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Qualitative Nature of Some Traditional Crude Drugs Available in Commercial Markets of Mumbai, Maharashtra, India

S. Ganesan* and Roopam Y. Bhatt

Welex Laboratories Pvt. Ltd.
Plot No. J - 134, M.I.D.C., Tarapur- 401506
Maharashtra, India.

E.mail: sganesan76@yahoo.com

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Abstract

Medicinal plants have been used since ancient times for the treatment of human ailments. Interest in medicinal plants has been shown throughout the world because of the safe and effective constituents of plant products. The increasing demand for herbal medicines, both in the developing and developed countries, has provided the stimulus for the workers in this field to maintain the quality and purity of their herbal raw materials and finished products. The standardization problem relating to herbal drugs arises from the complex composition of drugs that are used in the form of whole plants, plant parts or extracts obtained there from. To ensure reproducible quality of any herbal remedy, proper control of starting material is utmost essential. With this thought in mind, the present preliminary study was conducted in order to discover the qualitative nature of 20 Traditional crude drugs available in the commercial markets of Mumbai, Maharashtra, India.

Key words: Traditional medicine, crude drugs, markets, Mumbai.

Introduction

In India, the indigenous systems of medicine, namely Ayurvedic, Siddha and Unani, have been in existence for several centuries. These traditional systems of medicine together with Homoeopathy and Folklore medicine continue to play a significant role in the health care system of the population. Besides the demands for medicinal plants made by these systems as their raw material, the demand for medicinal plants made by the modern pharmaceutical industries has also increased manifold. Thus, medicinal plants constitute a group of industrially important crops which bring appreciable income to the country by way of export (Ganesan *et al*., 2006).

The majority of the medicinal and aromatic plants used by the herbal drug industry come from wild collections. In Madagascar, for example, over 80% of the medicinal plants in that are used in this trade come from wild collections. Similarly, about 90% of India's medicinal plants supply to

international market is from wild stocks. Hardly 10% of raw materials come from cultivated sources, which offer buyers more constituent quality and lower risk of adulteration than do their wild counterparts (Handa, 2005).

The adulteration of market samples is one of the greatest drawbacks in promotion of herbal products from India (Dubey, 2004). Plant samples in the market are stored under undesirable conditions over the years and often contain a mixture of other plant species (Khatoon et al., 1993), thus adversely affecting their bioefficacy. The efficacy of many of drugs is fading because of the adulterated, dried raw materials profusely available in the indigenous market (Anon, 1996). With this view in mind, the present preliminary study was conducted in order to discover the qualitative nature of Traditional crude drugs available in commercial markets of Mumbai, Maharashtra, India.

Materials and Methods

Collection and Identification of Crude drugs

The common and locally available Ayurvedic crude drugs were collected from Mumbai commercial markets. All samples were identified through comparison with standard references (The Ayurvedic Pharmacopoeia of India, Part I, Vol.I.1989; Vol. II. 1999; Vol. III. 2001; Selected Medicinal Plants of India, 1992; Nadkarni, 2002). Voucher specimens were photographed and archived at Welex Laboratories Pvt. Ltd, Tarapur- 401506.

Test for physiochemical parameters

All samples were pulverized with the help of a stone pestle and mortar and sifted with an IS/0.300mm sieve. Then, the fine powdered samples were stored in airtight polythene covers. The stored samples were tested for purity and strength following the procedure described in The Ayurvedic Pharmacopoeia of India.

Qualitative screenings of Phytochemicals

The qualitative screenings of powdered crude drugs for their active ingredients were carried out using the following standard procedures (Trease and Evans, 1983; Indian Pharmacopeias, 1996; Mukherjee, 2002; Horborne, 2005):

Test for Alkaloids

Extract 2g of powdered drug by warming for 2 min. with 20ml 1% sulphuric acid in a 50ml conical flask on a water bath, with intermittent shaking, centrifuge; pipette off the supernatant into a small conical flask.

Make an initial test for alkaloids by adding to 0.1 ml extract in a semi-micro tube, one drop of Meyer's reagent. It gives a cream precipitate with alkaloids.

Preparation of Mayer's reagent:

It is prepared by dissolving 1.36g of mercuric chloride in 60 ml distilled water (A) and 5g of potassium iodide in 60ml of distilled water (B). A and B are mixed together and the volume adjusted to 100ml with water.

