Full Length Research Paper

Quality assessment of frequently available mother tinctures in the market by employing standard values

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Received 10 February, 2021; Accepted 17 March, 2021

As general awareness for using herbal drugs in today’s health practice is increasing, the world is endorsing extraordinary increase in the consumption of herbal products. This increase in the demand has led to consumers’ dissatisfaction due to substandard products, making the herbal market risky. There is need to set standard that will help to evaluate herbal drugs to ensure formulation of high quality products. The present investigation is conducted to evaluate the physiochemical measures of accessible mother tinctures from Aconitum napellus, Kalmia latifolia, Crataegus monogyna, Atropa belladonna and Digitalis purpurea. Mother tinctures were collected from five manufacturers in Pakistan and evaluated for physiochemical parameters, % alcohol contents, weight in dry/ml, % non-volatile matter and pH. World synchronization, following WHO specific guidelines for herbal products standardization is needed. Also, regulation should be endorsed to control herbal market adoption of WHO guideline.

Key words: Herbal drugs, standardization, quality control, Aconitum napellus, Kalmia latifolia, Crataegus monogyna, Atropa Belladonna, Digitalis purpurea.

INTRODUCTION

For many eras, simpler herbal therapies and medical plants have been used in the world for the cure and improvement of various illnesses. Even though the use of medicinal plants is as ancient as mankind itself; in the early nineteenth century, their controlled use, isolation, and characterization of active substances begun. In the advancement of modern pharmacotherapy, the extractive plant isolates and isolated active substances played a vital role. Isolated compounds have assisted as a model for the synthesis of a large number of drugs (Veeresham, 2012).

In world health, therapeutic herbal remedies have significant importance. Plants have important impact on health care due to the great advances observed in modern medicine in recent decades (Calixto, 2000). Natural herbal products have been our most fruitful source of medicines. Each plant acts like a factory that has the ability to prepare infinite number of highly complex chemical substances (Kinghorn, 2002).

According to WHO, in developing countries, 75 to 80% of the population is hooked on medicinal remedies in the health care due to their better compatibility and adequacy with minor side effects on the human body (Kamboj, 2000).

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There are about 120 distinct chemical substances in the world that are present in plants and are utilized as medicines; many other drugs are simple modifications of these substances (Farooqi and Sreramu, 2010).

Synthetic drugs are authenticated by standard process for quality control, but no such standard is available for traditional drugs. According to American Herbal Products Association, “Standardization denotes the information and controls essential to create material of suitable consistency. This is done by reducing the intrinsic difference of natural product composition by the quality control processes applied (Gaedcke et al., 2003; Waldesch et al., 2003).

Standardization of herbal medicines is necessary to evaluate quality of drugs established by the concentration of their major component, their properties (in vitro, in-vivo), and quality assessment (Madhav et al., 2011).

World Health Organization (WHO) emphasizes adaptation of the qualitative and quantitative procedure for characterization of the samples, if active component is known. Herbal formulations should be standardized to those compounds where major constituents are identified. However, if the active components are still not identified, a specific plant substance should be selected for investigative purpose (Dixit and Yadav, 2008; WHO, 1992).

To get public confidence and to permit homeopathic product into health care system, the quality assurance regulatory agencies must emphasis on standard international procedures to guarantee the accuracy and reliability of the traditional homeopathic drugs and medicines (Wani et al., 2007).

New system of medicine that brings the standardization and regulation of homeopathic products is of supreme significance to assess validation and adequacy (Bauer et al., 1998).

A mother tincture is a liquid obtained from the extraction of a suitable plant or animal material with alcohol or water mixture within a specific ratio (Banerjee, 2002). Due to various factors, the international costs of mother tinctures are on the increase, as local manufacturers have become main providers. Therefore it is essential to frequently assess the quality of homeopathic products (Lee et al., 2007).

In present years, to help regulate the safety of homeopathic medicines, there have been a number of efforts put in by WHO (2009). Throughout the precedent few decades, the management of homeopathic medicines preparations is popular and is required (WHO, 2009).

