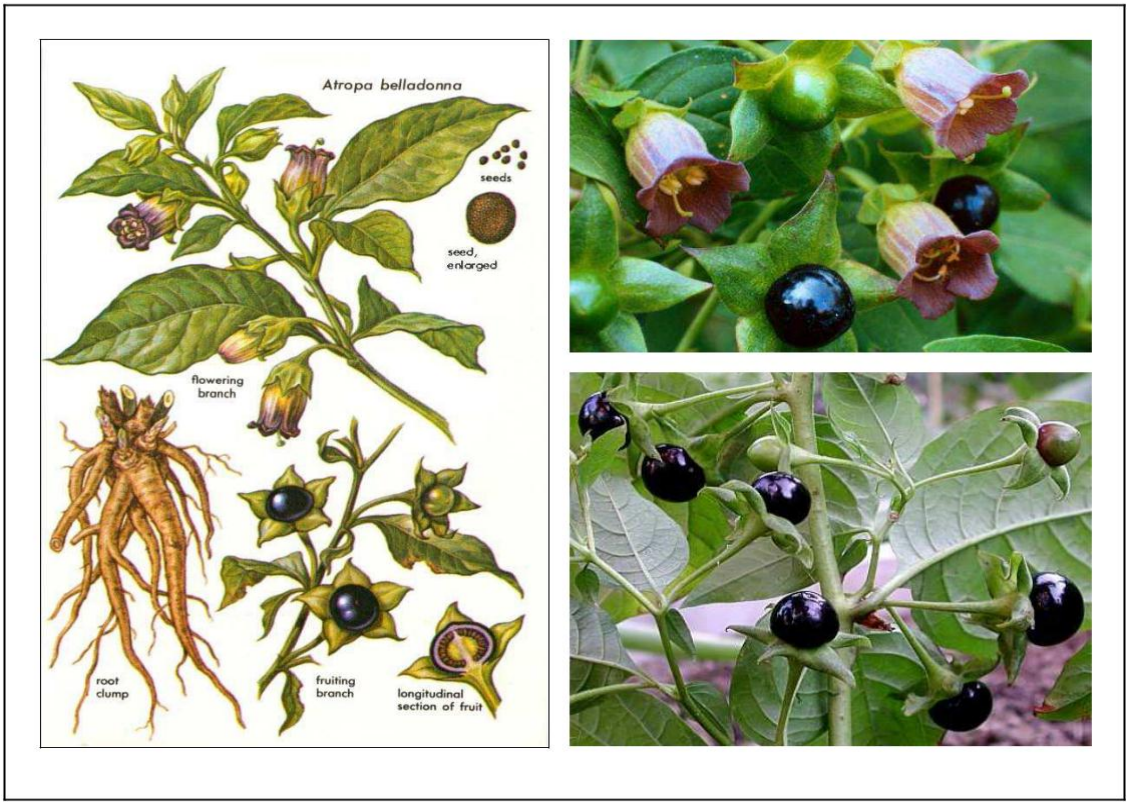

Figure 1: The features of the *Atropa belladonna* plant showing the leaves, roots, and berries Atropa belladonna - Deadly Nightshade.

MATERIALS AND METHODS

Collection and Quality assessment

Five mother tincture bottles were collected from main metropolitan cites (July, 2019) in Pakistan. All samples were put in storage by DCTMD,NIH, Islamabad by following WHO guidelines (GACP) and FCP (WHO, 2003). Samples were stored in new, well cleaned, colorless, neutral flint glass bottles. For glass stoppered

bottles, both the bottle and the stopper were of hard potash glass to avoid introduction of glass particles in the mother tincture. All containers were properly labeled with the proper mother tincture name, mentioning their strength/potencies and alcohol contained by % v.v., date of manufacture, Batch number, name of manufacturer, as far as possible while storing. The sign 'ø' was affixed after the name of each mother tincture, e.g., Atropa belladonna ø. Mother tinctures were kept at an even temperature of about 60ºF (15.6ºC) and stored in a dry, cool place in air tight, well closed, neutral flint glass bottles. Mother tinctures were well filtered before storing or when dispensing. Mother tinctures of Atropa Belladonna, manufactured by five homeopathic industries were collected from local distributor shops, then evaluated by using standard methodology (British Pharmacopoeia (BP), 1968).

1. % Alcohol contents
2. Weight on dry/ml
3. % Non-volatile Matter
4. pH

There are three methods given in BP (1968) and we had employed all three methods according to the need and requirement of parameters.

