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Full Length Research Paper

Pharmacognostic standardization of the stem of *Aerva persica* (Burm.f) Merrill (Amaranthaceae)

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***Aerva persica (Amaranthaceae*) is hoary-tomentose herb that possesses several biological activities like antiulcer, antimicrobial, hypoglycaemic, diuretic and demulcent. Important details like macroscopic and microscopic examination, physico-chemical parameters and high performance thin layer chromatography (HPTLC) profile of various extracts are presented in this paper. This study revealed that values for total ash, acid insoluble ash, water soluble ash, sulphated ash, swelling index, fibre content, bitterness, extractive values with petroleum ether, chloroform, ethyl acetate, ethanol and water were found to be 6.19, 1.42, 2.45, 6.77, 4%, 27%, 0.869, 0.5%, 1.25%, 11.25%, 2% and 1.75, respectively, are being reported for the first time. The standardisation studies help in the identification of *A. persica* from other *Aerva* species.**

Key words: *Aerva persica*, aflatoxins, Amarantheacae, antiulcer, standardisation.

INTRODUCTION

Herbal medicines are promising choice over modern synthetic drugs. They show minimum/no side effects and are considered to be safe. Generally, herbal formulations involve use of fresh or dried plant parts. Correct knowledge of such crude drugs is a very important aspect in preparation, safety and efficacy of the herbal product and standardization is a tool in the quality control process (Trease and Evans, 2002). Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carries an assurance of quality, efficacy, safety and reproducibility (Mukherjee, 2002).

Due to globalization, change in manufacturing and dispensing practice, use of synthetic pesticides and fertilizers, polluted climatic conditions, comparison with modern medicines, use of plant ingredient in modern medicine and legal requirements created the need of standardization (Harborne, 1988). A standardised extract means that the manufacturer has verified, this means that the active ingredient believed to be present in the herb, is really present in the preparation and also assured the potency, amount of the active ingredients in the preparation. With the ever-increasing use of herbal medicines and the global expansion of the herbal medicines market, safety has become a major concern (Bersani et al., 2000). Furthermore, the demand of 'readymade extracts' and for this dependence of manufactures on suppliers has necessitated the standardization of single plant drugs and their extracts. A well-defined and constant composition of the drug is therefore, one of the most important prerequisites for the production of a quality drug. World Health Organisation (WHO) Guidelines for Quality Standardized Herbal Formulations (1996a, b, 1992) includes quality control of crude drugs material, plant preparations and finished products, stability assessment and shelf life, safety assessment, documentation of safety based on experience or toxicological studies, assessment of efficacy by ethnomedical information and biological activity evaluations (Aboutabl et al., 1997). Though herbal products have become increasingly popular throughout the world, one of the impediments in its acceptance is the lack of standard quality control

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Figure 1. *Aerva persica.*

Figure 2. *A. persica* stem.

profile, so in this direction the standardisation of stem parts of *Aerva persica* were carried out. *A. persica* (Burm.f) Merrill (Amaranthaceae) is hoary-tomentose herb, up to 1 m in height (Maclenna et al., 1996). These are widely distributed in Srilanka, Myanamar, Rajasthan, Peninsular India and Gujarat. *A. persica* has many biological activities like antiulcer (Kushima et al., 2005, Vasudeva et al., 2012), antimicrobial (Chowdhury et al., 2002; Gehlot and Bohra, 1998), immunomodulatory effect (Nevin and Vijayammal, 2003), antidiarrhoeal (Joanofarc and Vamsadhara, 2003), antiplasmodic (Wassel et al., 1997; Simonsen et al., 2001), hypoglycaemic (Vetrichelvan and Jegadeesan, 2002), nephroprotective (Shirwaikar, 2004), antioxidant (Ahmed et al., 2006), diuretic (Majmudar et al., 1999; Vetrichelvan et al., 2000) and demulcent (Zapesochnaya et al., 1992), etc. The roots are chewed to form brush for cleaning teeth (Goonaratna et al., 1993). The woolly seeds and stem part are said to relieve headache (Radwan et al., 1999); they are also used against rheumatism (Jaswant et al., 2003). Vernacular names of *A. persica* are: Gujrati-Bur; Kannad-Dodda; Hindi-Gidda; Tamil-Perumpoolai; Telagu-Magavira; Delhi-Dholimundi; kamheda; Punjabi-Boi kalan; Rajastani-Buida. The major active constituents are alkaloids Canthin-6-one, 10-methoxycanthin-6-one (methylaervin), 10-hydroxycanthin-6-one (aervin), 10-β-d-glucopyraosyloxycanthin-6-one (aervoside), β-carboline-1-propionic acid, and 6-methoxy- β-carboline-1- propionic acid (aervolanin) reported in the roots and aerial parts of *Aerva lanata* (Zapesochnaya et al., 1992) (Figure 1).

