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Pharmacognostic and Physico-chemical Standardization of *Monotheca buxifolia* (Falc.) A. DC.

Maryam Ehsan[a]*, Muhammad Ibrar[a], Fazal Hadi[a] and Barkatullah[b]
[a]Department of Botany, University of Peshawar, KPK, Pakistan
[b]Department of Botany, Islamia College Peshawar, KPK, Pakistan

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Abstract. *Monotheca buxifolia* is an ethno-medicinally important plant of Pak-Afghan regions. The pharmacognostic standards of fruit, leaf, seed, barks of stem and root were set in present study. Microscopy revealed characteristic distinguishable powder drug fragments. Each part showed certain unique florescence behaviour with various reagents under light of various wavelengths. It has a broad spectrum phytochemical profile including amino acids and proteins, fats (fixed oils), sugars (both non reducing and reducing), alkaloids, flavonoids, glycosides, tannins, triterpenoids, phenolics, saponins, phytosterols and anthocyanins. The fruit had 15.5% moisture contents seed and leaf had 8.5% each and stem and root bark had 9.5% each. The values of total ash were 7.0, 6.0, 4.5, 8.25 and 11.75%; values of water soluble ash were 4.9, 3.5, 0.5, 4.2 and 6.75%, while the values of acid insoluble ash 1.5, 1.75, 4.0, 3.75 and 4.25% in fruit pulp, leaf, stem bark, root bark and seeds, respectively. The fixed oil yield of *M. buxifolia* was measured to be 8.33, 10.62 and 0.56% in fruit pulp, seeds and leaves, respectively. Palmitic acid, Oleic acid, Linolenic acid, Stearic acid and Myristic acid were the most frequently found fatty acids in each part. The plant is a rich source of phosphorus, nitrogen and potassium. The plant contained safe amounts of tested trace elements as directed by WHO except for cobalt in root bark (10.7±0.01) and lead in stem bark (22.48±0.33).

Keywords: pharmacognostic, *Monotheca buxifolia*, drug fragments

Introduction

Monotheca buxifolia is a short spiny tree widely distributed in the hilly areas of Pakistan and Afghanistan, locally called "gurguray". It has well known ethnobotanical uses as a source of edible fruit, fuel, fodder and medicinal benefits like diuretic, laxative, purgative, refrigerant and haematinic (Rashid and Khan, 2009). *M. buxifolia* has derived interest of various researchers for different medicinal aspects in the recent years. Anwar *et al*. (2018) reported the *in-vitro* antioxidant activity of Ag-capped *M. buxifolia* (Flac.). It has experimentally proven role in treatment and management of depression and free radicals based disorders (Burki *et al*., 2018), antipyretic and analgesic activities (Hassan *et al*., 2017), anti-proliferative activity against the human lung cancer (Javed *et al*., 2016), inhibitory actions against Urease enzyme activity and potentials of renal tissue protection; significant antipyretic, anti-inflammatory and anti-nociceptive properties and protective effects against hepatic damage induced by rifampicin and isoniazid (Ullah *et al*., 2016 a, b and c), hypoglycemic potentials (Javed *et al*., 2015), *in-vitro* antioxidant potential and radical scavenging activity (Jan *et al*., 2013; Rehman *et al*., 2013) anthelmintic, antiseptic and antimicrobial activities (Hazrat *et al*., 2013; Shah *et al*., 2012).

Keeping in view the immense increase in medicinal importance of this plant the present work is an effort to standardize *M. buxifolia*, as the purity and quality of any drug assures its effectiveness. By standardizing a drug, constant ratios of bio-active components, uniform potency, and adulteration free drugs are obtained (Fernandez-Bolanos *et al*., 2006). Pharmacognostic studies help in stepwise standardization of natural drugs especially those of herbal origin by evaluation of their morpho-anatomical and physico-chemical perspectives (Samuelsson and Bohlin, 2017).