Test for Essential oil / Volatile oil

Test 1.

Crush a small sample of the crude drug between the thumb and forefinger, and examine for the

presence of an odour.

Observation: Drug containing volatile oils have a strong odour.

Test 2.

Extract 1g of the powdered drug by warming with 10ml petroleum sprit (boiling point range 40-60 C.) in a boiling tube heated on a water bath. Do not let the solvent boil dry. Filter the mixture into an evaporating dish and concentrate the filtrate to about 1ml on a water bath. Using a pipette apply one drop of the extract to a filter paper. Expose the paper to a current of warm air and note the occurrence of any translucent area. If this observed, then oils are present.

Observation 1.

Place the paper in an oven at 105 C for 15min. and if the translucent spot can still be observed after that time, then a fixed oil is present.

Observation 2.

The presence of a volatile oil is detected by the disappearance or diminution of the translucent area.

Test for Flavonoids (flavone)

Test 1.

Prepare an aqueous filtrate of powdered drug, and take a portion of filtrate in a test tube, add 5 ml of dilute ammonia followed by add few drops of concentrated sulphuric acid. A yellow coloration is appears. Upon further standing, the yellow coloration disappears.

Test 2.

Take a small amount of powdered drug in a test tube, add 10 ml ethyl acetate and heat it over a steam bath for 3min. then filter the mixture, take 4 ml of the filtrate with 1ml of dilute ammonia solution. Observe the formation of yellow colouration. It is the indication of flavanoids compounds of drug.

Test for Glycosides

Test 1:

Extract 200 mg of the sample by warming in a test tube with 5ml of dilute (10%) sulphuric acid (Test with PH paper) on a water bath at 100C for 2min. centrifuge or filter, pipette off the supernatant or filtrate.

Neutralize the acid extract with 5% solution of NaOH (Noting the volume of NaOH added).

Add 0.1ml of Feling's solution 'A" and then Fehling's solution 'B' until alkaline (Test with PH paper) and heat on the water bath for 2min. Note the quantity of red precipitate formed and compare with that formed in Test 2.

Test 2:

Extract 200mg of the sample using 5ml of water instead of sulphuric acid. After boiling add a volume of water equivalent to the volume of NaoH used in Test 1.

Add 0.1ml of Fehling's solution A and then Feling's solution B until alkaline (test with PH paper) and heat on the water bath for 2min. and note the quantity of red precipitate formed (Test.2.)

Compare the quantity of precipitate formed in Test 2 with that formed in Test 1. If the precipitate in

Test 1 is greater than that in Test 2, then glycosides may be present, since Test 2 represents the amount of free reducing sugars already present in the crude drug, whereas Test 1 represents free reducing sugars plus those released on acid hydrolysis of any glycosides in the crude drug.

Tests for Potassium Salts

Dissolve 0.1g of substance being examined in 2 ml of water. Heat the solution with 1ml of sodium carbonate solution (10.6% w/v), no precipitate is formed. Add 0.05 ml of sodium sulphite solution (10%), no precipitate is formed, cool in ice , add 2 ml of a 15% w/v solution of tartaric acid and allow to stand, a white crystalline precipitate is produced.

Test for Saponin

Take 2g of the powdered sample and boil with 20 ml of distilled water in a water bath and filter it, 10 ml of filtrate is mix with 5 ml of distilled water and shake vigorously for a stable persistent froth. To this froth mix 3 drops of olive oil and shake vigorously, then observe for the formation of emulsion.

Tests for Starch

Take 1g of dry powder in 50 ml of water boil for one minute and cool, a thin and cloudly mucilage is produced, which gives thick and more transparent mucilage.

To 10 ml of the mucilage add 0.05 ml of 0.01M Iodine, a dark blue colour is produced, which disappears on heating and reappears on cooling.

Test for Tannins

Take 0.5g of the dried powdered sample in 20ml of water, boil on a water bath and filter it in a test tube. Add few drops of 0.1% ferric chloride and observe for brownish green or a blue-black colouration.

Test for Terpenoids (Salkowski test)

Take five ml of extract, mixed with 2 ml of chloroform, and concentrated H_2SO_4 (3ml) is added to form a layer. A reddish brown colouration on the inner face is formed. It is indicates the presence of terpenoids.