MATERIALS AND METHODS

Plant

A. persica was collected during July from Government Polytechnic, Hisar (Haryana), North India. The plant was taxonomically identified by Dr. H. B. Singh, Head, Raw Materials Herbarium and Museum Division of National Institute of Science Communication and Information Resources (Ref. no. NISCAIR/RHMD/Consult/-2007-08/893/77). The voucher specimen has been deposited in Pharmacognosy Division, Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar. The stem pieces were dried and made into powder. Standardisation of stem parts was carried out using standard methodology.

Preparation of ethanolic and aqueous extracts

500 g of powdered stem was extracted with 1.5 L of ethanol using Soxhlet apparatus for 4 h. Subsequently, 500 g of dried stem powder was boiled with 2.5 L of distilled water for 4 h. These extracts were filtered and the filtrate was evaporated to dryness under reduced pressure at 40 to 50°C and stored at 4°C until use.

Morphological studies

The dried stem parts were studied for its morphological characters using the appropriate techniques (Evans WC, 2005) (Figure 2).

Microscopical studies

The dried stem was soaked overnight in water to make it smooth enough for transverse section. Paraffin wax embedded specimens were sectioned using the rotatory microtome (Weswox Optik). The thickness of the section was 10 to 12 µm. The section was stained with safranin (1%) and light green (0.2%). Slides were mounted in Distyrene, plasticizer and Xylene (DPX) mountant. Photomicrographs were taken using trinocular microscope (Figure 3) (Olympus) (Mukherjee, 2002).

Histochemical colour reactions

The respective histological colour reactions of transverse section of stem part were done according to khan et al. (1993) and results are shown in Table 1.

Powder analysis

Microscopy

The powdered drug was bleached with 5% chloral hydrate and

Table 1. Histochemical color reactions of transverse section of stem.

S/N	Reagent	Test for	Nature of change in histochemical zone	Degree of change
1	Millon's reagent	Proteins	Yellow color	+
2	Iodine solution	Starch	Reddish blue	+
3	$FeCl_3$ solution	Tannin	Orange colour	+
4	Wagner's reagent	Alkaloids	Dark yellow color	-

+: Present; –: Absent.

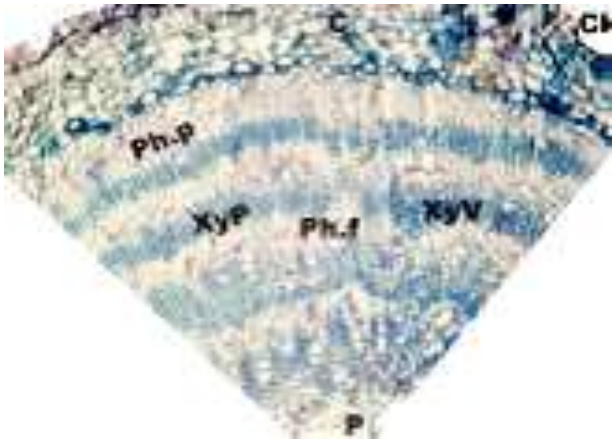

Figure 3. Transverse section of the stem. Ph.f: Phloem fibre; P: parenchyma; C: cortex; Pt: pith; XyV: xylem vessels; Ph.p: phloem parenchyma; XyP: xylem parenchyma.

Figure 4. Powder study of stem. Ck: Cork cells; E: epidermal cells; P: parenchyma; Pf: phloem fibres; T: trichomes; V: vessles; Xy: xylem fibres.

stained with phloroglucinol, concentrated hydrochloric acid and 50% glycerine for microscopical studies (Figure 4).

Colour reactions and fluorescence behaviour

Approximately 50 mg of the stem powder was treated with different chemical reagents (Table 2). The powdered drug after treatment was examined under ultraviolet (UV) light (254 and 366 nm) and visible light (Table 3) (Kokoski et al., 1958).

Fluorescence analysis

The fluorescence response of different extracts of 25 g powdered stem, namely, petroleum ether, chloroform, ethyl acetate, and ethanol were exposed to visible light, long UV (365 nm) and short UV (254 nm) was studied using the standard procedure (WHO, 1998) (Table 4).

Proximate analysis

Physico-chemical parameters like ash values, extractive values and moisture content were done (Table 6) (Indian Pharmacopoeia, 1996).