Materials and Methods

Collection and preservation. The plant collections were made from Paito Dara, Lower Dir, Pakistan. It was identified and authenticated from Peshawar University's Department of Botany and a voucher specimen was submitted to the same department (herbarium) for future reference. Rest of specimens were each (fruit, leaf, seeds, stem and root barks) separately washed, dried under shade, ground and packaged in air sealed bottles.

*Author for correspondence; E-mail: jerry_me111@yahoo.com

Powder drug studies. Shade dried powdered specimens of *M.buxifolia* leaf, fruit, seeds, stem and root barks were examined for physical features like colour, taste and odour. For microscopic study, a small amount of each was individually taken on slide and macerated by boiling in concentrated chloral hydrate solution. Various fragments of powdered drugs were then observed under microscope at 45X and 10X resolutions of the objective lens and photographed (Wallis, 1985).

Florescence study. The colour impressions produced by crude aqueous and methanol extracts and powdered drugs of *M. buxifolia* when observed under daylight and UV lights of different wavelengths (264 nm and 366 nm) both directly and after treating with various reagents (50% nitric acid (HNO_3), 50% hydrochloric acid (HCl), picric acid, 50% sulphuric acid (H_2SO_4), 10% $FeCl_3$ solution, NH_3 solution, iodine solution, NaOH in water and NaOH in ethanol) were noted following Nikam *et al*., (2009).

Preliminary phytochemical profiling. Preliminary qualitative phytochemical screening for proteins, carbohydrates, fats, flavonoids, tannins, alkaloids, glycosides, phenols, phytosterols, anthocyanins, saponins, and terpenoids in various parts of *M. buxifolia* was carried out following methods given by American Association of Clinical Chemistry (AACC), (2010) and Khandelwal (1998).

Determination of moisture content. Moisture content of the each powdered drug of *M. buxifolia* (leaf, fruit, seed and barks of stem and root) were determined following methods of Association of Official Agricultural Chemists (AOAC, 2016).

Determination of ash content. Ash values (including total ash, water soluble ash and acid insoluble ash) of the studied parts of *M. buxifolia* were determined as per the methods given by Jarald and Jarald (2007).

Fixed oil analysis. Following the detections of qualitative phytochemical screening, fixed oils were expressed from fruit, seed and leaves of *M. buxifolia* and each was subjected to GC-MS analysis to know their fatty acid composition as per methods given by AOAC (2016) and American Oil Chemist's Society (AOCS, 2013).

Elemental analysis. Elemental profile of the studied parts of *M. buxifolia* was determined through atomic absorption spectrophotometry to detect the amounts potassium (K), nitrogen (N) and phosphorus (P), manganese (Mn), iron (Fe), zinc (Zn), copper (Cu), lead (Pb), chromium (Cr), cobalt (Co), cadmium (Cd) and nickel (Ni) following AOAC, (2016) and Tuzen *et al*. (2003).

Results and Discussion

Powder drug studies. *Fruit and seed*. The dark blackish brown powder of fruit pulp had a sweet fruity odour. It has fragments of epidermis with few stomata and starch granules, fragments of endocarp packed with starch grains and oil globules, prismatic crystals of calcium oxalate and rounded starch grains. The light brown powder of seed had an indistinct odour and bitter taste. Major fragments included isodiametric sclerenchyma cells of testa, parenchyma cells of endosperm with polygonal outlines and aggregated masses within, spiral vessels, parenchyma cells of cotyledons, aggregated grains of starch and variously formed crystals of calcium oxalate (Fig. 1-2).

***Leaf*.** The powdered drug prepared from leaf was dull green in colour and had fragments of upper epidermis having polygonal cells without intercellular spaces, few actinocytic stomata and the attached cylindrical, elongated cells of palisade layer, isodiametric parenchyma cells of spongy mesophyll tissue containing crystals of calcium oxalate, fragments of lower epidermis with trichomes and actinocytic stomata, unicellular non-glandular trichomes (both simple and branched), cuboid and prismatic crystals of calcium oxalate (Fig. 3).