Method-I

Measure 25 ml of mother tincture in a graduated flask at 20°C, shifted to a 500 ml flask. The flask was connected to distillation assembly using a condenser. The concentrate was brought to 20.0°C and diluted with water to 100 ml with at the same temperature. Measure specific gravity at 20.0°C and the refractive index of the solution at 20.0°C. Ethyl alcohol content was determined by reference (refractive index does not differ by more than 0.00007).

Method II

But if the refractive index varies by more than 0.00007, 75 ml of the distillate is treated with powdered NaCl and petroleum (boiling-range, 40 to 60°), condensed to about 70 ml and diluted to 75 ml. then note specific gravity and refractive index

Method III

After applying method 1 and 2, refractive index still does not resemble the specific gravity, the distillate comprises contamination. Concentrate contains steam-volatile substances other than alcohol (it will be turbid/oily drops). In case of steam volatile acids, the solution is made alkaline with N/I sodium hydroxide, by using phenolphthalein as indicator, before the final distillation. (BP, 1968)

IDENTIFICATION OF ATROPINE

Melting Point

Melting point of atropine was found to be 113-116°C.identical to the reported value (114-116°C). (Scheinmamn., 1970).

Ultraviolet absorption Spectra

Ultraviolet spectra were measured using SHIMADZO 1085 spectrophotometer (Japan) and wavelength scaned between 200-1100 nm using ethanol as solvent.

FTIR

KBr disc of materials were measured with SHIMADZO fourier transforms infrared model FTIR 8300 (Kyoto, Japan).

High performance liquid chromatography (HPLC) measurements

HPLC model shimadzu LC-6A (Japan) was used for separation with reversed phase DB C-18 (250×4.6 mm) column silica as stationary phase. At ambient temperature flow rate was 1ml/min, detection UV at 254 nm.

Atomic absorption Spectrophotometer

Using Varian Tectron AA-775 atomic absorption spectrophotometer elemental concentrations measurements were done for the determination of Cd,Cu,Mn,Fe,Mg,Ca,K and Zn.

RESULTS

5 sample bottles of Atropa Belladonna mother tinctures manufactured by 5 leading homeopathic industries were collected from different location of Pakistan i.e, Islamabad, Rawalpindi, Lahore and physicochemical parameters % alcohol contents, Weight on dry/ml, % non-volatile matter, and pH were evaluated.

Atropa Belladonna (Results in Table 1) shows alcohol content observed range 31.76 - 50.19% (Range = 41 to 50) with the exception of BM and WH alcohol content observed range is 31.76 and 34.99 that are below the range. Weight on dry/ml observed range from 0.918 - 0.955 (range = 0.926 - 0.948) with the exception of BM Weight on dry/ml observed range is 0.918 that are below the range and RL and WH Pvt. Ltd whose result are 0.953 and 0.955 that are above the range, Non-volatile matter varies from 0.628 to 1.44% (range greater than 1.4% according to GHP)except of RL and WH Pvt. Ltd whose results are 1.44 and 1.43% all other below the range, pH observed range is 5.75 - 6.97 (range = 6.4 - 7.0) with the exception of WH and MH pH observed range is 6.97 and 6.53all other are below the range.

Table 1: Mother tincture (Atropa Belladonna).

Mother tincture	Alcohol content (%)		Weight on dry/ml (g/ml)		Non-volatile matter (%)		pH		Manufacturer
	Observe	Range	Observe	Range	Observe	Range	Observe	Range	
Atropa Belladonna	41.62	41-50	0.935	0.926-0.948	1.15	>1.4	6.34	6.4-7.0	KL
	48.45		0.953		1.44		5.75		RL
	31.76		0.918		0.628		5.89		BM
	34.99		0.955		1.43		6.97		WH
	50.19		0.931		1.13		6.53		MH

KL= Kamal Laboratories, Rawalpindi Pakistan; RL= Rax Laboratories, Homeopathic, Lahore Pakistan; BM= BM (private) Limited Lahore Pakistan; WH= Warsan homeopathic Laboratories, Lahore Pakistan; MH = Masood homeopathic Stores & Hospitals, Lahore, Pakistan.