Pharmacological studies

The different pharmacological parameters like swelling index in water, foaming index, tannins fraction, bitterness value and haemolytic activity on powdered stem was studied according to standard procedures (WHO, 1998) (Table 6).

Table 2. Behaviour of stem powder with different chemical reagents.

S/N	Treatment	Color
1	Powder as such	Light brown
2	Powder + 1 N HCl	Light brown
3	Powder + 1 N NaOH	Brown
4	Powder + Acetic Acid	Greenish brown
5	Powder + 5% Ferric chloride	Brown
6	Powder + Picric acid	Greenish brown
8	Powder + 5% Iodine	Yellowish brown
9	Powder + 1 N HNO_3	Reddish brown

Toxicological parameters

Powdered drug was checked for the determination of arsenic and heavy metals like lead, cadmium and arsenic and the presence of total viable aerobic count, pathogens like *E. coli*, *Salmonella typhi*, *Pseudomonas aeruginosa, Staphylococcus aureus* and the presence of aflatoxins was determined by chromatographic methods using standard aflatoxins B_1, B_2, G_1, G_2 mixtures (WHO, 1998; Zapesochnaya et al., 1992) (Tables 5 and 6).

Table 3. Fluorescence nature of stem powder under ultra violet (UV) and visible radiations.

S/N	Treatment	Long UV (365 nm)	Short UV (254 nm)	Visible
1	Powder	Brown	Cream	Light green
2	Powder + 1 N HCl	Green	Light green	Light brown
3	Powder + 1 N NaOH	Greenish brown	Dark brown	Brown
4	Powder + 50% HNO_3	Green	Green	Reddish brown
5	Powder + 50% H_2SO_4	Green	Green	Red
6	Powder + Methanol	Green	Green	Sandy color
7	Powder + Acetic acid	Brown	Light green	Brown
8	Powder + Picric acid	Fluorescent green	Dark green	Light brown
9	Powder + 1 N NaOH in methanol	Greenish brown	Brown	Dark brown
10	Powder + 5% $FeCl_3$	Brown	Brown	Brown
11	Powder + 1 N NaOH in methanol + Nitrocellulose in amyl acetate	Brown	Dark brown	Rock color
12	Powder + 1 N HCl + Nitrocellulose in amyl acetate	Brown	Light brown	Brown
13	Powder + 1 N NaOH + Nitrocellulose in amyl acetate	Rock color	Dark green	Rock color
14	Powder + Nitrocellulose in amyl acetate	Greenish brown	Greenish brown	Light color
15	Powder + 5% iodine	Greenish brown	Greenish brown	Reddish brown

Table 4. Fluorescence nature of different solvent extracts of stem under ultra violet (UV) and visible radiations.

S/N	Extract	Long UV (365 nm)	Short UV (254 nm)	Visible
1	Petroleum ether	Green	Green	Yellow
2	Chloroform	Greenish brown	Brown	Green
3	Ethyl acetate	Dark green	Green	Light green
4	Ethanol	Greenish brown	Brownish green	Brownish yellow
5	Aqueous	Black	Brown	Blackish brown

Table 5. Microbial determination in various extracts.

S/N	Extract	Total bacterial count	Total fungal count	*Escherichia coli*	*Salmonella typhi*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
1	Alcoholic	Within the limits	-	+	-	-	-
2	Aqueous	Within the limits	-	+	-	-	-

+: Present; -: Absent.

High performance thin layer chromatography (HPTLC)

About 25 g of alcoholic and aqueous extracts of the stem was first suspended in hot distilled water and then shaken with different solvents in increasing order of polarity, that is, petroleum ether, chloroform, ethyl acetate and methanol in 500 ml of separating funnel. For each extract, that is, petroleum ether (PE), chloroform (Ch), ethyl acetate (EA) and methanol (Me), TLC was performed on silica coated plates to develop a suitable solvent system and then visualised in UV at 254 and 366 nm. The solvent systems for petroleum ether fraction 6:2:1 (PE:EA:Me), for chloroform fraction 25:2 (Ch:Me), for ethyl acetate 100:11:11:27 (EA:FA:AC:Water) and for methanol fraction 2:10:5 (PE:EA:Me). After selecting solvent system for each fraction, HPTLC studies was done by using 10×10 cm of glass plates, CAMAG TLC Scanner3 "Scanner3_130716 (1.14.26) attached with UV detector of D2 and W lamp with 281 V, second order optical fibre at 366 nm and controlled by win CATS software. HPTLC data and results are shown in Table 7 and Figures 5 to 8.

RESULTS AND DISCUSSION

Morphology

Stem part is greenish, cylindrical shape and solid with 3 to 7 mm diameter. Outer surface is smooth without any striations. It has brittle fracture with characteristics taste and odour (Figure 2).