***Stem bark*.** The powdered drug prepared from stem bark of *M. buxifolia* was light brown in colour and had an indistinct odour and a slightly bitter taste. It had fragments of dark brown, compactly arranged cork cells having thick walls and attached fragments of phallogen, polygonal, brown, thick walled cells of cortex having crystals of calcium oxalate and starch aggregates, isolated thick lignified phloem fibres and some fibres attached with the parenchyma of medullary rays (Fig. 4).

***Root bark*.** The greyish brown powder drug of root bark of *M. buxifolia* was bitter to taste and had no distinct odour. It had rectangular, dark brown pigmented, thick walled cells of periderm, fragments of cortical cells which were polygonal and pigmented and contained plenty of starch grains, parenchyma cells of medullary ray, perforated xylem vessels and crystals of calcium oxalate (Fig. 5).

Florescence studies. The observations of florescence behaviour of *M. buxifolia* under lights of different wavelengths (nanometres (nm)) are summarized in Table 1.

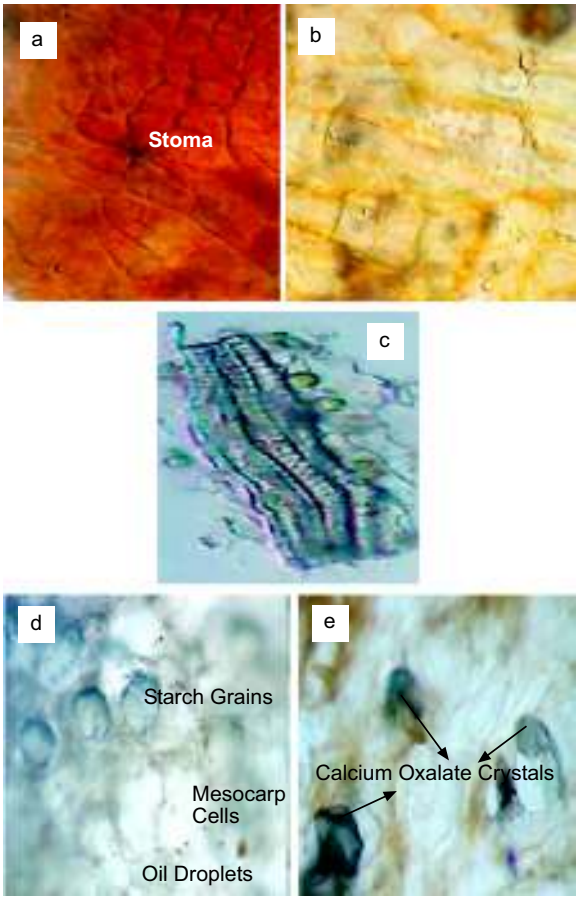

Fig. 1. Characteristic fragments of *M. buxifolia* fruit powder. (a) Fragments of epidermis with stomata, (b) Fragments of pigmented epicarp filled with oil droplets and starch granules, (c) Spiral vessels, (d) Fragments of mesocarp cells with starch granules and oil globules and (e) Crystals of calcium oxalate.

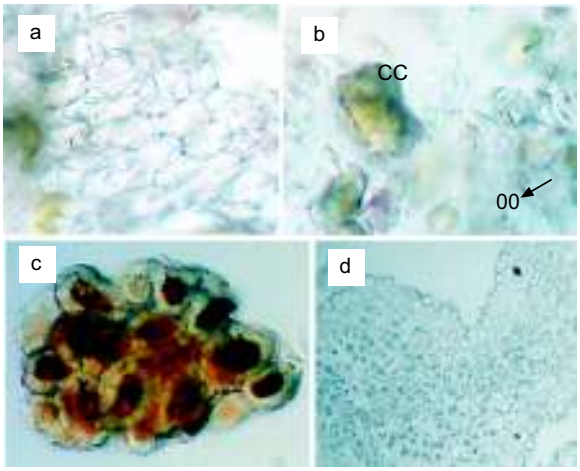

Fig. 2. Characteristic fragments of *M. buxifolia* seed powder. (a) Thin walled parenchyma cells of cotyledons, (b) Oil droplets (OD) and crystals of calcium oxalate (CC), (c) Sclerenchyma cells of seed coat and (d) Fragments of endosperm.