Test for Vitamin C or Ascorbic Acid

To 2ml of 2% w/v solution, add 2ml of water, 0.1g of sodium bicarbonate and about 20mg of ferrous sulphate, shake and allow stand; a deep violet colour is produced. Add 5ml of 1M sulphuric acid, the colour disappears.

Results and Discussion

Identification of plants with botanical verifications is essential as contamination due to misidentification of plant species or parts is common. Characterizing compound or biomarker is identified from the plant part to assure the identity and quality of the preparation, this need not be responsible for the therapeutic activity. Details including various names (binomial, vernaculars, etc.) with collection conditions, and part to be used should be documented to ensure proper identification (WHO, 2001).

In the present study, a total of 20 crude drugs were analyzed, 8 from root origins, 6 from fruits, 3 from leaves, 2 from stem bark and one from an insect gall. The details regarding the names of the plants (Binomial), local name (Hindi), appearance, organoleptic test and location collection are enumerated in

Table-1.

The therapeutic potentials of plant and animal origins have been used from ancient times by a simple process without the isolation of pure compounds (i.e. in the form of crude drugs or the galenicals prepared from them). The pharmacological action of crude drug is determined by the nature of its constituents (Mukherjee, 2002), such as alkaloids, terpenoids, flavonoids, glycosides, saponins, tannins, etc.

A test requirement for foreign matter would ensure the extent of contamination of extraneous matters, such as filth and other parts of botanicals not covered by the definition of the herbal drug. Since sand and soil are predicable contaminations of botanicals, test requirements for total ash, water-soluble ash, acid insoluble ash, residue on ignition and sulphated ash would be expected to limit such contaminants (Handa, 2005).

In the present study, among 20 crude drugs tested against purity and strength, all were soluble in water and ethanol in different degree. Higher levels of water-soluble extractives were noticed only in 5 crude drugs, viz., *Emblica officinalis* (50%), *Pistacia integrima* (40.8%), *Picrorhiza kurroa* (35.20%), *Abies webina* (34%) and *Glycyrrhiza glabra* (30%). The lowest levels were found in *Helecteres isora* and *Tecoma undulata* 8% in each. In ethanol soluble extractives, *Emblica officinalis* (40%) and *Pistacia integrima* (32.8%) resulted in maximum levels of solubility (Table-2.). It may be due to the presence of soluble nature of the compounds.

In the total ash contents, *Tribulus terrestris* (14%) and *Adhatoda vasica* (12%) having higher level of ash content and remaining drugs in the range of 4-10% of total ash. In acid insoluble contents of crude drugs, *Boerhaavia diffusa* and *Cyperus rotundus* contain more amount (4%) and remaining drugs having 0.1 to 2% range (Table-2.).

The identification of biologically active compounds is an essential requirement for quality control and dose determination of plant based drugs. A medicinal herb can be viewed as a synthetic laboratory as it produces and contains a number of chemical compounds. These compounds responsible for medical activity of the herb are secondary metabolites (Dubey *et al*., 2004).

In the qualitative phytochemical screening, all the crude drugs have the main constituents (Table-3). Among 20 drugs screened, 65% of drugs have tannins, 40% of drugs contain volatile or essential oils, 35% of drugs have glycosides and saponins. Alkaloids and starch are also present in 30% of drugs as a main component. Mostly these are the very common ingredients of Ayurvedic medicinal drugs. Further to confirm the presence of these active principles, quantitative analysis is essential. Hence at the time of sampling of crude drugs from commercial markets, this qualitative analysis is necessary one.

The difference in the purity and strength of the crude drugs may be due to quantitative and qualitative difference in the active principles or presence of compounds. As well as the nature of drugs (young or mature parts), geographical location, condition of harvest, processing of drugs (sun dry, shade dry, oven dry), storage etc., the amount and nature of active constituents is not constant throughout the year. The age of the plant is also of considerable importance and governs not only the total quantity of the active constituents produced, but also the relative proportions of the active principles (Evans, 2002 and Horonok, 1992).

It has been reported that the content of Taxol in *Taxus baccata* leaves and extracts stored at room

temperature for one year decreased by 30-40% and 70-80% respectively, while storage in a freezer and out of direct sunlight produced no adverse deterioration (Das *et al*., 1998).

