

Pharmacognostic Evaluation of Dammul Akhawain with Reference to Standardization

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Abstract

In view of probability of adulteration especially in unorganized drugs, this study was designed for standardization of *Dammul Akhawain* (Dragon's blood) to generate data for future reference. The study was carried out on a samples of Dragon's blood obtained from the plant *Pterocarpus marsupium* Roxb, considered as the standard sample. The study comprised of morphology, physicochemical study, physical constants, preliminary phytochemical study and spectrophotometry. Since in literature parameters for this have not been mentioned sufficiently, therefore, the findings of this study may be considered as standard for quality assessment of available sample of Dragon's blood.

Keywords: Pharmacognosy, Unorganized drug, Standardization, Phytochemistry.

Introduction

Adulteration, substitution, misidentification, quality inconsistency, and controversy pertaining to herbal drugs are challenging the wide acceptability of traditional systems of medicine. Therefore, it is imperative to determine authenticity of crude drugs used for the preparation of medicaments, because it is associated with the safety of consumers. However, it becomes challenging when two or more plants are claimed to be the source of one drug especially in case of unorganized drugs, such as gum, resin, oleo-resin and oleo-gum-resin, as in such circumstances herbarium and drug museum, which are the main sources of information, become of little use (Bonakdar, 2002). Eventually, important drugs compromise their efficacy in spite of possessing significant effects.

Among the various steps to be taken for solving these problems, quality assessment of samples of all herbal drugs appears to be of utmost importance. Conventional methods of standardization substantiated with analytical techniques are considered most reliable tools for quality assessment of herbal drugs (Shinde, and Dhalwal, 2007). Most of the regulatory guidelines also suggest macroscopic, microscopic, physicochemical and phytochemical standardization of medicinal plants materials. HPLC, HPTLC, and spectrophotometry etc. may further reinforce the above methods (Xing *et al.*, 2010).

Dragon's blood (DB), as known in trade, is a bright red resin obtained from a number of different taxa such as *Croton*, *Dracaena*, *Daemonorops*, *Calamus* and *Pterocarpus* (Shinde, Dhalwal, 2007). Hence, a number of plants such as *Croton draconoides* Müll Arg, *Pterocarpus marsupium* Roxb, *Calamus rotang* Linn, *Croton draco* Schltdl & Cham, *Croton lechleri* Müll Arg, *Croton urucurana*

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Baill, *Croton xalapensis* Kunth, *Daemonorops draco* Blume, *Daemonorops didymophylla* Becc, *Daemonorops micranthus* Becc, *Daemonorops motleyi* Becc, *Daemonorops rubra* (Reinw ex Blume) Mart, *Daemonorops propinquus* Becc, *Dracaena cinnabari* Balf.f, *Dracaena cochinchinensis* Hort ex Baker, and *Pterocarpus officinalis* Jacq etc. have been accounted as the source of DB (Xing *et al.*, 2010). Such a big list of sources for one drug creates enormous degree of uncertainty. Even if two or more sources, as happens in case of some drugs, are considered the problem persists as it is not clear which source the available sample belongs to.

DB is a red resin and the name refers to reddish resinous product applied to many red resins described in literature (www.en.wikipedia.org, 2010). However, red gum resin of some of the above mentioned sources have the official status in their respective countries which they are indigenous to, such as red gum resin of *Pterocarpus marsupium* Roxb., family Fabaceae is known as Indian Dragon's blood (www.thefullwiki.org, 2010), red gum resin of *Dracaena cinnabari* is considered as Socotra Dragon's blood. Similarly, *Dracaena cochinchinensis* is said to be the source of Chinese Dragon's blood (www.aseanbiodiversity.info, 2010).

DB is an important drug used in Unani Medicine as Qabiz (astringent), Habis (styptic), Muqawie meda (stomachic) , Mohallil-e-Auram (resolvent), Dafe-Zaheer (anti dysentery) (Ibn Sina, 2007). Its liniment is useful in anal fissure, prolapsed rectum and inflammation of rectum. It is styptic and astringent for stomach whether used alone or with other astringent drugs, when used as enema it causes constipation (Khan,1314 AH).

In this study, red gum- resin of *Pterocarpus marsupium* Roxb. (DB) has been considered as the standard sample viewing that the same is commonly available in Indian crude drug markets. Therefore, it was obtained from the plant *Pterocarpus marsupium* Roxb, and was evaluated pharmacognostically to establish standard parameters for further studies.