Phytochemical profile. Both aqueous and methanol extracts of *M. buxifolia* Fruit pulp, leaf, stem bark and root bark gave positive indications for presence of amino acids, proteins, carbohydrates, flavonoids, phenolic compounds, alkaloids, glycosides, phytosterols, triterpenoids, tannins, anthocyanins, and saponins. Volatile oils were not indicated in any of the tested extracts while fixed oils and fats were detected in fruit pulp, leaf and seed only. Glycosides, anthocyanins and flavonoids were also lacking in the seed extracts. Only the aqueous seed extract gave positive detection for sterols.

Moisture content, ash contents and fixed oil yield. ***Fixed oil analysis.*** Seeds of *M. buxifolia* yielded 10.62% fixed oils which is the highest percentage among the tested parts, the fruit yielded 8.33% followed by leaf 0.56%, which was the least of all. GCMS analysis revealed that the fixed oil of seed was composed of 34 fatty acids, major being Linolenic acid (20.73%), Oleic acid (20.30%), Palmitic acid (5.52%) and Stearic acid (2.074%). The fixed oil of fruit also had 34 component fatty acids including Myristic acid, Palmitic acid and Oleic acid in 2.53, 2.52 and 1.828%, respectively. Fatty acids of leaf oil included Palmitic acid, Linoleic acid, Oleic acid, Stearic acid and Myristic acid in following percentages 5.008, 3.892, 2.864, 1.506 and 1.163%, respectively.

Elemental analysis. The parts per million (PPM) concentrations of various macro and micro-nutrient elements of *M. buxifolia* are given in Table 4.

Plants are natural drug industries providing chemical solutions against various health disorders since the start of human civilization. The modern pharmaceutics derive their core potential formulas from chemical essentials of plants (Singh *et al*., 2002). However, while exploiting a plant as an active drug, manufacturers may come across adulterations, substitutions and false identities. Standardization of crude drugs is a mean to avoid such discrepancies. It helps in identifying the adulterations, impurities and mistaken identities (Pferschy-Wenzig and Bauer, 2015). There are a number of methods for

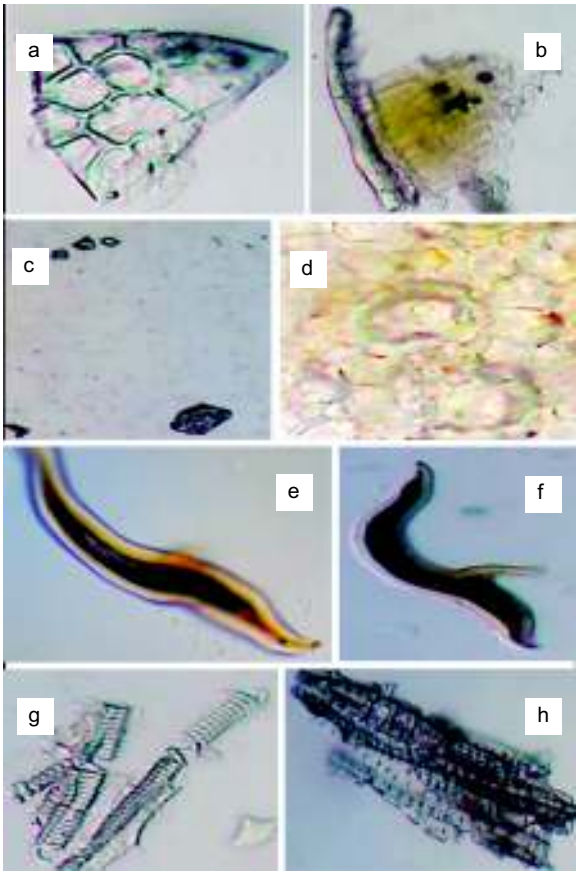

Fig. 3. Fragments of powder drug of *Monotheca buxifolia* leaf. (a) Upper epidermis, (b) Upper epidermis with palisade cells, (c) Calcium oxalate crystals, (d) Fragments of lower epidermis with actinocytic stomata, (e) Simple unicellular trichome, (f) Forked trichome, (g) Spiral xylem vessels and (h): Simple pitted vessels.