Other reports indicated that the stored drug samples harbour mycotoxin producing fungi in high frequency. Degradation of alkaloids and medicinally valuable secondary metabolites of stored plant drugs due to fungal infestations has been reported (Horie et al., 1979; Narita et al., 1980; Roy and Chaurasia, 1989). It would be, therefore, advisable to treat plant drugs with non-toxic chemicals at various stages of storage and processing (Dubey et al., 2004).

Ideally the exact geographical source of a herbal material so much so the condition under which it has been grown, harvested, dried and stored should be known, the chemical treatment such as pesticide or fumigants used during harvesting or storing should be known. However, in many cases since the herbal raw materials are obtained from varied geographical and commercial sources, the above conditions may not be always known, for these reasons, therefore appropriate level of testing in addition to the monograph criteria of any official book should be carefully assessed, based on various factors including the nature of material, knowledge of its batch history and test result from previous batches (Mukherjee, 2002). Further test also carried out for the identification and quantification of active principles using TLC and HPTLC methods with their marker compounds, comparison of these active compounds with crude drugs collected from different geographical regions of India.

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Table 1. List of Crude Drugs and their Description

S. No.	Name of the Sample	Local Name	Appearance	Organoleptic Test	Location of Market
1.	***Abies webina***	Talispatra	Dried leaves with small stem pieces	Mild astringent with Characteristic odour	Mumbai
2.	***Adhatoda vasica***	Vasaka	Dried pieces of leaves	Bitter	Mumbai
3.	***Boerhaavia diffusa***	Punarnava	Dried entire Root	Astringent with characteristic odour	Mumbai
4.	***Chichorium indipus***	Kasini	Dried seeds	Astringent	Mumbai
5.	***Clerodendrum***	Bharangimul	Dried stem	Mild	Mumbai

	indicum		pieces	astringent	
6.	***Cyperus rotandus***	Motha	Dried entire tubers	Mild bitter with characteristic odour	Mumbai
7.	***Emblica officinalis***	Avla	Dried cut pieces of Fruits	Mild sour with astringent	Mumbai
8.	***Glycyrrhiza glabra***	Yastimadhu	Dried Rhizome Pieces	Sweetish	Mumbai
9.	***Hedychium spicatum***	Kapurkachri	Dried cut pieces of rhizome	Mild bitter with Characteristic odour	Mumbai
10.	***Helecteres isora***	Marodfali	Dried entire Fruits	Oily with characteristic odour	Mumbai
11.	***Holarrhena antidysentrica***	Kutaj	Dried pieces of Bark	Strong bitter	Mumbai
12.	***Picrorhiza kurroa***	Kutki	Dried pieces of Rhizome with Root	Strong Bitter	Mumbai
13.	***Pimpinella anisum***	Saunf	Dried Fruits	Mild sweet with characteristic odour	Mumbai
14.	***Pistacia integerrima***	Kakdashringi	Dried entire Gall	Astringent with mild bitter	Mumbai
15.	*Smilax China*	Chopchini	Dried cut pieces of root	Astringent with characteristic odour	Mumbai
16.	***Tecoma undulata***	Rohitak	Dried bark pieces	Astringent with characteristic odour	Mumbai
17.	***Trachyspermum ammi***	Ajwayan	Dried fruits	Mild hot with characteristic odour	Mumbai
18.	***Tribulus terrestris***	Gokhru	Dried entire Fruits	Astringent	Mumbai
19.	***Withania somnifera***	Ashwagandha	Dried entire Root	Mild bitter	Mumbai
20.	***Zingiber officinalis***	Sonth	Dried Rhizome	Mild hot with Characteristic odour	Mumbai

Table 2. Purity and Strength of Crude Drugs.