Table 6. Physico-chemical parameter of the stem of *A. persica.*

S/N	Parameter	Results
1	Bitterness value	0.869
2	Loss on drying (%)	12
3	Swelling index	4
4	Foaming index	100
5	Tannins (%)	75
6	Aflatoxins (B_1, B_2, G, G_2)	Absent
7	Fibre content (%)	27
8	**Extractive values (%)**	
	a. Petroleum ether (60-80^{o}C)	0.5
	b. Chloroform	1.25
	c. Ethyl acetate	11.25
	d. Ethanol	2.0
	e. Aqueous	1.75
9	**Ash values (%)**	
	a. Total ash	6.19
	b. Acid insoluble ash	1.42
	c. Water soluble ash	2.45
	d. Sulphated ash	6.77
10	**Heavy metal concentration (ppm)**	
	a. Arsenic	0.7159
	b. Cadmium	0.0068
	c. Lead	0.0638
11	**Haemolytic activity**	
	a. Aqueous extract	Absent
	b. Ethanolic extract	Absent

Microscopical characters

The outermost layer consisted of 2 to 3 layers of flattened cork cells. Below the cork, six to seven layers of normal parenchymatous cells of the cortex were observed. The phloem lied externally, composed of parenchymatic cells and fibres. The xylem vessels were observed in few radial rows. The smaller vessels constituting the protoxylem lied towards the centre and the bigger ones constituting the metaxylem lied away from the centre. The xylem parenchyma was observed surrounding the xylem vessels. The pith consisted of polygonal thin walled cells with conspicuous inter cellular spaces (Figure 3).

Powder analysis

Microscopy

The powder of the stem showed the presence of phloem fibres, isodiametric cork cells, and normal parenchyma cells (Figure 4).

Histochemical tests

The sections of stem treated with different reagents showed the presence of proteins, starch, tannins and alkaloids.

Physicochemical studies

The physicochemical constants such as ash values showed total ash of 619 and water soluble ash of 1.42

Conclusion

The pharmacognostic standardization for the stem part of *A. persica* is laid down for the first time in this study. Morphological and anatomical studies of the stem will

Table 7. HPTLC data of various extracts of *A. persica* stem.

S/N	Name of the Extractives	Adsorbent	Spot No.	Stem values	
				R_f value	Area (%)
1	Petroleum ether	Silica gel	1	0.10	83.17
			2	0.61	1.80
			3	0.71	2.45
			4	0.87	3.42
			5	0.99	1.31
			6	1.23	3.80
			7	1.35	4.06
2	Chloroform	Silica gel	1	-0.47	61.23
			2	-0.03	10.41
			3	0.14	5.39
			4	0.33	3.67
			5	0.44	10.00
			6	0.71	2.86
			7	0.86	1.14
			8	1.42	1.56
			9	1.64	2.42
			10	1.91	1.32
3	Ethyl acetate	Silica gel	1	0.01	32.58
			2	0.15	7.96
			3	0.25	14.44
			4	0.57	18.85
			5	0.82	11.40
			6	1.09	18.78
4	Methanol	Silica gel	1	-0.05	50.34
			2	0.15	37.87
			3	0.60	5.78
			4	0.76	6.02

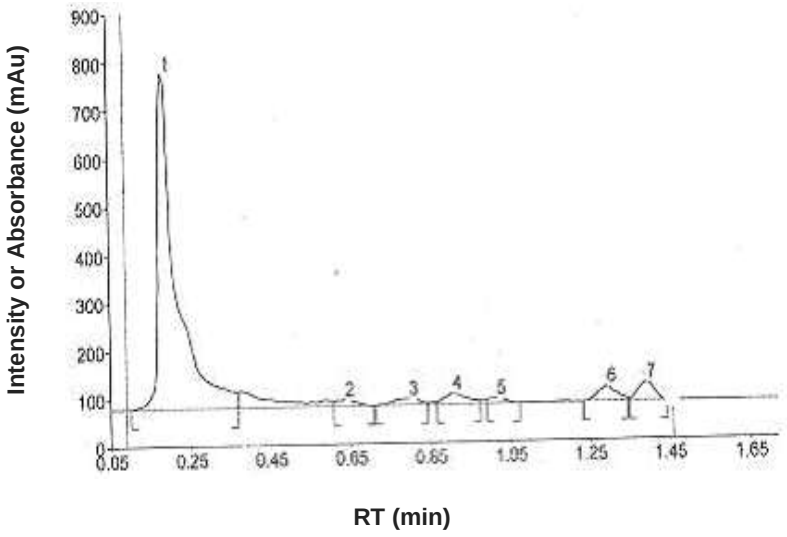

Figure 5. HPTLC profile of petroleum ether extract of stem of *A. persica*.

