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Physico-chemical and biological properties of seed powder of flannel weed

Manish Kumar, Aradhita Ray, Akshma Berwal and Ashok K. Pathera*
Department of Food Technology, Guru Jambheshwar University of Science and Technology, Hisar, Haryana 125001

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ABSTRACT

Assessment of various physico-chemical and biological properties of seed powder of *Sida cordifolia* was done. This plant grows along roadsides and in open land in tropical climates. Mainly useful plant parts are seeds, leaves and roots. The loose bulk density, tapped bulk density, hausner ratio and angle of repose of sample were found to be 0.31g/cm^3±0.1, 0.38 g/cm^3± 0.2, 1.22%± 1.6 and 30.96°± 0.7, respectively. Moisture content of sample was found to be 10.00±1.6. Total ash, fat, total protein and non protein nitrogen was 3.80% ± 0.5, 10.56% ± 0.3, 22.00% ± 0.9 and 11.20% ± 0.4, respectively. Thermal stability of seeds was up to 200 °C. Low molecular weight protein bands ranging 10-60 kDa were identified. The demonstration of antimicrobial activity against microbes may be indicative of the presence of broad spectrum antimicrobial compounds. Amide I and amide II bands were two major bands of the infrared spectrum. This powder has very low water absorption capacity.

Key words: Antimicrobial activity, Infrared spectra, Physico-chemical characters, *Sida cordifolia*, Thermal stability

Sida cordifolia (bala, country mallow or flannel weed), native of India is a perennial shrub of mallow family *Malvaceae*. It has naturalized all over the world, and is considered a persistent weed in Africa, Australia and the southern United States (Pole 2006). *Sida cordifolia* grows along road sides and in open land in tropical climates of India and Sri Lanka. It is found mainly in North-Western part of India particularly Haryana, Punjab, Maharashtra and Rajasthan. It grows well in the plains of India, especially, in damp climates. The seeds contain much larger quantities of alkaloids than the leaves and roots. The weed is useful in treating colic disorders and gonorrhoea (Agharkar 1991).

Traditionally the plant has been used as CNS depressant, fat reducer, analgesics, anti-inflammatory, hypotensive, hepatoprotective and antiasthmatic (Mediros *et al.* 2005). Presence of ephedrine has highlighted the utility of this plant. Conventionally, nutrition and food companies were using plants such as *Ma-Huang* (*Ephedra* plant), because it contained relatively large amounts of ephedrine, in their weight loss products. However, since this product was banned in many countries including the USA and UK, they are now looking for alternatives. *Sida cordifolia*, with its ephedrine and pseudoephedrine has gained a set of attention and is now sold by many of these companies (Ghosal *et al.* 1975). It is almost odourless with slightly bitter taste (Rangari 2000). Some work has already been carried out using various components mostly with regard to pharmaceutical (Franco *et al.* 2005, Mediros *et al.* 2005). Not much work has been conducted in relation to various properties of this plant to use it in food formulation to treat diseases. So an attempt has been made to characterise *Sida cordifolia* for further processing. Various examinations like physical, proximate, thermal, surface, protein characterisations and antimicrobial activity are carried out.

***Corresponding author:** apathera@gmail.com

MATERIALS AND METHODS

Seeds of *Sida cordifolia* were collected from their natural habitat *i.e.* Mahendargarh (Southern Haryana), India and were authenticated by Department of Botany and Plant Physiology, CCS Haryana Agriculture University, Hisar, Haryana. All the chemicals used in analysis were of analytical grade supplied by HiMedia, Qualigens and central drug house. Sample prepared by crushing seeds into fine powder using mechanical blender for analysis and were stored in brown coloured glass bottles at average temperature of 25 °C.

Physical characterisation

Various physical characters like size, bulk density, angle of repose, hausner ratio and color were determined. Length and breadth of seeds were observed by using digital vernier calliper (A2583) (AOAC, 1995). For bulk density, sample powder (20 g) was taken in a 100 ml measuring graduated cylinder. The cylinder was put on horizontal surface and volume occupied by sample was recorded. Ratio of mass by volume was determined as loose bulk density. Volume

was again determined after tapping the cylinder three times at an interval of 2 seconds that gives tapped bulk density (Phani *et al.* 2011). The hausner ratio (H) is a number that is correlated to the flow ability of a powder or granular material (Beddow 1995).

$$\text{Hausner ratio (H)} = \frac{\text{Tapped bulk density}}{\text{Loose bulk density}}$$