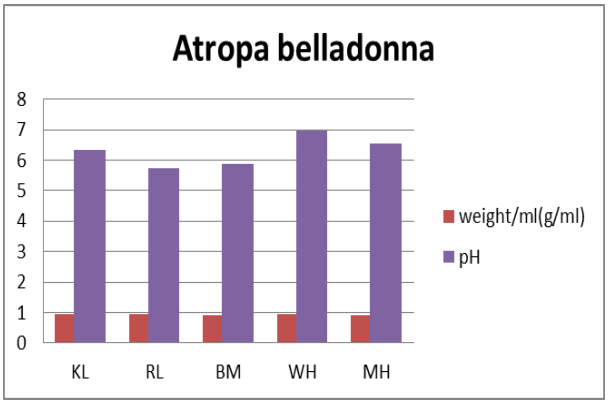

Figure 2: Comparison of pH and Weight on dry/ml of A.Belladonna.

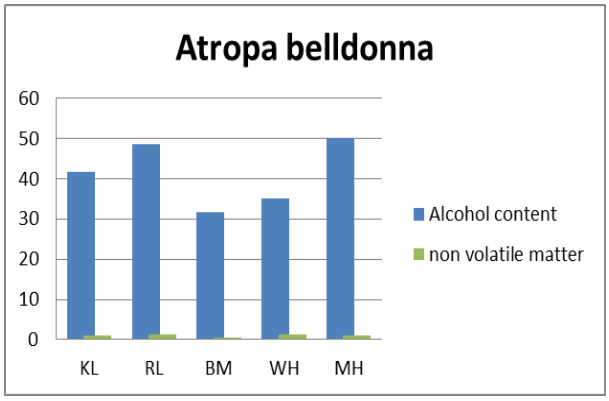

Figure 3: Comparison of % Alcohol content and % non-volatile matter of A.Belladonna.

UV-Vis measurements

Ultra-violet spectrum was measured in ethanol as solvent. Fig.(5) shows the maximum absorption for atropine which is equal to 283.1 nm indicates a difference with that of standard solution peak in fig.(4) that is equal to 285 nm

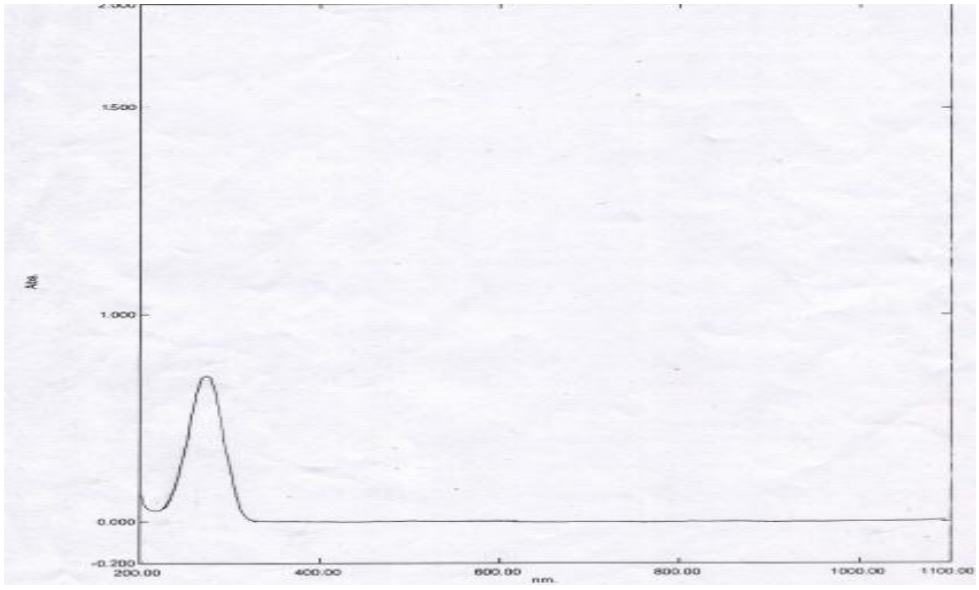

Figure 4: UV spectra for standard atropine solution.