Materials and Methods

Materials

The sample of DB was collected from *Pterocarpus marsupium* Roxb. grown in Erepalya, Bidadi, Hobli, District Ramnagar, Karnataka, India. It was authenticated by Dr. Shiddamallayya N and Dr. V. Rama Rao, vide Drug Authentication/ SMPU/NADRI/BNG/2010-11/961. A voucher specimen has been deposited in the Drug Museum of National Institute of Unani Medicine (NIUM), Bangalore, vide Voucher specimen No.01/IA/Res./2011.

Methods

Organoleptic evaluation

The organoleptic characters like color, odor, taste, luster, texture, fracture, consistency and cut surface of were examined by naked eye (Anonymous, 1968).

Physicochemical evaluation

For estimation of extractive values and ash values, methods described in British Pharmacopoeia (Anonymous, 1968) were applied. Moisture content was determined by TGA method (Anonymous, 1992).

Determination of pH value

The pH value of 1% and 10% aqueous solution was estimated by the method described by (Khandelwal, 2008; Brewster and Mcewen, 1971).

Determination of melting point

Melting point was estimated by melting point apparatus model C-LMP-1, Campbell electronics.

Solubility test

Solubility was tested by the method described in British Pharmacopoeia (Anonymous, 1968).

Qualitative Phytochemical evaluation

For preliminary phytochemical studies, powder of the DB was extracted in different solvents viz. petroleum ether, di-ethyl ether, chloroform, ethanol, acetone, benzene and distilled water. The extracts were subjected to various qualitative phytochemical tests for estimation of alkaloids, glycosides, carbohydrates, phenol compounds, tannins, phytosterols, proteins and amino acids etc.

Alkaloid was tested by Dragendorff's test, Mayer's test, Hager's test and Wagner's test (Anonymous, 1992). Protein and amino acids were tested by Ninhydrin test, Biurette's reaction, Million's reaction and Xanthoproteinic reaction (Khandelwal, 2008; Brewster, and Mcewen, 1971). Glycosides were tested by Molish's test (Paech and Tracey, 1955). Cardiac glycosides were tested by Keller-killiani test. Bufadenoloids were tested by Liebermann's test. Flavonoids were tested by Ammonia test (Anonymous, 1992). Saponin was tested by

Honey comb frothing test (Arthur and Chan, 1962). Tannins were tested by Ferric chloride test (Brewster and Mcewen, 1971). Phenols were tested by Ferric chloride test and Lead acetate test (Khandelwal, 2008). Phytosterols / Terpenes were tested by Hosse's reaction, Liebermann Burchard's reaction, and Moleschott's reaction (Khandelwal, 2008).

Test for Inorganic constituents

Ash of DB was prepared. To the ash 50% v/v hydrochloric acid and 50% v/v Nitric acid were added, and kept for an hour and then filtered. Various tests were performed with the filtrate for qualitative estimation of inorganic constituents such as sulphate, phosphate, iron, chloride, carbonate and nitrate (Brewster and Mcewen, 1971).

Spectrophotometry

The alcoholic extract was subjected to Spectrophotometry by using UV-VIS Spectrophotometer. The test was performed at room temperature with the following settings: Number: 18-1885-01-0259; Spectral Bandwidth: 2.00 nm; scan Range: 190.00 to 360.00 nm; Measure Mode: Abs; Interval: 1.00 nm, Speed: Medium.

Results

The results of macroscopic evaluation are shown in table 1 and figure 1&2. The extractive values taken in ethanol, chloroform, diethyl ether, pet. ether, benzene, acetone, and distilled water; mean percentage of total ash, acid insoluble ash, water insoluble ash and water soluble ash; moisture content as obtained by TGA method; mean percentages of pH value in 1% and in 10% aqueous solution, and the melting point are given in table 2. The Preliminary

Table 1: Morphology of Dragon's blood

S.No.	Characteristics	Result
1.	Color	Yellowish red
2.	Luster	Lustrous
3.	Fracture	Transverse
4.	Texture	Brittle
5.	Consistency	Liquid
6.	Odor	Odorless
7.	Taste	Astringent
8.	Cut surface	Smooth