Angle of repose in general is "The angle formed between the horizontal plane and a slope line extending along the face of a heap formed by pouring material onto a horizontal surface." The height and diameter of heap of powder was noted (Lachman 1991).

$$\tan é = h/r$$

The color of the powder was measured with Hunter Lab Colorimeter (Color Tec- PCM™). The color values L^*, a^*, b^* were measured directly from digital colorimeter that works on the principle of illumination. The dimension L^* means lightness, with 100 for white, 0 for black; a^* indicates redness when positive and greenness when negative, b^* indicates yellowness when positive and blueness when negative (Khouryieha and Aramounib 2012). The hue angle and chroma were calculated from a* and b^* values, as follows:

$$\text{hue angle} = \tan" (b^*/a^*)$$

$$\text{chroma} = "[(a^*)^2 + (b^*)^2]$$

Chemical characterisation

Moisture, ash, crude fat content of seed powder was determined by methods given in AOAC (1995). Crude protein and nitrogen was analyzed by DUMAS based protein analyzer. This procedure is faster and less complicated than Kjeldahl method (ICC 2001). Non protein nitrogen was determined by using Kjeldahl method with slight modification in preparation of sample (AACC 2000).

Surface characterisation

Surface properties were determined by using FTIR (Fourier Transform Infrared Spectroscopy) and obtained spectra was comparatively analyzed and interpreted with a chart for Characteristics IR absorption frequencies of organic functional groups and carbonyl containing functional group (Demirdroven *et al.* 2004).

Thermal analysis

Thermal analysis was carried out with the help of DSC (differential scanning colorimeter) - TGA (thermo gravimetric analysis) model (SDT Q600 V20.9). Powdered sample (4.5 g) was run in sample pan from room temperature to 400°C with slope of 10°C/min. It is a type of testing performed on samples that determine changes in weight in relation to a temperature program in a controlled atmosphere. Such analysis relies on a high degree of precision in three measurements: weight, temperature, and temperature change. Weight changes during increase in temperature were recorded. A plot of weight changes versus temperature and heat flow versus temperature was recorded (Wunderlich 1990).

Estimation of anti-microbial activity

Powdered sample was subject to aqueous extract. The resulting filtrate was then concentrated in a rotary evaporator and subsequently lyophilized to dryness. Antimicrobial activity was determined against *E.coli* and *Aspergillus heteromorphus*. Antimicrobial activity was evaluated by the bore well method. For determination of antibacterial activity bacterial cultures were inoculated on to nutrient agar. For determination of antifungal, fungal isolates of 10^6 cfu/ml were inoculated on potato dextrose agar. Well were made and impregnated with 50 µl of extract dilutions reconstituted in minimum amount of solvent at concentrations of 50, 100, 200 mg/ml were applied over each of the culture plates seeded with 0.5 McFarland and 10^6 cfu/ml cultures of bacteria and fungi respectively. Bacterial cultures were then incubated at 37°C for 18 hours while fungal culture was incubated at 30-32°C for 48 hours. Wells impregnated with 50 µl of 50, 100, 200 mg/ml of sodium benzoate (standard antimicrobial agent) were used for comparison. Antimicrobial activity was determined by measuring the zone of inhibition around each well. For each concentration three replicates were conducted against each organism (Sarangi *et al.* 2011).

Rheological analysis

Mixing behaviour of dough was determined by using Perten micro- dough LAB. The micro-dough LAB is a small scale (4g) dough mixer and analysis system to determine the quality and processing characteristics of flour and dough (Dang *et al.* 2007).

Protein characterisation

Sample was prepared by grinding 1 g sample in 1 ml chilled phosphate buffer (0.1 M, pH 7.0) along with 50 mg insoluble polyvinyl pyrrolidone. These were then centrifuged at 10,000 x g at 4 °C for 15 min. The pellet was discarded and protein in the supernatant was quantified according to Bradford (Bradford 1976). The protein extract was transferred to an equal volume of 2 x sample buffer, heated at 100° C for 3 min, cooled and used for SDS-PAGE. An aliquot containing 50 µg of sample protein was loaded in each well. Electrophoresis was carried out using vertical slab gel electrophoresis apparatus following standard method of electrophoresis (Laemmli 1970).