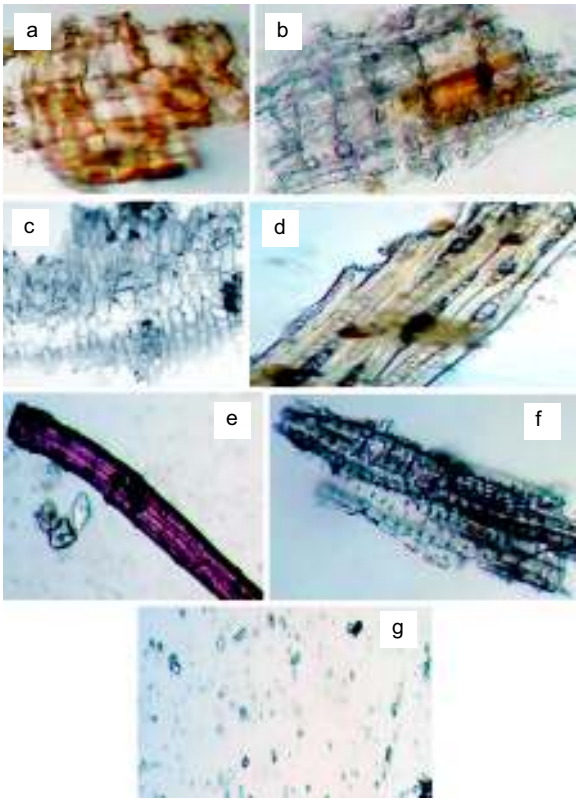

Fig. 4. Fragments of powder drug of *Monotheca buxifolia* stem bark. (a) Cork cells, (b) Cortical cells containing starch grains, (c) Fragment of periderm, (d) Fibres attached with parenchyma cells of medullary rays, (e) Thick lignified phloem fibre, (f) Vessels and (g) Aggregates of calcium oxalate and Starch granules.

standardization of crude drugs however WHO recommends the pharmacognostic studies to be most reliable in this regard (WHO, 1995). Pharmacognosy provides improved techniques for identification, structural studies and bioassays (Samuelsson and Bohlin, 2017).

A drug of uniform potency which has the same chemical makeup and produces the same pharmacological effect in repeated testing is said to be a standardized drug (Heinrich *et al*., 2018; Holiman, 1989).

Microscopic studies of powdered drugs also help in finding adulterants and substituents of crude drugs (whether added accidentally or intentionally). These are irrelevant substances including fragments of exhausted parts or other plants, microbial spores, dust particles and faecal deposits of small insects etc. (Jarald and Jarald, 2007).

Study of fluorescence behaviour of crude drugs is yet another mean for their standardization since the cut surfaces of such drugs, their extracts and powders have unique phytochemical constituents which produce different characteristic colour hues in UV light. If they are not themselves florescent, re-orientation of the chemical structures into florescent derivatives can be attained by treating them with various chemical reagents. This phenomenon can be used as a standard reference for qualitative identification and indication of adulteration to natural drugs (Zhao *et al*., 2011; Wallis, 1985).

