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Preliminary Pharmacognostical Standardisation of *Lawsonia inermis* Linn. Seeds

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Abstract: Commonly known as Henna or Mehndi, *Lawsonia inermis* Linn. (Lythraceae) is an important medicinal plant of Indian Systems of medicine. Henna leaf powder is used for staining hair, nails and beard. Seeds have been reported to possess deodorant action and are used in cases of menorrhagia, vaginal discharge and leucorrhoea. Few phytochemical studies have been carried out on seeds. However, there has been no report on standardisation of henna seeds. In view of the ethnopharmacological use, preliminary pharmacognostical standardisation of *L. inermis* seeds was carried out. In the present investigation, seeds had typical pyramidal shape and dark brown in color. The average dimensions of the seed were 1.51 mm (thickness) X 2.202 mm (length) X 1.887 mm (width). The powder was odorless and tasted slightly bitter. The transverse section of seed showed 2 or 3 layered yellowish brown testa, thick endosperm and a small embryo. Successive extractive value was highest in case of aqueous extract. Mean ash values (%) were 3.056 (total), 0.76 (acid insoluble ash) and 0.84 (water soluble ash). Loss on drying was 9.09%. The average pH of aqueous extract was 7.04. Seeds had a bitterness value of 125, foaming index 1080 (units/g), resins (5.53%) and fixed oil content (1.429%). Screening of alcoholic and aqueous extracts indicated the presence of carbohydrates, protein, phenolic and triterpenoids. Preliminary TLC finger prints of the seed extracts were also developed.

Key words: Henna seeds, standardisation

INTRODUCTION

Lawsonia inermis Linn. (Lythraceae) is an important medicinal plant of Indian Systems of Medicine. It is commonly known as Henna or Mehndi. Henna leaf powder is used for staining hair, nails and beard. Seeds have deodorant properties and are useful in menorrhagia, vaginal discharge and leucorrhoea (Anonymous, 2006; Nawagish, 2005; Zafar *et al*., 2006). Few phytochemical studies have been carried out on seeds (Zafar *et al*., 2006). However, there has been no report on standardisation of seeds. As the seeds are used by females for suppressing the body odor during gynecological problems, it was thought worthwhile to standardize them. The use of relatively simple parameters such as moisture content, extractive value, ash value, swelling index, tannin content, bitterness and R_f values etc. is quite handy in development of preliminary standards (Evans, 1982). Accordingly, the standardization of henna seeds was carried out.

MATERIALS AND METHODS

Materials

Seeds were collected from *L. inermis* shrubs growing naturally in Hamdard Nagar, New Delhi in the months of November and December, 2004 and identified by an expert taxonomist in Department of Botany, Faculty of Science, Jamia Hamdard. Voucher specimen of the seed (No. SA-001/2004) has been retained in the laboratory for reference purpose.

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Methods

Morphological examination of seed was followed by microcopy of transverse section of the seed. The extractive values were determined for cold, hot and successive extraction according to the Pharmacopoeial method (Anonymous, 1996).

The total, acid-insoluble and water-soluble ash values were determined according to the method described in Pharmacopoeia (Anonymous, 2002). The percentage of foreign matter in the crude drug was determined. pH of 1 and 10% aqueous extracts was measured with a calibrated digital pH meter. Loss on drying was also noted. Bitterness value of the drug sample was determined with reference to quinine hydrochloride as described by WHO (Anonymous, 1998). Swelling and foaming indices were also studied by standard procedures. Resin, fat (fixed oil) and microbial load were determination as per WHO guidelines.

The seeds were powdered and the powder was subjected to flouresence analysis after being separately treated with water, NaOH, H_2SO_4, HCl, HNO_3, ethyl acetate, chloroform petroleum ether and examined under UV light and daylight. Color reactions of powder were observed after being treated separately with NaOH, H_2SO_4, HCl, HNO_3, glacial acetic acid, Iodine, chloroform and petroleum ether.

The petroleum ether, alcohol, chloroform and aqueous extracts of the seeds were prepared and residues obtained after separate evaporation of the above mention extracts were subjected to preliminary phytochemical screening (Nawagish, 2005) for the detection of plant constituents: sterols, alkaloids, saponins, tannins, flavonoids, proteins, amino acids, carbohydrates (including sugars), resins, lipids.

TLC profiling was done as per the method described by Stahl (1969). Three extracts; namely chloroform, methanol and petroleum ether extracts; obtained from the seeds of *L. inermis* were subjected to thin layer chromatography to find out the nature and approximate number of compounds present.

RESULTS AND DISCUSSION

Keeping in view of the ethnopharmacological importance of henna seed in gynecological disorders, preliminary studies were undertaken for standardisation of *Lawsonia inermis* Linn (Lythraceae) seeds.

Seeds of *L. inermis* Linn. were smooth in texture, pyramidal in shape and dark brown in color. The average dimensions of the seed were found to be 1.51 mm (thickness) × 2.202 mm (length) × 1.887 mm (width). The powder was odorless and slightly bitter in taste. Morphological studies on the seed are in agreement with previous findings (Anonymous, 1996).