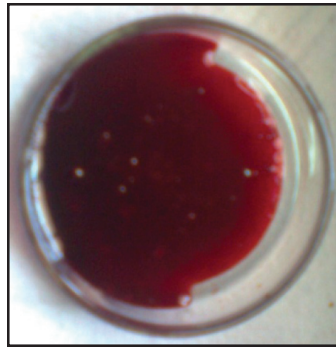


Figure 1: Samples of Dragon's blood



Figure 2: Powder of dried sample of Dragon's blood

Table 2: Physical constants of Dragon's blood

S.No.		Parameters	Values (Mean \pm SEM)	
1.	Extractive Values	Solvents	Pet. ether	0.00
			Di-ethyl ether	0.00
			Chloroform	0.00
			Benzene	0.00
			Acetone	28.26 \pm 0.86
			Ethanol	63.33 \pm 1.46
			Distilled water	68.44 \pm 2.25
2.	Ash Values	Total Ash	4.03 \pm 0.19	
		Acid insoluble Ash	2.59 \pm 0.14	
		Water insoluble Ash	12.94 \pm 0.16	
		Water soluble Ash	0.45 \pm 0.08	
3.	Moisture content		0.00%	
4.	pH	1% solution	4.66 \pm 0.17	
		10% solution	4.78 \pm 0.05	

Phytochemical screening of the different solvent extracts was done systemically for phytochemical constituents. Glycoside, amino acid and phytosterol were positive. Iron, nitrate and phosphate were also detected. Spectrum scanning gave three peaks and three valleys (Figure 3).

Discussion

Due to crude nature of herbal drugs, traders often take advantage of it and indulge in malpractices; however, it may happen due to ignorance as well. Usually it is noticed that commercial samples of some drugs do not match with their description in literature (Kartik *et al.*, 2010).

Table 3: Solubility of Dragon's blood in different organic solvents at different temperature

S.No.	Solvents	Result
		Temperature°C
		20 40 60 80
1.	Ethanol	Soluble
2.	Pet. Ether	Insoluble
3.	Di-ethyl ether	Insoluble
4.	Chloroform	Insoluble
5.	Benzene	Insoluble
6.	Acetone	Insoluble
7.	Distilled water	Insoluble