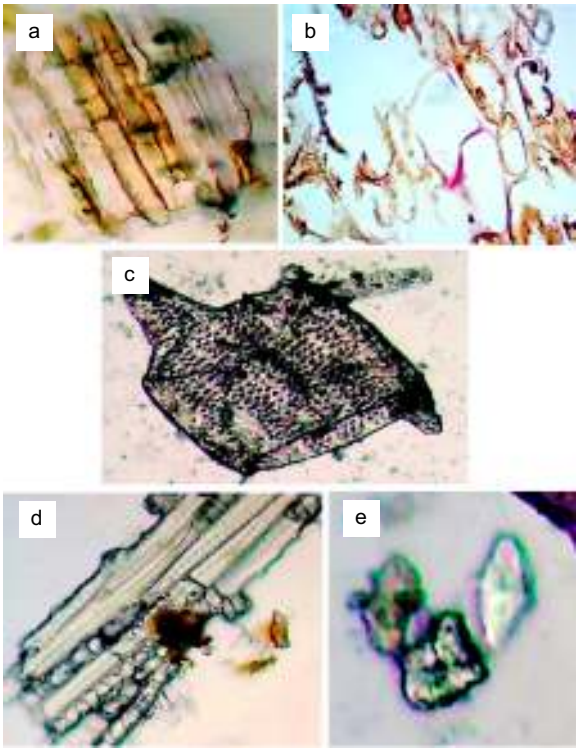

Fig. 5. Characteristic fragments of *M. buxifolia* root bark. (a) Cork cells, (b) Fragments of cortical cells, (c) Vessel with pitted walls, (d) Fibers attached with Medullary rays and (e) Crystals of calcium oxalate.

During various physiological processes of plants a large number of intermediate secondary metabolites are produced which have potential pharmaceutical actions (Chung *et al*., 1998). Alkaloids are one such intermediate phytochemicals with preventive potentials against a broad range of cancer types (Jiang *et al*., 2016). Tannins have remarkable antioxidant and antimicrobial potentials (Chung *et al*., 1998), saponins help in maintaining levels of sugars and cholesterol in blood. They are also effective against cancers and prevent dental caries (Shi *et al*., 2004), flavonoids are potential antioxidants, free radical scavengers, antihypertensive and antitumor agents. They also prevent HIV infestations and cardiac disorders (Xiao *et al*., 2011), terpenoids are natural antiulcer, antimalarial, antimicrobial, anti-hepaticidal, anticancer and diuretic agents (Asadi-Samani *et al*., 2016; Dudareva *et al*., 2004), Cardiac glycosides and steroids are used against selective proliferative disorders, congestive cardiac failure and have known anti-arrhythmic actions (Newman *et al*., 2008). Evaluation of the biochemical profile of crude drugs may also help in standardization as altered biochemical parameters indicate incidence of substitution, adulteration and low grade drug constituents (Jarald and Jarald, 2007). The present study reveals that *M. buxifolia* contains a broad spectrum of active phytochemical constituents which may individually or in various combinations with other secondary phyto-constituents produce tremendous pharmacological actions.

Moisture contents directly affect the shelf life of dried crude drugs as it supports microbial infestations which need moisture for colonising host surfaces and carrying out their enzyme reactions (Chanda, 2014). Since all the dried powdered drugs prepared from *M. buxifolia* had low moisture contents (Table 2), they can be safely stored without microbial deteriorations for longer periods during study or for therapeutic purposes.

Determination of ash values is a useful quantitative standard for purity and quality of a drug. They also help in finding adulterated drugs or drugs with mixed identities. Total ash values are evaluated to indicate presence of inorganic earthy matters like lime, chalk or siliceous substances in a drug. Acid insoluble and water soluble ash values indicate the proportions of exhausted drug material and calcium oxalate in a drug, respectively (Jarald and Jarald, 2007). Ash analysis of *M. buxifolia* revealed that all the parts had low amounts of inorganic, non-physiological matter in them. Seed had highest ash values followed by root bark, fruit, leaf and the lowest of the stem bark (Table 2). These ash constants may be consulted in future for authentic choice and selection of this plant.