RESULTS AND DISCUSSION

Physical characterisation

The properties such as loose bulk density, tapped density, hausner ratio and angle of repose are often referred to as the derived properties of sample which depend mainly on size distribution, shape and tendency of the particles to adhere together. The loose bulk density, tapped bulk density, hausner ratio and angle of repose of sample were found to be 0.31g/cm^3±0.1, 0.38 g/cm^3± 0.2, 1.22%± 1.6 and 30.96^o± 0.7 respectively as shown in (Table 1). When angle of repose is less than 30°, it indicates that material is free flowing and values greater than 40° suggest a poorly flowing material. The static angle of repose value for *Sida cordifolia* seeds was found to be 30.96° indicating good flow properties. The Hausner ratio is used in a wide variety of industries as an indication of the flow ability of a powder. A Hausner ratio greater than 1.25 is considered to be an indication of poor flow ability. From the result it was seen that Hausner ratio and angle of repose was 1.22± 1.6 and 30.96^o± 0.7 respectively. It was concluded from the values that the sample has good flow properties for future application (Beddow *et al.* 1969, Conesa 2004).

Colour analysis

The colour of the material plays a major role in consumer's perception and acceptability of the product. The colour parameters L^*, a^*, b^*, hue angle and chroma for the powder (Table 2). Lower value of L^* represent darker color. Positive values of a^* and b^*indicates redness and yellowness of sample. Overall the color results indicated substantially darker and browner appearance of sample. Hue angle and chroma both relate to color perception. Hue is the attribute of color that is related to the perceived colors. Chroma represents color intensity of a surface judged in comparison to a pure white. Chroma vary from gray (chroma = 0) to brilliant red (chroma = 104). From the results it was concluded that sample was darker in color and had more color intensity (Khouryieha and Aramounib 2012).

Table 1. Physical characterisation of *Sida cordifolia* powder (put table in upper paragraph)

Characteristic	Result
Loose bulk density	0.31 g/cm^3±0.1
Tapped bulk density	0.38 g/cm^3± 0.2
Size	3.50 mm ± 0.2
Hausner ratio	1.22%± 1.6
Angle of repose	30.96^o± 0.7

Values are mean ±SD of three replicates

Table 2. Colour analysis of *Sida cordifolia* powder

Characteristic	Result
*L**	05.05±0.5
*a**	16.60±1.5
*b**	35.54±2.0
hue angle	64.95±0.4
chroma	39.23±2.0

Values are mean ±SD of three replicates

Chemical characterisation

Moisture content of sample was found to be 10.00%±1.6 .Total ash, fat, total protein and non proteinic nitrogen was 3.80% ± 0.5, 10.56% ± 0.3, 22.00% ± 0.9 and 11.20 ± 0.4 respectively. Moisture is an indicator of storage stability. Powder with high moisture content (over 14.5%) attracts mold, bacteria, and insects, all of which cause deterioration during storage. Flour with low moisture content is more stable during storage. Ash in powder can affect color, imparting a darker color to finished products. Protein content of the powder was found to be high so it can be used as a fortifying material to make protein enriched products. Results obtained were similar to that obtained by other investigators in *Hibiscus sabdariffa* plant (Adebayo-Tayo *et al.* 2000).

Surface characterisation

FTIR spectra of sample were shown in the 4000–400/cm range. The spectrum is quite complex and contains several bands arising from the contribution of different functional groups belonging to protein, lipids and carbohydrates. Amide I and amide II bands are two major bands of the protein infrared spectrum. The infrared of the protein is characterized by a set of absorption regions known as the amide region and the C–H region. The most widely used modes regions are amide ^{2}, amide ^{22} and amide ^{222}. Amide ^{2} band arises principally from C=O stretching vibration of the peptide group. Amide ^{22} band is primarily N–H bending with a contribution from C–N stretching vibrations. The amide ^{222} absorption is normally weak and arises primarily from N–H bending and C–N stretching vibrations. The amide I band (between 1600 and 1700/cm) is mainly associated with the C=O stretching vibration (70-85%) and is directly related to the backbone conformation.

Amide II results from the N-H bending vibration (40-60%) and from the C-N stretching vibration (18-40%). This band is conformationally sensitive. Amide III (1300-1400/cm) and IV (1200-1300/cm) are very complex bands resulting from a mixture of several coordinate displacements (Cakmak *et al.* 2006). The bands observed at 3700-3950/cm are due to hydroxyl

Table 3. Chemical analysis of *Sida cordifolia* powder

Characteristic	Result (%)
Moisture	10.00±1.6
Total ash	03.80±0.5
Fat	10.56±0.3
Total protein content	22.00±0.9
Non protein nitrogen Content	11.20±0.4

Values are mean ±SD of three replicates

(O-H) vibrations and band observed at 3292.49/cm is due to O-H stretching. Banding pattern observed at 3024.38/cm and 2926.01/cm are due to alkene and CH_2 asymmetric stretch mainly lipid respectively. The bands in the 1500–1200/cm regions arise mainly from the C-H bending vibrations of CH_3, CH_2 and CH functional groups. In our study these are observed at 1238.30, 1323.17, 1402.25 and 1446.61/cm. Information on phosphodiester functional groups can be obtained in the region between 1250 and 1200 cm^{-1} which corresponds to > P = O asymmetric stretching frequencies (Dumas and Miller 2003, Yee *et al.* 2004).

