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PHYSICO-CHEMICAL, PHYTOCHEMICAL EVALUATION AND DPPH-SCAVENGING ANTIOXIDANT POTENTIAL IN MEDICINAL PLANTS USED FOR HERBAL FORMULATION IN PAKISTAN

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Abstract

The active parts of 11 medicinal plants were analyzed for physico-chemical evaluation, phytochemical determination and antioxidant activity. The physico-chemical evaluation revealed that highest water soluble extractive was from *Origanum vulgare* (38%), highest chloroform extractive was from *Psoralea corylifolia* (21%); highest ethanolic extractive was that of *Acorus calamus* (11%) and the highest hexane extractive value was for *Arnebia nobilis* (9.8%). The total ash content evaluation indicated that *Achillea millefolium* yielded (20.2 %) and *Rauvolfia serpentina* yielded (41.6%); these values are much higher than the standard ash values for these plants indicating that these drugs are highly adulterated and substandard. The highest essential oil was yielded by *Acorus calamus* (3.2%). The highest saponin percentage was analyzed in *Acorus calamus* (8.9%), while the alkaloids percentage was determined at 21% in *Peganum harmala*. Among all the plants assessed for DPPH free radical scavenging activity, the maximum activity was shown by *Paeonia emodi* (85.8%), followed by *Achillea millefolium* (81.7%) and *Origanum vulgare* (80.3%).

Introduction

The use of herbal medicines for the treatment of diseases and infections is a safe and traditional therapy (Najafi & Deokule, 2010). Plants in the genus *Achillea* are used for treatment of fever, asthma, bronchitis, cough, skin inflammation, jaundice, liver disease (Yassa *et al*., 2007), healing of wounds, menstrual regulation, flatulence, dyspepsia and haemorrhage treatment (Candan *et al*., 2003; Cavalcanti *et al*., 2006; Dokhani *et al*., 2005); they also have antioxidative and anticancer properties. The rhizome of *Acorus calamus* is locally used for abdominal pain, dyspepsia, flatulence and dysentery (Ahmad *et al*., 2006). The roots of *Arnebia nobilis* are used as an anthelmintic, antipyretic, antiseptic and are also useful against the diseases of the eye, bronchitis, abdominal pain and itching (Arora *et al*., 2009; Khatoon *et al*., 1993). The decoction of *Fumaria indica* is used for constipation and is helpful in liver obstruction, with hepatoprotective effects. It is a central nervous system depressant, diuretic, diaphoretic, anthelmintic, laxative, blood purifier, antipyretic, antidiarrhoeal, hypoglycemic, smooth muscles relaxant, hydrocholeretic, anti-inflammatory and anti-nociceptive (Rao *et al*., 2007). The leaves of *Gymnema sylvestre* are used in diabetes and are chewed to reduce glycosuria (Chopra *et al*., 1956). Shoots of *Origanum vulgare* are chewed for toothache and used as a flavoring agent. The roots of *Rauvolfia serpentina* are used to treat malaria, high blood pressure, bowel infections and spleen diseases; they also have hypnotic and sedative effects, and are used to treat insanity (Mia *et al*., 2009). The stem of *Paeonia emodi* is dried, powdered and a paste is applied for joint pain and as a plaster on bone fractures. The tubers of *Paeonia emodi* are useful for uterine diseases, colic, bilious obstructions, dropsy, epilepsy, convulsions and hysteria, and are also given to children as a blood purifier (Chopra *et al.,* 1956). *Peganum harmala* is locally used as an anthelmintic, narcotic, and given for fever and colic. Its roots are boiled and used to kill lice, and the extract is useful in wound healing (Derakhshanfar *et al.,* 2010). *Psoralea corylifolia* seed oil is beneficial in scabies and ringworm infestations (Uikey *et al*., 2010), and recommended orally with betelnut leaf for leprosy (Sun *et al.,* 1998). *Vetiveria zizanioides* is effective to shorten labor (Lans, 2007), its oil is used in the treatment of infantile hyperhidrosis (Chatterjee *et al.,* 2005), and is used as an anti-microbial and anti-fungal agent in the pharmaceutical industry for perfumery and aromatherapy (Bowles *et al.,* 2002; Bhuiyan *et al.,* 2008; Chaudhury *et al.,* 2007; Weyerstahl *et al.,* 1996).

These eleven plants produce valuable secondary metabolites that combat and detoxify free radicals released in the body during metabolism. Antioxidant constituents of plant materials are important in the maintenance of health and protection from coronary disease because they possess the ability to protect the body from damage caused by toxic free radical induced oxidative stress (Ahmad *et al*., 2010a, 2010b; Ahmad *et al*., 2011a, 2011b).