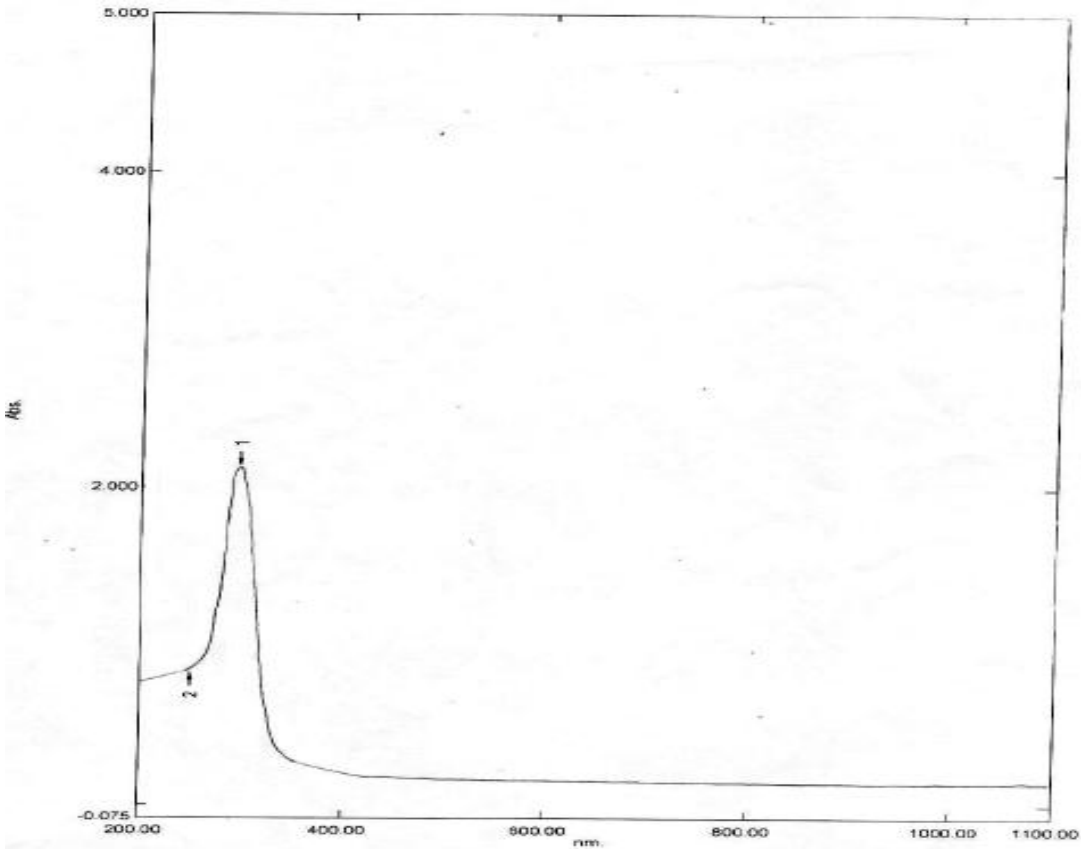

Figure 5: UV spectra for atropine that extracted from *Atropa belladonna.*

FTIR

The values of peak taken from standard spectrum is shown in fig.6 as reprted by (Scheinmamn., 1970).Fig.7 shows spectrum for extracted atropine.

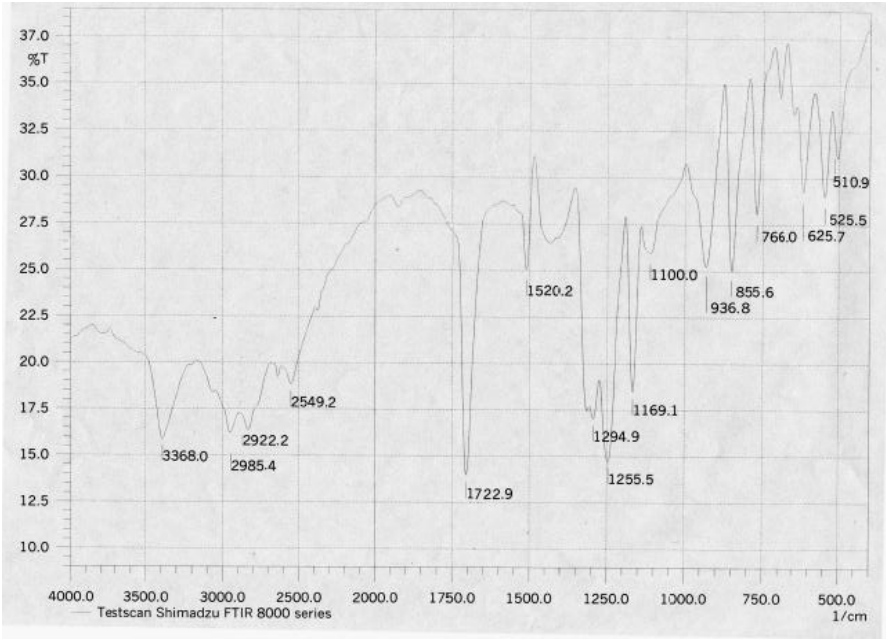

Figure 6: FTIR spectrum of standard atropine.