Fats and oils store the highest amounts of energy among the major biomolecules. They form a protective seal in body against heat and moisture loss and keep the organs lubricated. Cosmetic, food and pharmaceutical industries use fixed oils along with aromatic oils in a variety of their commercial products particularly related to body and skin care. Fixed oils are composed of a huge range of different fatty acid combinations in varying proportions which impart unique properties and pharmacological potential to each of them (Mohammed and Jorf-Thomas, 2003). These include cure to diseases as complex as chronic hepatic damage to the simple constipation. Inadequately low intake of these may result in

Table 1. Fluorescence studies of of *M. buxifolia*

Reagents used	Light	Plant parts				
		Fruit	Seed	Leaf	Stem bark	Root bark
Powder as such	Visible daylight	Dark blackish brown	Almond brown	Dull green	Light brown	Ashy cinnamon brown
	UV light (254 nm)	Maroon	Dark brown	Brownish green	Dull brown	Light brown
	UV light (366 nm)	Reddish maroon	Florescent green	Greyish green	Greyish brown	Greyish brown
Powder drug+50 %HCl	Visible daylight	Dark reddish brown	Reddish brown	Dull green	Cinnamon brown	Dark brick red
	UV light (254 nm)	Yellow brown	Dark brown	Dark yellowish green	Yellowish brown	Dark brick red
	UV light (366 nm)	Greenish brown	Greyish green	Mud brown	Greyish brown	Light maroon
Powder drug+ 50% H_2SO_4	Visible daylight	Maroonish brown	Caramel brown	Dark brownish green	Dark brown	Blackish brown
	UV light (254 nm)	Dark Amber	Dark yellowish brown	Yellowish brown	Dark brown	Blackish brown
	UV light (366 nm)	Dark greenish brown	Dark greenish brown	Greenish brown	Light maroon	Burgundy
Powder drug +50% HNO_3	Visible daylight	Cinnamon brown	Amber	Orange brown	Dark yellowish brown	Dark amber
	UV light (254 nm)	Yellowish brown	Dark brown	Yellowish brown	Dark brown	Dark brown
	UV light (366 nm)	Dark reddish brown	Florescent green brown	Dark brown	Orange brown	Brownish Maroon
Powder drug+ Picric acid	Visible daylight	Dark brown	Orange brown	Dull yellowish green	Light orange	Mustard
	UV light (254 nm)	Maroon	Yellowish brown	Yellowish green	Brown	Yellowish brown
	UV light (366 nm)	Dark maroon	Maroon	Dark yellowish green	Dark brown	Greenish brown
Powder drug+ glacial acetic acid	Visible daylight	Reddish brown	Brick red	Dull green	Light brown	Amber
	UV light (254 nm)	Yellowish brown	Dark brown	Yellowish green	Yellowish brown	Amber
	UV light (366 nm)	Cinnamon brown	Dark green	Dull brown	Greenish brown	Greyish green
Powder drug+ NaOH	Visible daylight	Dark brown	Brick red	Dull yellowish green	Cinnamon brown	Greyish brown
	UV light (254 nm)	Maroon	Dull brown	Brownish green	Mud brown	Dull brown
	UV light (366 nm)	Dark maroon	Greenish brown	Yellowish brown	Light greenish brown	Dark greyish brown
Powder drug + Ethanol	Visible daylight	Dark brown	Brick red	Dark ashy green	Light brown	Reddish brown
	UV light (254 nm)	Dark brown	Brown	Yellowish green	Light brown	Dark reddish brown
	UV light (366 nm)	Dark grey	Dull florescent green	Ash grey	Dull green	Greenish brown
Powder drug+ Iodine	Visible daylight	Dark burgundy	Yellowish orange	Dark brown	Dark Yellowish orange	Dark brown
	UV light (254 nm)	Yellowish brown	Dull green	Greenish yellow	Light brown	Orange brown
	UV light (366 nm)	Greenish brown	Cinnamon brown	Dark greenish brown	Dark brown	Dark greenish brown
Powder drug +$FeCl_3$	Visible daylight	Greenish maroon	Orange green	Yellowish green	Yellowish green	Greenish maroon
	UV light (254 nm)	Brown	Brown	Dark brown	Dull green	Dull brown
	UV light (366 nm)	Brown with florescent green hue	Orange maroon	Purplish brown	Dark greenish brown	Dark purplish brown