The overall thrust of the present research was to investigate the physico-chemical parameters, characterize the phytochemicals and assess 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging antioxidant activity.

Materials and Methods

Phytochemical Screening: Plant parts including roots, bark, leaves, rhizomes, seeds, stems and tubers of *Achillea millefolium, Acorus calamus, Arnebia nobilis, Fumaria indica, Gymnema sylvestre, Origanum vulgare, Paeonia emodi, Peganum harmala, Psoralea corylifolia, Rauvolfia serpentina* and *Vetiveria zizanioides* (Table 1) were procured from the local market in Peshawar. The botanical identities were confirmed and voucher specimens were deposited in the PES herbarium, PCSIR Laboratories Complex, Peshawar, Pakistan.

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Table 1. Medicinal plants with active parts, market price and flowering period.

Species	Part used	Price per kg	Flowering period
Achillea millefolium	Stems & leaves	192	June-September
Acorus calamus	Rhizomes	120	June-July
Arnebia nobilis	Root/Root bark	96	May-June
Fumaria indica	Stems & leaves	60	March-June
Gymnema sylvestre	Leaves	128	April-November
Origanum vulgare	Stems & leaves	160	June-October
Paeonia emodi	Tubers	48	May-June
Peganum harmala	Seeds	60	April-October
Psoralea corylifolia	Seeds	80	August-December
Rauvolfia serpentina	Roots	720	July-September
Vetiveria zizanioides	Whole plant	160	September

Determination of alkaloids: Dried plant material was ground into powder and 100 g of powder was extracted with 100% ethanol (150 ml). The ethanol was allowed to evaporate and 20 g of dried extract was dissolved in 5% HCl (50 ml). The mixture was centrifuged and the aqueous portion was transferred to a new tube and basified with NH_4OH (pH 8-10). The aqueous (basic) portion was extracted with $CHCl_3$ three times, and then concentrated under reduced pressure. Each sample was dried and weighed to determine the amount of alkaloid residues.

Determination of saponins: Ten grams of ground plant material were defatted with 30 ml hexane, extracted three times with 30 ml methanol and concentrated to one third of the original volume. Cold acetone (100 ml) was added to the extract, which was then refrigerated for 50 minutes. The extract was filtrated by pressure filtration using pre-weighed filter paper (Whatman No. 1). The weight of the saponins was determined by subtracting the original weight of the filter paper.

Determination of volatile oil: Plant parts were ground in a blender and subjected to hydro-distillation for 4 h for extraction of oils following the procedure of Bhuiyan *et al*., (2008). The oil samples were extracted three times with hexane (30 ml) and treated with anhydrous sodium sulfate (5-10g). The supernatant was collected with a pipette and concentrated by rotary evaporator under reduced pressure to obtain the essential oil.

Determination of extractive values: Ten to 30 grams of dried powdered material of each plant were placed in four flasks. Distilled water, chloroform, hexane and ethanol in a quantity of 30 ml were added to them. The soluble compounds were then filtered using double filter paper after 5-6 days. The filtrate was concentrated on a rotary evaporator under reduced pressure. The extracts obtained were reweighed and the percentage yield of the extract was determined as per Banso & Adeyemo, (2007).

Determination of total ash value: One to two grams of the plant material was poured in pre-weighed crucibles and placed over a flame to burn the plant material completely to ash. These crucibles were covered with lids, than placed in a furnace at 600°C for 2 hours. After cooling, the crucibles were placed in desiccators and reweighed. The percentages of ash values were then calculated following the procedures in Pharmacopoeia of India (Anonymous, 1955).

Determination of antioxidant activities: To determine antioxidant activities, 1,1-diphenyl-2-picrylhydrazyl (3.96 mg) was dissolved in 20 ml of methanol to make a stock solution. Plant material (5 g) was extracted with 50 ml methanol for 5 days with periodic shaking. The extract was filtered and concentrated by rotary evaporator. 5 mg of this extract was dissolved in 20 ml methanol to make a stock solution. The procedure was repeated for each plant, and 0.5 ml of sample solution was added to 1 ml of DPPH solution separately. These solutions were kept at room temperature in the dark for 30 min (incubation period). The absorbance was measured in a spectrophotometer at 517 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity using the equation (Ahmad *et al*., 2010a):

$$\% \text{ Scavenging DPPH free radical} = 100 \times (1\text{-AE/AD})$$

where AE is absorbance of the sample solution and AD is the absorbance of the DPPH solution with nothing added (blank, without extract).

Statistical analysis: For statistical analysis, three replicates were conducted for each activity and the experiments were repeated twice. Analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) was used for comparison among treatment means, using Statistix 8.1.