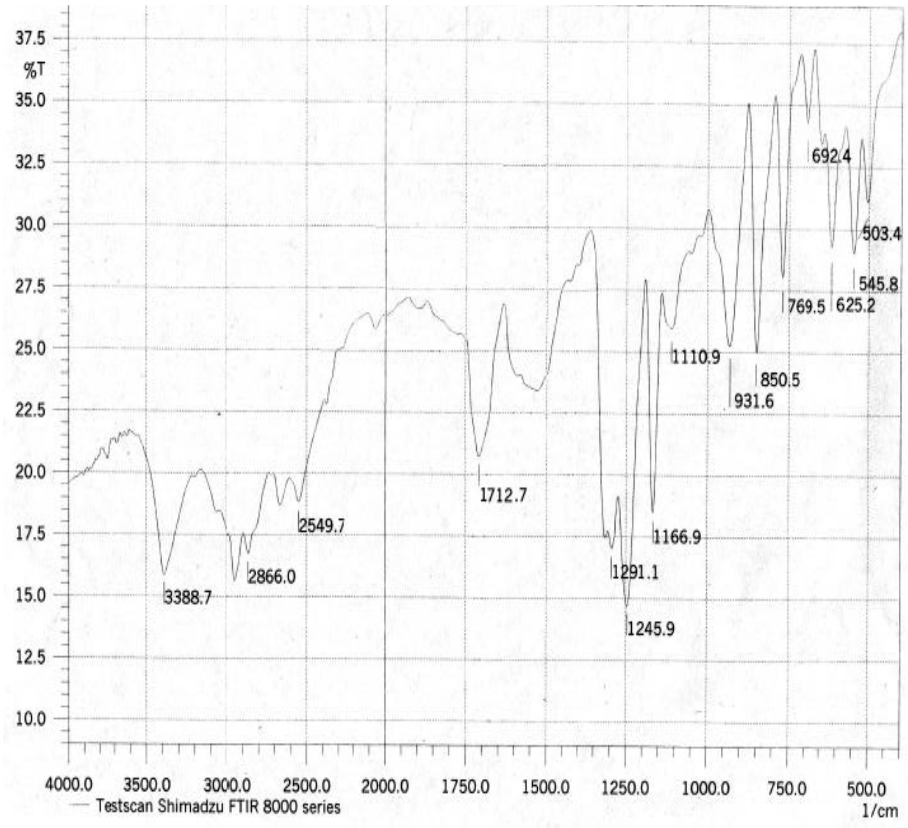

Figure 7: FTIR spectrum of extracted atropine.

HPLC

Chromatographic separation was done by HPLC using THF:deioinzed water:acetic acid (40:60:1) v/v as mobile. Atropine standard concentration was injected at 5 µg/ml. The retention time was reported at 11.18 min. The retention time for extracted atropine was recorded at 11.17 min with peak area 114084.

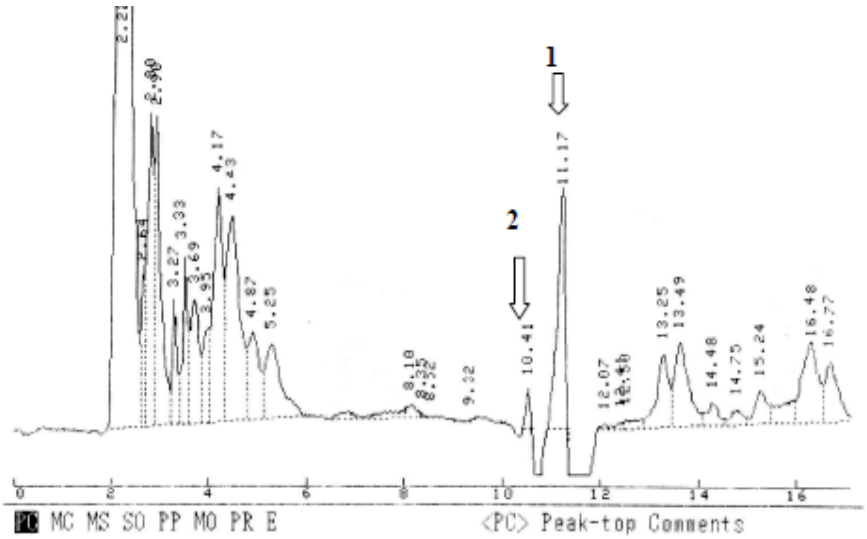

Figure 8: HPLC spectrum for total extract of *atropa belladonna* 1. (Atropine) 2. (Catechin).