During homeopathic treatment, adverse events taking place are rarely recognized. Contamination of available substance and flaws of good manufacturing practice should be considered in safety assessment. Sufficient and authentic information on herbal products is progressively significant and purchased with consultation of a healthcare provider. Homeopathic medicines are benign, for its expansive use in children, pregnant women and the elderly; thus, its regulation, and standardization should be taken into consideration.

The current study was done to assess the physiochemical factors of Aconitum napellus, Kalmia latifolia, Crataegus monogyna, Atropa Belladonna and Digitalis purpurea manufactured by local homeopathic industries using standard procedures. These are available in different formulation to treat various illnesses.

MATERIALS AND METHODS

Collection and quality assessment

Twenty five mother tincture bottles were collected from Lahore and Rawalpindi (July, 2019) in Pakistan. All samples were put in storage by DCTMD, NIH, Islamabad 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, while storing. The ‘a’ sign was affixed after the name of each mother tincture, for example, Kalmia latifolia a. Mother tinctures were kept at an even temperature (stored between 10-20°C) of about 60°F (15.6°C) and stored in a dry, cool place in air tight, well closed, neutral flint glass bottles. Mother tinctures were well filtered to preserve them before storing or when dispensing. Mother tinctures of Aconitum napellus, Kalmia latifolia, Crataegus monogyna, Atropa belladonna and Digitalis purpurea, manufactured by five homeopathic industries were collected from local distributor shops and then evaluated for % Alcohol contents, weight on dry/ml, % non-volatile matter and pH using standard methodology (British Pharmacopoeia (BP), 1968).

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

Method-I

25 ml of mother tincture was measured in a graduated flask at 20°C, and was later 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 at same temperature. Measurements of the specific gravity at 20.0°C and the refractive index of the solution at 20.0°C were carried out. Ethyl alcohol content was determined by reference (refractive index does not differ by more than 0.00007).

Method II

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 specific gravity and refractive index were noted.

Method III

After applying method 1 and 2, and the refractive index still does not resemble the specific gravity, then the distillate is contaminated.
That is the 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 using phenolphthalein as indicator, before the final distillation (BP, 1968) Figure 1.

RESULTS

Twenty five sample bottles of Aconitum napellus, Kalmia latifolia Crataegus monogyna, Atropa Belladonna, Digitalis purpurea mother tinctures manufactured by 5 leading homeopathic industries were collected from different location in Pakistan that is, Islamabad, Rawalpindi, Lahore and physicochemical parameters % alcohol contents, weight on dry/ml, % non-volatile matter, pH were evaluated.

A. napellus shows alcohol content observed range of 5 mother tinctures 59.09 - 66.38% (limit= 57 - 85%), Weight on dry/ml observed range was 0.88 - 0.92 g/ml (limit = 0.890 to 0.920), with the exception of BM Weight on dry/ml which is 0.88; all other were within the range (Table 1). Non-volatile matter varies from 0.94 to 2.76% (range greater than 2.0% according to GHP) except for RL and WH Pvt. Ltd whose results are 2.34 and 2.76%. All other are out of range and are below 2.0% and pH observed range of 5.10 - 6.16 (limit = 5.5 to 6.5), with the exception of WH and MH pH whose observed range is 5.10 and 5.40, all other are within the range and are above 5.5. Alcohol content % ranges were given by manufacturers. pH and weight on dry/ml ranges were given in EHP, except % Non-volatile matter, which was specified in GHP (Figure 2 and 3).

K. latifolia shows alcohol content observed range from 59.09 to 70.10% (range 55 - 81%) (Table 2). Weight on dry/ml shows observed range from 0.895 to 0.916 (Range = 0.900 - 0.950) with the exception of MH Weight on dry/ml (0.895), all other are within the range. Non-volatile matter shows observed range from 0.76 to 2.6% (range greater than 4.5% according to GHP), none was greater than 4.5%. pH varies from 4.60 to 5.77 (Range = 4.5 to 5.5) except RL and MH Pvt. Ltd whose results are 5.64 and 5.77, all other are within the range. Range of Non-volatile matter is given in GHP (Figure 2 and 3).