The region from 1200 to 900/cm is mainly dominated by a sequence of bands due to C-O, C-C stretching vibrations of polysaccharides. These groups mainly occur in carbohydrates and polysaccharides (Wolkers 2004). *Sida cordifolia* showed polysaccharide bands at 1064.71/cm. It can be concluded that this plant showed the presence of oligosaccharides, phosphates, proteins, carbohydrates and carotenoid. This work offers scope for further research on biological activity of this medicinal plants. In the present study, it was seen that FTIR spectroscopy can be used for easy and rapid identification of various functional groups responsible for medicinal properties.

Thermal analysis - A loss in weight up to 70 °C was observed due to presence of moisture. But after that weight was constant up to 200 °C. This indicates that the sample was stable up to 200 °C. After 200 °C, decomposition (pyrolysis oxidation) started. But in present study, weight was constant up to 200 °C that means sample used was free from impurities.

Anti-microbial activity

Results indicated that seed extract was effective against both microbes (Table 4). The highest inhibitory activity (11 mm) was noticed with the concentration of 200 mg/ml with *E. coli.* In both cases *i.e* with *E. coli* and *Aspergillus heteromorphus,* the inhibitory activity with extract was less than that of sodium benzoate at all the concentrations (50, 100 and 200 mg/ml). The inhibitory activity of seed extract was about seventy per cent of activity exhibited by standard sodium benzoate.

Table 4. Anti-microbial activity of extract of *Sida cordifolia* powder

	Zone of inhibition (mm)					
	Water extract of seeds			Sodium benzoate		
Concentration (mg/ml)	50	100	200	50	100	200
E.coli	6	8	11	9	12	15
Aspergillus hetromorphus	8	13	17	10	17	24

The demonstration of antimicrobial activity by water extract provides the scientific basis for the use of this plant in traditional treatment of disease, since most traditional medicine men use water extract as their solvent in which decoctions are prepared (Sarangi *et al.* 2011).

Rheological analysis

Results indicated that this powder has very poor mixing character as graph can reach to 250 BU. Generally 500 BU is standard in case of wheat and other cereals. Therefore, it can be estimated that the powder of *S. cordifolia* alone can not be used in formulations as it has poor mixing behaviour. However it can be used to supplement with wheat powder, following observations were made:

1 Peak resistance - The maximum torque attained, as measured from the middle curve. It was observed 229.0 mNm

2 Development time - The time taken for the dough to reach the peak resistance. It was 1.3 min.

3 Arrival time - Time required for the top curve to reach the peak resistance. It was observed 1.1 min.

4 Departure time - The required time for the top curve to fall below the peak resistance. It was observed 1.7 min.

5 Stability - The difference between the arrival and departure times. It was observed 0.6 min.

6 Mixing tolerance index - The difference between the top-curve torque at the development time and the top-curve torque at a specified time after the development time (typically 5 minutes) (Dang *et al.* 2007).

Protein characterisation - The SDS-PAGE analysis showed approximately three major protein bands, with relative molecular masses ranging from 10 to 60 kDa. In order to obtain good resolution of proteins of low molecular weight by gel electrophoresis, 10% polyacrylamide gel was used. Such a gel system gave good resolution of proteins of low molecular weight. It was seen that LMW proteins were present in sample. Effect of LMW protein was correlated with very short

gap in peaks that was observed during study of mixing behaviour (Hussein 2010).

Sida cordifolia powder has good flow properties and storage stability. It was observed that the protein and fat content of powder was very high so it can be used as a fortifying material. The color intensity of powder was found high. FTIR spectra observed was quite complex and contains several bands arising from the contribution of different functional groups belonging to protein, lipids and carbohydrates. The demonstration of antimicrobial activity against microbes may be indicative of the presence of broad spectrum antimicrobial compounds. DSC-TGA thermogram was used to study thermal behaviour of powder and found that weight was constant up to 200 ^{o}C which indicate that *Sida cordifolia* was free from impurities. Rheology analysis indicared that powder has very poor mixing character but it can be easily mixed with other cereals. The SDS-PAGE analysis showed approximately three major protein bands, with relative molecular masses ranging from 10 to 60 kDa.

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