Results

Keeping in view the medicinal importance of *Achillea millefolium, Acorus calamus, Arnebia nobilis, Fumaria indica, Gymnema sylvestre, Origanum vulgare, Paeonia emodi, Peganum harmala, Psoralea corylifolia, Rauvolfia serpentina* and *Vetiveria zizanioides,* these plants were thoroughly investigated for their physico-chemical characters including their solvent extractive values (Table 2), total ash values and major active constituents to analyze their quality, safety and standardization for their safe use. The extracts of these plants were then evaluated for antioxidant activity with DPPH. The physico-chemical evaluation was performed for the standardization of these crude drugs, as they are openly sold in the market. This study revealed that highest water soluble extractive was from *O. vulgare* (38%), the highest chloroform extractive was from *P. corylifolia* (21%), the highest ethanolic extractive was from *A. calamus* (11%) and the highest hexane extractive value was from *A. nobilis* (9.8%) (Table 2).

Table 2. Mean percentage (%) of extractive values of eleven medicinal plants in four different solvents (chloroform, water, ethanol and hexane).

Species	Chloroform%	Water%	Ethanol%	Hexane%
Achillea millefolium	6.8^{b*}	8.4^{bc}	9.6^{ab}	1.7^{b}
Acorus calamus	1.3^{bc}	21.0^{ab}	11.0^{a}	1.2^{b}
Arnebia nobilis	12.1^{ab}	3.2^{bc}	6.1^{bc}	9.8^{a}
Fumaria indica	2.6^{bc}	15.0^{b}	4.8^{c}	2.7^{b}
Gymnema sylvestre	2.3^{bc}	18.3^{b}	7.3^{b}	3.5^{b}
Origanum vulgare	4.3^{bc}	38.0^{a}	5.4^{bc}	2.2^{b}
Paeonia emodi	2.5^{bc}	21.0^{ab}	4.9^{c}	1.1^{b}
Peganum harmala	12.3^{ab}	19.0^{b}	7.1^{b}	6.8^{ab}
Psoralea corylifolia	21.0^{a}	16.7^{b}	9.0^{ab}	3.3^{b}
Rauvolfia serpentina	2.04^{bc}	22.0^{ab}	7.3^{b}	1.1^{b}
Vetiveria zizanioides	2.7^{bc}	0.01^{c}	3.0^{c}	1.2^{b}

*Letters within each column indicate means that are significantly different at $p<0.05$

The total ash content evaluation indicated that *A. millefolium* yielded 20.2% and *R. serpentina* yielded 41.6%, both of which are much higher than the standard ash values given in the World Health Organization (2005) monographs for these plants, indicating that these samples were highly adulterated and substandard (Table 3). Screening of these plants for their major active constituents revealed the presence of essential oils, saponins and alkaloids. The highest essential oil percentage was yielded by *A. calamus* (3.2%), followed by *V. zizanioides* (0.43%), *O. vulgare* (0.3%), *A. millefolium* (0.045%), and *P. corylifolia* (0.034%). Saponin percentage was highest in *A. calamus* (8.9%), followed by *P. emodi* (2.34%), and *A. millefolium* (1.7%), while alkaloids percentage was highest in *P. harmala* (21%), followed by *Acorus calamus* (11%), *Arnebia nobilis* (9.4%), *G. sylvestre* (1.33%), *R. serpentina* (0.58%) and *F. indica* (0.4%) (Table 3).

Table 3. Antioxidant potential, ash value, essential oils, saponins and alkaloids mean percentage (%) in eleven medicinal plants.

Species	Antioxidant Activity (%)	Ash Value (%)	Essential Oils (%)	Saponins (%)	Alkaloids (%)
Achillea millefolium	81.7^{a*}	20.2^{b}	0.045^{c}	1.7^{b}	--
Acorus calamus	69.8^{bc}	2.20^{c}	3.2^{a}	8.9^{a}	11^{a}
Arnebia nobilis	53.9^{d}	10.6^{c}	--	--	9.4^{a}
Fumaria indica	61.8^{c}	15.3^{bc}	--	--	0.4^{bc}
Gymnema sylvestre	54.4^{d}	7.90^{c}	--	--	1.33^{b}
Origanum vulgare	80.3^{a}	9.60^{c}	0.3^{ab}	--	--
Paeonia emodi	85.8^{a}	6.01^{c}	--	2.34^{b}	--
Peganum harmala	65.7^{bc}	6.40^{c}	--	--	--
Psoralea corylifolia	36.1^{e}	14.5^{bc}	0.034^{c}	--	--
Rauvolfia serpentina	61.8^{c}	41.61^{a}	--	--	--
Vetiveria zizanioides	61.8^{c}	4.92^{c}	0.43^{b}	--	--