The transverse section of seed showed presence of testa, small endosperm and embryo. Testa was 2 or 3 layered and appeared yellowish brown. Endosperm was composed of thick walled polygonal cells. Embryo appeared as innermost structure surrounded by endosperm cells. The cells of embryo were small in size and polygonal in shape. Results of microscopic studies of the seed are in agreement with previous findings (Anonymous, 1996).

The mean values of different solvent extractives have been indicated in the Table 1. Individual hot extractive values are higher than cold extractives one-obviously indicating the superiority of hot extraction process. Successive extractive value is highest in case of water, indicating the possibility of considerable content of polar compounds in seeds.

Mean ash values (%) were found to be 3.056 (total), 0.76 (Acid insoluble Ash) and 0.84 (Water soluble ash). Total ash value is relatively low due to low content of carbonates, phosphates, silicate and silica. Foreign matter in seeds was 5.36%. Low ash value and foreign matter may also be

attributable to first-hand collection of plant material from a non-polluted area (interior of Hamdard Nagar, New Delhi) with sufficient green cover. Loss on drying turned out to be 9.09% on account of loss of water and/or volatile chemicals. The average pH of aqueous extract was 7.04. Seeds had a bitterness value of 125. No swelling was observed possibly due to absence of mucilage in seeds. Foaming index was found to be 1080 (units g^{-1}). Resin (5.53%) and fixed oil content (1.429%) was observed. Fixed oil content is low on account of small endosperm region which contains cells with fixed oils. Forty one white circular colony forming units were detected in 1:100 dilution of seeds on nutrient agar medium. The results of fluorescence analysis and color reactions of powder with different chemicals have been shown in Table 2 and 3, respectively.

Preliminary phytochemical investigation was undertaken for the identification of different type of chemical constituents present in the seed. Screening of alcoholic and aqueous extracts indicate the presence of carbohydrates, protein, phenolic and triterpenoids (Table 4). Preliminary TLC finger prints of the seed extracts were developed and reported in Table 5.

Table 1: Extractive values of henna seeds with different solvents

Extraction method	Petroleum ether	Chloroform	Alcohol	Water
Cold	0.8730	0.724	1.446	1.524
Hot	2.3365	2.313	6.600	30.370
Successive	2.2410	1.633	4.780	10.340

Table 2: Florescence analysis of henna seed powder

Solvent used	UV light (254 nm)	UV light (366 nm)
NaOH in methanol	Light green	Dark green
NaOH in water	Clear	Light pink
Benzene	Brick red	Dark green
Acetone	Light brown	Dark green
Ethyl acetate	Light brown	Pink
Chloroform	Light brown	Pink
Dil. H_2SO_4	Light green	Light green
Conc. HCl	Brick red	Dark brown
Distilled water	Light brown	Light green
50% HCl	Greenish yellow	Yellow
Dil. HNO_3	Brick red	Dark green
Conc. H_2SO_4	Black	Black

Table 3: Powdered drug reactions of henna seed extracts with different reagents

Treatments	Observation
Conc. HCl	Dark brown
Conc. HNO_3	Brick red
Conc. H_2SO_4	Black
Glacial acetic acid	Brownish yellow
Iodine solution	Reddish brown
NaOH in methanol	Griyesh green
NaOH in water	Dark brown

Table 4: Preliminary phytochemical tests of henna seed extracts

Tests	Pet ether extract	Alcoholic extract	Chloroform extract	Water extract
Alkaloids	-	-	-	-
Phenolics	-	+	-	+
Flavonoids	-	-	-	-
Sterols	-	-	-	-
Carbohydrates	-	+	-	+
Tannins	-	-	-	-
Proteins	-	+	-	+
Triterpenoids	-	+	-	-

+ = Present; - ve = Absent

Table 5: TLC fingerprint of henna seed extracts

Extract	Solvent system	Reagent used	No. of spots	R_f-value
Methanol	Chloroform: Methanol (9:1)	Anisaldehyde	7	0.21, 0.27, 0.47, 0.54, 0.60, 0.71, 0.73
Pet. Ether (60-80)	Pet. ether (60-80): Chloroform (50:50)	Anisaldehyde	4	0.07, 0.12, 0.47, 0.50
Chloroform	Chloroform: Methanol (95:5)	Anisaldehyde	6	0.09, 0.19, 0.28, 0.43, 0.63, 0.80

CONCLUSIONS

Fresh henna seeds have been subjected to preliminary pharmacognostic standardisation including phytochemical screening. The present investigation adds to the existing knowledge of henna seed and will be quite useful for development of a formulation for treating cases of body odor associated with diseased female reproductive system. Heavy load of microbe might have resulted from contamination during storage. There is a need to compare the microbial load of fresh and stored seeds. HPTLC fingerprinting w.r.t. class-specific marker compounds and their estimation in seeds needs to undertaken. Pharmacological studies are also required to validate the utility of seeds in gynecological problems.

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