Table 2. Percent moisture contents, ash values (w/w) and fixed oil contents of *M. buxifolia*

Plant part	Moisture contents	Total Ash	Acid insoluble Ash	Water soluble Ash	Fixed oil yield
Leaf	8.5%	6.0%	1.75%	3.5%	0.56%
Fruit	15.5%	7.0%	1.5%	4.9%	8.33%
Seed	8.5%	11.75%	4.25%	6.75%	10.62%
Root bark	9.5%	8.25%	3.75%	4.2%	-
Stem bark	9.5%	4.5%	4.0%	0.5%	-

retarded growth and various deficiency disorders of skin and reproductive system (Benatti *et al*., 2004; Riechart, 2002). The present study revealed that *M. buxifolia* contains plenty of fatty acids of huge commercial importance in its seed, fruit and leaves, especially w.r.t cosmetic and pharmaceutical industry. The unsaturated fatty acids were higher in proportion as compared to saturated fatty acids. Saturated fatty acids increase the risks of cardio-vascular disorders due to elevation of blood cholesterol levels. Thus fixed oils obtained from *M. buxifolia* are safer for medicinal uses (Abed, 2007).

The organic structures of plants contain various non-metallic and metallic ions as their component parts. These may be associated with enzyme molecules or in the form of metallo-proteins. Together with phyto-chemicals they enhance the nutritional and pharma-cological significance of plants. These compounds are involved in a number of vital body functions and their deficiency leads to abnormal development of the organism (Morabad *et al*., 2012). The trace elements despite their low amounts play key role in a plant's nutritional significance and therapeutic potentials. However their excess or prolonged use may pose various health risks due to toxic accumulations in the body (Ibrar *et al*., 2013). To avoid such encounters WHO (1998) recommends safe doses of heavy metals for human intake. Elemental profile of *M. buxifolia* analyzed in this work showed that all the parts of this plant contain large amounts of potassium, nitrogen and phosphorus which are component parts of mojor bio-molecules (Table 4). Fruit, seeds and leaves are good source of iron and may be used in anemic conditions. The plant had all the trace elements in safe limits directed by WHO except for cobalt (10.7±0.01 ppm) in root bark and lead (22.48±0.33 ppm) in stem bark (Table 4). This must be taken care of while consuming these parts.

Conflict of Interest. The authors declare they have no conflict of interest.

Table 4. elemental profile of *M. buxifolia*

Element detected	Plant part				
	Leaf	Fruit	Seed	Root bark	Stem bark
P (ppm)	140.63±0.01	359.4±0.04	343.8±0.03	609.4±0.06	125.0±0.01
N (ppm)	2800±1.6	3000±0.91	2300±1.62	1200±0.52	1600±0.21
K (ppm)	3041.0±0.30	9711.6±0.97	2648.6±0.26	3531.5±0.35	2158.1±0.22
Fe (ppm)	1.16±0.05	1.07±0.006	2.47±0.01	0.84±0.03	0.32±0.01
Mn (ppm)	0.04±0.003	0.06±0.005	0.12±0.002	0.19±0.009	0.06±0.006
Cu (ppm)	0.065±0.001	0.24±0.01	0.24±0.004	0.68±0.005	1.58±0.009
Cr (ppm)	1.02±0.07	1.01±0.19	1.014±0.12	1.04±0.03	1.007±0.05
Zn (ppm)	0.39±0.02	0.19±0.009	0.15±0.01	2.36±0.008	0.99±0.006
Pb (ppm)	0.36±0.18	0.82±0.19	2.80±0.33	22.48±0.33	0.18±0.03
Co (ppm)	-0.13±0.00	-1.57±0.14	-0.156±0.04	-1.23±0.16	10.7±0.01
Ni (ppm)	0.70±0.03	0.75±0.06	1.16±0.23	1.58±0.33	1.05±0.02
Cd (ppm)	ND	0.02±0.009	0.01±0.012	0.01±0.001	0.016±0.02

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