C. monogyna shows alcohol content observed range from 63.38 - 70.10% (range 63 - 70%). Except for RL alcohol content is 63.38%, all other are out of range and are above 63.38% (Table 3). All observed range weight on dry/ml is 0.90 - 0.94 (Range = 0.904 - 0.926) except for MH Weight on dry/ml which is 0.94 (above expected limit). Non-volatile matter varies from 1.08 to 5.12 % (range greater than 3.0% according to GHP) except for RL Pvt. Ltd whose result is 5.12, all results are below the range. pH varies from 4.95 to 5.77 (range = 5.4 to 5.9) except for KL and WH Pvt. Ltd whose result are 4.95 and 5.25 that are below the range (Figure 6 and 7). A. belladonna shows alcohol content observed range to be between 31.76 and 50.19% (Range = 41 to 50) with the exception of WH alcohol content with an observed range of 31.76 - 34.99 which is below the range (Table 4). Weight on dry/ml has an observed range of 0.918 - 0.955 (range = 0.926 - 0.948), with the exception of BM Weight on dry/ml with an observed range of 0.918 that are below the range as well as RL and WH Pvt. Ltd whose results are 0.953 and 0.955, which are above the range. Non-volatile matter varies from 0.628 to 1.44% (range greater than 1.4% according to GHP) except for RL and WH Pvt. Ltd whose results are 1.44 and 1.43%, which are below the range. The pH observed range is
Table 1. Mother tincture of Aconitum napellus.

| Mother tincture | Alcohol content (%) | Weight on dry/ml (g/ml) | Non-volatile matter (%) | pH   | Manufacturer | City           |
|-----------------|---------------------|--------------------------|-------------------------|------|--------------|----------------|
| Aconitum napellus | 66.38               | 0.89                     | 1.4                     | 5.91 | KL           | Rawalpindi     |
|                 | 59.09               | 0.918                    | 2.34                    | 5.90 | RL           | Lahore         |
|                 | 70.10               | 0.88                     | 1.4                     | >2.0*| BM           | Lahore         |
|                 | 62.72               | 0.92                     | 2.76                    | 5.10 | WH           | Lahore         |
|                 | 62.72               | 0.91                     | 0.94                    | 5.40 | MH           | Lahore         |

*Observe value limit.

Figure 2. Comparison of pH and Weight on dry/ml of Aconitum napellus. 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 3. Comparison of % alcohol content % non-volatile matter of Aconitum napellus.
Table 2. Mother tincture (*Kalmia latifolia*).

| Mother tincture | Alcohol content (%) | Weight on dry/ml (g/ml) | Non-volatile matter (%) | pH | Manufacturer | City       |
|-----------------|---------------------|-------------------------|-------------------------|----|--------------|------------|
| *Kalmia latifolia* | 62.72               | 0.908                   | 2.6                     | 4.78 | KL           | Rawalpindi |
|                  | 62.72               | 0.916                   | 1.52                    | 5.77 | RL           | Lahore     |
|                  | 59.09               | 0.916                   | 1.9                     | >4.5*  | BM           | Lahore     |
|                  | 62.72               | 0.910                   | 1.10                    | 4.60  | WH           | Lahore     |
|                  | 70.10               | 0.895                   | 0.76                    | 5.64  | MH           | Lahore     |

*Observe value limit.

Figure 4. Comparison of pH and weight on dry/ml of *K. latifolia*.