Atomic absorption spectrophotometer

Elemental concentration in leaves were determined by atomic absorption spectrophotometry. The very low level heavy metals (Cu,Mn,Zn) and the highly level of nutrients (Ca,Fe,K,Mg) make it valuable for popular medicine. Shown in Table 2.

Table 2: Atomic absorption results in extracted atropa belladonna.

Element	Concentration in ppm
Mg	50.5
Ca	480
K	540
Mn	1.5
Zn	2.0
Fe	50.3
Cd	0.00
Cu	0.7

DISCUSSION

Natural merchandises occupied a significant and beneficial part to cure several human diseases due to their safety and efficiency. In drug discovery, the foremost outstanding feature of natural products is their long-lasting significance and their structural versatility (Yuan et al., 2016).Till now, in developed countries plant derived products has been great demand. These products are greatly used as medicinal products, makeups and nutraceuticals. There must have a good harmonization between the quality of raw materials, in process materials and the final products, it has become essential to develop consistent and specific quality control methods using a combination of traditional and current instrumental method. Standardization is an essential measurement for ensuring the quality control of the herbal drugs (Kumari et al., 2016). In recent years, as an outcome of comprehensive development of science and technology, capability to form high-quality herbal medicines is greatly improved. In public the acceptance of herbal medicine as a natural and mild substitute to synthetic drugs is very high in developed countries and, from a worldwide perspective, unit sales of herbal medicines is persistently growing. However, there are still many obstacles in this regard (Đorđević et al., 2013). In order to obtain high quality herbal raw material there is a need to aware people in the cultivation, collection of medicinal plants. The concept of organic production of herbal medicines should be encouraged. Manufacturers should be required to produce only quality-assured herbs, herbal materials, herbal preparations and finished herbal products. Herbs consist of raw plant material, such as leaves, seeds, flowers, fruit, stems, roots, wood, bark, rhizomes or other plant parts, that may be whole, powdered or fragmented. Finished herbal products may include powdered or comminuted herbal materials, extracts, tinctures and fatty oils. Numerous methods are used for their production i.e. extraction, purification, fractionation, concentration, or other biological or physical processes. When more than one herb is used, the term "mixture herbal product" can be used. Mixture herbal products and finished herbal products may contain excipients besides the active ingredients. If chemically defined active matters have been added, including synthetic compounds or isolated constituents they are not considered to be herbal. In traditional Chinese medicine, Ayurveda, Unani, Naturopathy, Osteopathy and Homeopathy, herbal medicines are most often used as health remedy (WHO, 2000) (Biswas et al., 2014).

In developing or developed countries plant materials are used as home remedies and as raw material for the pharmaceutical industry, they make up a major proportion of the universal drug market. Many factors contribute directly or indirectly to the safety, effectiveness, acceptability and quality of herbal product. Nowadays, the field of herbal medicines is progressing very fast but standardization of herbal drugs remains unexplored. While synthesizing herbal products, it is vital to have all the pivotal knowledge of that particular drug including all its pharmacological action, to phytoconstituents, to its standardization via several methods in respect to numerous parameters.

There is utmost need for more advanced techniques of standardization of herbal medicines. Many reservations regarding the standard and quality of mother tincture have been seen with the incredible increase in use of traditional herbal medicines. The development of new analytical techniques will provide a specific and prompt tool in the herbal research, so as to get rapid marketing approval from regulatory authorities. New standard method development is obligatory to establish quality control and to analyze the active constituents in herbal products, also good manufacturing practice (GMP), good agricultural practice (GAP) etc should be considered. This will encourage the practitioners to enhance the quality and also standardization process of the drug. Need of the hour is to develop techniques that comprises both modern methods and traditional methods of evaluation. The DC&TMD,NIH, Islamabad with partnership of WHO thought-out many workshops, trainings and few booklets as well to edify the shareholders of herbal and homoeopathic products to familiarize and contrivance standard GMP, quality assurance and implement procedures (Malik et al., 2013). The need of hour is a grim requisite to appliance these endorsements, derived from these events. The current study should be comprehensive to assess quality and standard of various used herbal mother tinctures.

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