S. No.	Name of the Sample (Binomial)	pH*	Loss On Drying* (%)	Water Soluble Extractive* (%)	Ethanol Soluble Extractive* (60%)	Total Ash* (%)	Acid Insoluble Ash* (%)
1.	*Abies webina*	5.37	8	34	16.8	6	1
2.	*Adhatoda vasica*	8.82	18	28	13.5	12	1
3.	*Boerhaavia diffusa*	6.48	10	9.6	10	10	4
4.	*Chichorium indipus*	6.20	10	9.6	3.2	8	1.5
5.	*Clerodendrum indicum*	6.48	14	12	6	4	1
6.	*Cyperus rotandus*	5.94	12	20	8	4	4
7.	*Emblica officinalis*	3.28	18	50	40	7	2.5
8.	*Glycyrrhiza glabra*	5.71	16	30	22	10	2
9.	*Hedychium spicatum*	6.47	20	20	11	8	1
10.	*Helecteres isora*	6.68	16	8	10	10	1.5
11.	*Holarrhena antidysentrica*	5.37	12	22	18	6	1
12.	*Picrorhiza kurroa*	5.22	16	35.20	17.60	7	1
13.	*Pimpinella anisum*	6.16	16	15	8	8	1
14.	*Pistacia integerrima*	4.01	16	40.8	32.8	7	0.1
15.	*Smilax China*	5.63	8	20	7	10	2
16.	*Tecoma undulata*	5.22	12	8	6.6	8	1
17.	*Trachyspermum ammi*	5.90	10	20	12.8	9	0.1
18.	*Tribulus terrestris*	6.43	6	18.4	12	14	2

19.	*Withania somnifera*	5.83	10	20.8	15	7	1
20.	*Zingiber officinalis*	3.51	22	25	15	6	1

* = Each value is a mean of Triplicates

Table 3. Screening of Active Principles of Crude Drugs.

S. No.	Name of the Sample (Binomial)	Active principle	Qualitative Tests									
			Alk1	**Es/Vo**	**Fla**	**Gly**	**Pos**	**Sap**	**Sta**	**Tan**	**Ter**	**Vit.C**
1.	*Abies webina*	Tannins, Essential oil	+	+	-	-	-	-	-	+	-	-
2.	*Adhatoda vasica*	Vasicine	+	-	-	-	-	-	-	-	-	-
3.	*Boerhaavia diffusa*	Alkaloid	+	-	-	-	-	-	-	-	-	-
4.	*Chichorium indipus*	Starch	-	-	-	-	-	-	+	-	-	-
5.	*Clerodendrum indicum*	Glycosides, Tannin, Terpenoids	-	-	+	-	-	+	-	+	-	-
6.	*Cyperus rotandus*	Starch, Saponins, potassium salts	-	+	-	-	+	+	+	-	-	-
7.	*Emblica officinalis*	Tannin, Vitamin-C	-	-	-	-	-	-	-	+	-	+
8.	*Glycyrrhiza glabra*	Glycyrrhizin	-	-	+	+	+	+	-	+	-	-
9.	*Hedychium spicatum*	Volatile oil, Starch	-	+	-	-	-	-	-	+	-	-
10.	*Helecteres isora*	Saponin, Tannin	-	+	-	-	-	+	-	+	-	-
11.	*Holarrhena antidysentrica*	Conessine	+	-	-	+	-	-	-	+	-	-
12.	*Picrorhiza kurroa*	Kutkin, Picrorhizin	-	-	-	+	-	-		-	-	-
		Flavonoids,										

13.	*Pimpinella anisum*	Saponin, Tannin	-	+	+	-	-	-	-	+	-	-
14.	*Pistacia integerrima*	Tannin	+	+	-	+	-	-	-	+	-	-
15.	*Smilax China*	Saponin, Glycosides	-	-	-	+	-	+	+	+	-	-
16.	*Tecoma undulate*	Tannin, Saponin	-	-	-	+	-	+	-	+	-	-
17.	*Trachyspermum ammi*	Volatile oil	-	+	-	-	-	-	-	+	-	-
18.	*Tribulus terrestris*	Saponin, Alkaloids	+	-	-	-	+	+	+	+	-	-
19.	*Withania somnifera*	Withanolides	+	-	-	+	-	-	+	-	-	-
20.	*Zingiber officinalis*	Gingerol	-	+	-	-	-	-	+	-	-	-

Alk. - Alkaloids, Es/Vo. - Essential oil/Volatile oil, Fla. - Flavonoids, Gly. - Glycosides, Pos. - Potassium salts, Sap. - Saponin, Sta. - Starch, Tan. - Tannins, Ter. - Terpenoids, Vit.C. - Vitamin-C.

+ = Presence of active principle.