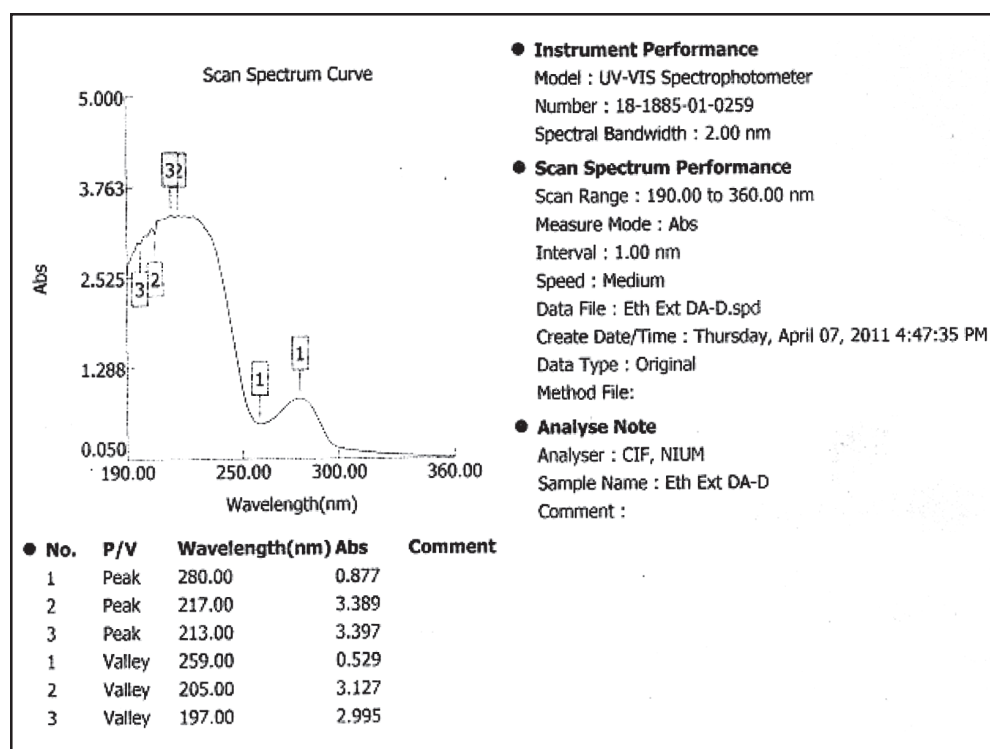


Figure 3: Spectrum Scan curves of Dragon's blood

So literature survey can give preliminary ideas about probable sources of drugs to draw some conclusion. Most of the literature consulted revealed that gum-resin of *Pterocarpus marsupium* (www.en.wikipedia.org), *Daemonorops draco* (www.botanical.com), *Dracaena cinnabari* (Al-Awthman *et al.*, 2010), and *Dracaena cochinchinensis* (www.aseanbiodiversity.info, 2010; www.aseanbiodiversity.info, 2010) may be considered as the sources of DB for different countries. It

was also concluded that gum-resin of *Pterocarpus marsupium* Roxb is the Indian Dragon's blood.

Morphological studies of crude drugs give some idea at very first sight. Color, odor, taste, luster, fracture, and consistency etc. (Evans, 2008; Pearson and Prendergast, 2007) are some prominent characters, therefore, these characters were observed.

It is important to note that physical constants of a drug are good criteria for identification. These constants are extractive values, ash values, and moisture content. These parameters are widely accepted for checking purity of drugs (Anonymous, 1992). These parameters too were applied in this study.

The constituent of a particular drug sample can be estimated in terms of % with respect to air dried weight by extracting in various solvents known as extractive value which was applied to the sample. In literature gum resin of *Pterocarpus marsupium* has been reported to be 80 - 90 % soluble in alcohol. Our finding demonstrated no yield in diethyl ether, pet. ether and benzene. However the yield % was 28.26 ± 0.86 , 63.33 ± 1.46 , 68.44 ± 2.25 in ethanol, acetone and distilled water, respectively.

Ash value is taken in terms of total ash, acid insoluble, water insoluble and water soluble ash. In some cases there may be considerable difference in total ash value in the same drug which may either be due to variation in the amount of oxalate or some adulteration with metallic and the like materials or earthy material. In such cases acid insoluble ash is taken into consideration. This parameter was also applied in our study.

The water content (moisture) of crude drugs is another important parameter for checking purity of drugs. In this study, Thermo gravimetric Analysis (TGA) method was applied for estimation of moisture content. This method is suitable for all types of substances as it provides quantitative measurement of mass change in materials. It records changes in mass from dehydration, decomposition and oxidation of a sample with time and temperature (El-Sayd and Makawy, 2010). We found 0.00% moisture in the sample.

DB is a gum-resin and for these types of drugs, melting point, pH and solubility may be considered important parameters. Gum-resin is insoluble in water and petroleum ether but more or less soluble in alcohol, chloroform, and ether. Crude drugs containing mixed chemicals are described with certain range of melting points. pH of solution of a substance at 1 % w/v and 10% w/v of water soluble portion can give accurate estimation of purity of a drug. These parameters were also. No data regarding melting point and pH of gum resin of *Pterocarpus marsupium* are available in literature; therefore, we considered our findings as stand. In literature melting point of gum resin of *Calamus draco*

is shown to be 76°C. (www.henriettesherbal.com, 2010). The melting point of standard sample did not coincide with the reported finding. Similarly, pH and solubility were estimated at 20, 40, 60, and 80°C. Except solubility, no data on gum- resin of *Pterocarpus marsupium*, which is 80-90 % soluble in cold water and almost soluble in alcohol, are available (Kokate, 2007), we considered our results as standard.

The analysis of physiologically active compounds is important parameter for checking the authenticity of a drug. These compounds are alkaloids, glycosides, flavonoid, phytosterol, essential oil, resin, tannin, etc. These were also estimated. Analysis of inorganic constituents may also be considered parameter for checking the authenticity of drug. In literature *Pterocarpus marsupium* is shown to contain flavonoids, tannins, and phytosterol. Our findings are in confirmation with the report.

Spectrophotometry may be a sophisticated tool for standardization of crude drugs. Spectrum scanning curves were obtained to get preliminary information. It demonstrated peaks and valleys of different absorbance and wave length, indicating presence of different constituents.

Conclusion

Detailed data on DB regarding physical, chemical and other properties are not available to compare our findings, therefore, our findings may be considered as standard for Indian Dragon's blood for future reference.