Figure 5. Comparison of % alcohol content and % non-volatile matter of *K. latifolia*.
Table 3. Mother tincture (Crataegus monogyna).

| Mother tincture | Alcohol content (%) | Weight on dry/ml (g/ml) | Non-volatile matter (%) | pH     | Manufacturer | City     |
|-----------------|---------------------|-------------------------|-------------------------|--------|--------------|----------|
| Crataegus monogyna | 70.10               | 0.90                    | 2.84                    | 4.95   | KL           | Rawalpindi|
|                  | 63.38               | 0.917                   | 5.12                    | 5.41   | RL           | Lahore   |
|                  | 66.38 (43-65*)      | 0.91                    | 0.904-0.926*            | 2.16   | BM           | Lahore   |
|                  | 70.10               | 0.92                    | 1.08                    | 5.61   | WH           | Lahore   |
|                  | 66.38               | 0.94                    | 2.16                    | >3.0*  | MH           | Lahore   |

*Observe value limit.

Figure 6. Comparison of pH and Weight on dry/ml of C. monogyna.

Figure 7. Comparison of % alcohol content and % non-volatile matter of C. monogyna.
Table 4. Mother tincture (A. belladonna).

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

*Observe value limit.

Figure 8. Comparison of pH and Weight on dry/ml of A. belladonna.

5.75 - 6.97 (range = 6.4 - 7.0), with the exception of WH and MH pH observed range which is 6.97 and 6.53; all others are below the range (Figure 8 and 9).

Digitalis shows alcohol content observed range as 41.62 - 59.09% (limit= 39 - 50%) with the exception of WH and MH Pvt. Ltd whose result is 41.62 and 48.45%; all other are out of range and are above 50% (Table 5). Weight on dry/ml observed range from 0.943 - 0.948 (limit = 0.930 - 0.950), Non-volatile matter varies from 0.76 to 3.46% (range is greater than 2.5 but less than 3.8% according to GHP) except for KL and BM Pvt. Ltd whose results are 2.5 and 3.46%, all other are out of range and are below 2.5%. The pH observed range is 4.78 - 6.64 (limit = 5.5 - 6.5), with the exception of RL observed value which is 5.63; but all other results are out of range (Figure 10 and 11).

DISCUSSION

Natural merchandises play 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 of great demand. These products are greatly used as medicinal products, makeups and nutraceuticals. To have a good harmonization between the qualities of raw materials, processing of the materials and the final products, it is 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 herbal drugs (Kumari and Kotecha, 2016). In recent years, as an outcome of comprehensive development of science and technology, capability to form high-quality herbal medicines has 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.
Figure 9. Comparison of % Alcohol content and % non-volatile matter of *A. belladonna*.

Table 5. Mother tincture (*D. purpurea*).

| Mother tincture | Alcohol content (%) | Weight on dry/ml (g/ml) | Non-volatile matter (%) | pH          | Manufacturer | City      |
|-----------------|---------------------|--------------------------|-------------------------|-------------|--------------|-----------|
| Digitalis       | 59.09               | 0.948                    | 2.5                     | 5.20        | KL           | Rawalpindi |
|                 | 51.94               | 0.930-0.950              | 1.2                     | 5.63        | RL           | Lahore    |
|                 | 39-50               | 0.943                    | 3.46                    | >2.5 but <3.8 | BM           | Lahore    |
|                 | 41.62               | 0.944                    | 0.76                    | 4.78        | WH           | Lahore    |
|                 | 48.45               | 0.946                    | 1.86                    | 6.64        | MH           | Lahore    |

*Observe value limit.

Figure 10. Comparison of pH and Weight on dry/ml of *D. purpurea*.
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 enlighten people in the cultivation and 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 that is 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 and Biswas, 2014).

In developing or developed countries, plant materials are used as home remedies and as raw material for the pharmaceutical industry, making 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 from its pharmacological action, 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 the 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 standardize the process of the drug making. The need of the hour is to develop techniques that comprise both modern methods and traditional methods of evaluation. The DC&TMD, NIH, Islamabad in partnership with WHO has thought-out many workshops, trainings and few booklets to edify the shareholders of herbal and homoeopathic products to familiarize and contrivance standard GMP, quality assurance and implement procedures (Malik et al., 2013). It should be made mandatory to apply these commendations from such events and booklets.

**CONFLICT OF INTERESTS**

No conflict of interests.

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