*Letters within each column indicate means that are significantly different at $p<0.05$

DPPH-scavenging antioxidant activity of ethanolic extracts of all the plants were measured in terms of hydrogen donating or radical scavenging ability using the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH). Among all the plants assessed for DPPH free radical scavenging activity, the maximum activity was shown by *Paeonia emodi* (85.8%), followed by *Achillea millefolium* (81.7%), *Origanum vulgare* (80.3%), *Acorus calamus* (69.8%), *Peganum harmala* (65.7%), *Vetiveria zizanioides* (61.8%), *Rauvolfia serpentina* (61.8%), *Fumaria indica* (61.5%), *Gymnema sylvestre* (54.4%), *Arnebia nobilis* (53.9%) and *Psoralea corylifolia* (36.1%).

Discussion: Determination of extractive values, ash residues and active components (saponins, alkaloids and essential oil content) plays a significant role for standardization of the indigenous crude drugs. The purposes of standardized extraction procedures for crude drugs are to attain the therapeutically desired portion and to eliminate the inert material by treatment with a selective solvent known as *menstruum*. The extract formed can directly be used as a tincture or fluid extract, or be further processed for incorporation into tablets or capsules, or it may be fractionated to isolate individual chemical entities such as ajmalicine, hyoscine and vincristine, which are modern drugs (Thomson, 2007). Thus, standardization of extraction procedures contributes significantly to the final quality of the herbal drug (Handa *et al*., 2008).

The British Herbal Pharmacopoeia (1976) indicated that *Acorus calamus* yielded not more than 6% total ash, and our results yielded 2.2% ash contents. The permissible limit for *Fumaria officinalis* is 16%, and we found *F. indica* ash contents to be 15.3% (Table 3). The upper permissible limit of ash for *Paeonia lactiflora* is 6.5% (World Health Organization 2005), and we found that *Paeonia emodi* yielded 6.01% total ash contents, so the material can be recommended for use as medicine. In contrast, the permissible ash limit of *A. millefolium* is 10%, but we found 20.2% ash residue. The total ash

contents of *R. serpentina* also must not exceed 10%, but our sample showed 41.6% ash contents.

In the present study, the highest essential oil yield was from the rhizome of *A. calamus* (3.2%) (Table 3); this is in line with the results of Chopra *et al.*, (1956), which indicated that the dry rhizome of *A. calamus* contained 1.5-3.5% of a yellow aromatic essential oil. The second highest oil percentage was yielded by the whole plant of *V. zizanioides* (0.43%), which is within the range of results (0.2-1.7%) documented by Chopra *et al.*, (1956). The percentage of oil yielded by the aerial parts of *O. vulgare* was 0.3%, which is closest to Chopra *et al.*, (1956), who reported yield of 0.45-0.525%. However, Derwich *et al.*, (2010) recently reported that the yield of *O. vulgare* essential oil to be 1.15%. The fourth highest yield was from aerial parts of *A. millefolium* (0.045%), which was similar to the results (0.47% oil) of Chopra *et al.*, (1956).

Alkaloids have detoxifying and antihypertensive properties (Trease & Evans, 1982). According to Chopra *et al.,* (1956), different varieties of *R. serpentina* root yielded different percentages of alkaloids: Bihar variety contained 0.8%-1.3% total alkaloids while Dehra variety yielded 1-1.3% alkaloids. The World Health Organization (2005) reported that *R. serpentina* contained least 1% total alkaloids, but we only recorded 0.58% from this species. The alkaloidal yield of *F. indica* (0.4%) was in accordance with the results of Chopra *et al.,* (1956), who found that *F. officinalis* contained 0.13% alkaloids in the form of protopine.

Studies conducted on free radical scavenging activity of medicinally important plants have shown that the efficiency of each plant species differs depending on the particular assay methodology, reflecting the complexity of the mechanisms involved in total antioxidant capacity (Matkowski *et al.,* 2008). In this study antioxidant activity of all the plants was determined using the DPPH method. Among these eleven plants, maximum radical scavenging activity (RSA) was recorded from *Paeonia emodi,* which showed 85.8% activity (Table 3), while the lowest activity (36.1%) was shown by *Psoralea corylifolia*. The result for *P. corylifolia* agrees with that of Anwar (2006), who found 34.59% RSA at a concentration of 500 µg/ml. According to Rachh *et al.,* (2008), significant antioxidant activity of an alcoholic extract of *G. sylvestre* was found to be due to the presence of acidic compounds, flavonoids, phenols, saponins, tannins and triterpenoids. They found maximum scavenging activity of 57.1% at 100 µg/ml concentrations; we found that *G. sylvestre* showed 54.4% RSA at concentration of 250 µg/ml.

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