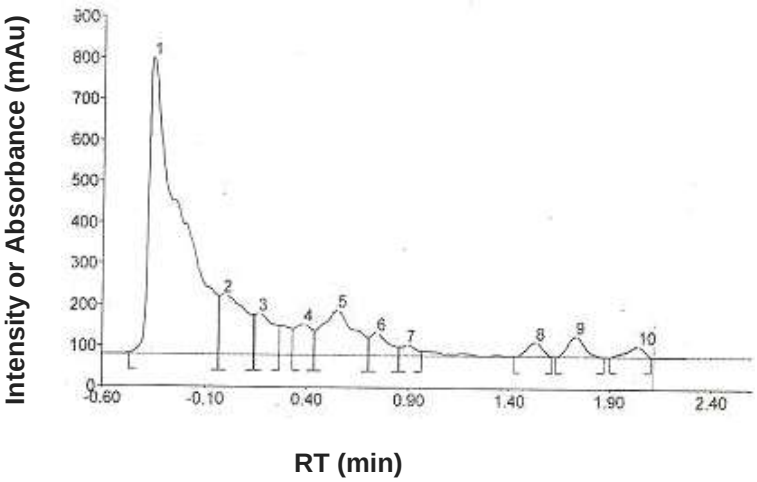

Figure 6. HPTLC profile of chloroform extract of stem of *A. Persica*.

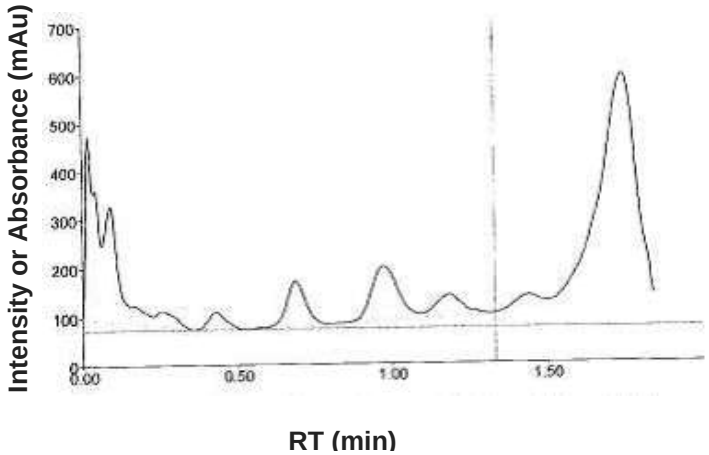

Figure 7. HPTLC profile of ethyl acetate extract of stem of *A. persica*.

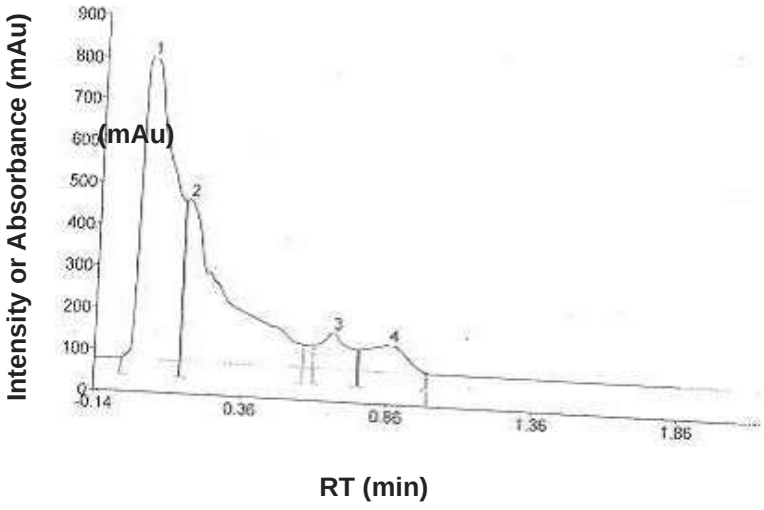

Figure 8. HPTLC profile of methanol extract of stem of *A. persica*.

enable the identification of crude drug. The information obtained from HPTLC studies will be useful in finding out the genuity of the drug. Ash values, extractive values, bitterness values, aflatoxin analysis, tannin, foaming and swelling index, and heavy metal detection can be used as reliable aid for detecting adulteration. These are simple, but reliable standards will be useful to a layperson in using the drug as a home remedy. Also, the manufacturers can utilize them for identification and selection of the raw material for drug production.

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