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References

- Al-Awthan, Y.S., Abu Zarga, M. and Shtaywy A., 2010. Flavonoids Content of *Dracaena cinnabari* Resin and Effects of the Aqueous Extract on Isolated Smooth Muscle Preparations, Perfused Heart, Blood Pressure and Diuresis in the Rat. *Jordan J Pharmaceuti Sci.* 3 (1):8 -17.
- Anonymous, 1968. British Pharmacopoeia, General Medical Council. Pharmaceutical Press, London, pp.11276-77; 1285- 88.
- Anonymous, 1992. Quality Control Methods for Medicinal Plant Materials, rev. 1, Organisation Mondiale De La Sante. World Health Organisation, p.159.
- Arthur, H.R. and Chan, R.K.R., 1962. A Survey of Hong Kong Plants testing for Alkaloids, Essential oil, and Saponin. *Trop-sci.* 4: 147.

- Bonakdar, R.A., 2002. Herbal cancer cures on the Web: noncompliance with the Dietary Supplement Health and Education Act. *Family Medicine* 2002, July - Aug.; 34(7):522-527. [cited 2010Aug.5] <http://www.ncbi.nlm.nih.gov/pubmed/12144007>.
- Brewster, R.C. and McEwen, W.E., 1971. *Organic Chemistry*, 3rd ed. Prentice-Hall of India private Ltd, New Delhi, p. 406.
- El-Sayd, N.I., and Makawy, M.M., 2010. Comparison of Methods for Determination of Moisture in Food, *Research J Agriculture and Biol Sci.* 6(6): 906-11.
- Evans, W.C., 2008. *Trease and Evans Pharmacognosy*, Elsevier, A Division of Reed Elsevier India Private Ltd., New Delhi, pp. 57-60, 525.
- Ibn Sina, 2007. *Al-Qanun Fit Tib.* Part 1st, Vol.2nd. (Urdu translation by Kantoori, H.G.). Idara Kitab-ul-Shifa, New Delhi, p. 80.
- Kartik, C.P., Surendra, K.P., Ranjit, K.H., Kumar, K.J., 2010. Traditional Approaches towards Standardization of herbal Medicines-A review. *J Pharmaceut Sci and Technol* 2 (11): 372-379.
- Khan, M.A., 1314H. Muheete Azam, Part 2, Vol. 2nd. *Matabae Nizami*, Kanpur, pp. 18-19.
- Khandelwal, K.R., 2008. *Practical Pharmacognosy-Techniques & Experiments*, Nirali Prakashan, Pune, pp. 146-59,193.
- Kokate, C.K., 2007. *Practical Pharmacognosy*, Vallabh Prakashan, Delhi, p. 96.
- Paech, K. and Tracey, M.V., 1955. *Modern Methods of Plants Analysis*, 3rd Vol. Springer & Verlag Gottingen. Hidelbrg, Berlin,pp. 295,334,471.
- Pearson, J. and Prendergast, H.D.V., 2007. Collection corner Daemonorops, *Dracaena* and other Dragon's Blood. *Economic Botany*; 96.
- Shinde, V. and Dhalwal, K,2007. Pharmacognosy: The Changing Scenario. *Pharmacognosy Reviews* 1 (1): 1-5.
- Wallis, T.E., 2005. *Text book of Pharmacognosy*, 15th ed. CBS Publication, New Delhi, pp. 556-58, 578-79.
- www.aseanbiodiversity.info/Abstract/51009754.pdf [cited 2010 March 18].
- www.aseanbiodiversity.info/Abstract/51009754.pdf [cited 2010 March 18].
- www.botanical.com/botanical/mgmh/d/dragon20.html [cited 2010 March 17].
- www.en.wikipedia.org/wiki/Dragon%27s_blood Categories: Incense | Magic (paranormal) | Pigments | Resins). [Cited 2010March 17].
- www.en.wikipedia.org/wiki/Pterocarpus_marsupium [cited 2010 Dec. 30].
- www.henriettesherbal.com/eclectic/bpc1911/calamus-drac.html [cited 2010 July 13].

www.thefullwiki.org/Dragon%27s_blood#wikipedia_Name_and_source. [cited 2010 Oct. 24].

Xing Hong Wang, Changhe Zhang, Ling Ling Yang, Xiao-Hong Yang, Ji-Dong Lou, Qiue Cao and Jose Gomes – Laranjo, 2010. Enhanced dragon's blood production in *Dracaena cochinchinensis* by elicitation of *Fusarium oxysporum* strains. *J. Med. plants Res.* (24): 2633-2640.

