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Physicochemical and phytochemical standardization of fruit of *Sesbania grandiflora*

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ABSTRACT

The World Health Organization encourages the use of medicinal plants especially in countries where conventional treatment of major diseases seems to be insufficient. India has good biodiversity and Indian medicinal plants are used in successful management of various diseases. Sesbania grandiflora (SG), family – Leguminosae, is a well known Indian medicinal plant used in traditional system for various diseases. SG comprises of various phytochemical constituents. The extracts of this plant have been pharmacologically evaluated for hypolipidemic activity, wound healing activity, anti-ulcer activity, antioxidant activity, hepatoprotective activity etc. The therapeutic activity is due to the phytochemicals present in the plant. So, it is highly essential to standardize the medicinal plant on the basis of physicochemical and phytochemical aspects to identify the genuine plant part. The results demonstrated that the physico-chemical parameters such as ash value, extractive value, foreign matter, loss on drying, foaming index and crude fiber content determination are the suitable parameters in the standardization of the fruit of SG. The fluorescence analysis of the powder drug further authenticated its validation. The preliminary phytochemical screening of MSG and its fractions revealed that the plant part contained alkaloids, glycosides, saponin, tannins, flavonoids, carbohydrates, proteins and sterols. Thus, it can be assumed that these are the standard parameters in the standardization of fruit of SG. The benefit of the study would be to the group of researchers who will be working in the identification and use of the fruit of SG.

Key words: *Sesbania grandiflora*, ash value, fiber content, phytochemical screening, T.L.C.,

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INTRODUCTION

Traditional medicine practice involves the prescription of medicinal plants and their extracts to prevent, diagnose and eliminate the physical, mental and social illness [1]. The prevalence of use of traditional folk medicines obtained from plant resources is still more than 80% of the total population in developing countries like India [2]. Medicinal plants and its formulations have served the human society from ancient time in curing various diseases [3]. Due to multiple factors the use of medicinal plants as medicines around the globe still exceeds than the use of modern synthetic drugs. This ancient approach of treatment is unchallengeable [4]. To establish the scientific basis of the effectiveness of medicinal plants in the Ayurveda, the standardization procedure is mostly adopted. India uses nearly7, 000- 7,500 medicinal plants in various forms to treat the various diseases and the potency of these products mainly based on the quality of the raw materials used [5]. The medicinal plants contain several phytomolecules and certain phytomolecules are toxic in nature [6]. In order to reduce the incidence of toxicity, adulteration, indiscriminate use of medicinal plant and its formulation, the quality of the plant and its formulation is highly

essential. Systematic evaluation of the quality control parameters of the plants material and its formulation using modern analytical techniques like chromatography and spectroscopy can be effective in validation of herbal drugs. Thus, standardization is a prerequisite step to follow in the consideration of herbal medicines for the betterment of health.

Sesbania grandiflora (SG), family: Leguminosae-Fabaceae, is a well known Indian medicinal plant which is used in traditional Indian medicines for various diseases. It is reported that SG rich with various phytoconstituents such as saponin, flavonoids, phenolics, alkaloids, tannins, carbohydrates, proteins and glycosides are pharmacologically active [7]. The different parts of the plant such as leaves, roots, bark, flower and fruit are reported to possess several pharmacological activities in experimental conditions [8]. The various pharmacological activities of this plant includes hypolipidemic [9], wound healing [10], anti-ulcer [11], antioxidant [12], antiurolithiatic [13], anticancer and chemo-preventive [14], hepatoprotective [15], anxiolytic and anticonvulsive [16], antimicrobial [17], anti-tuberculosis [18], analgesic and antipyretic activity [19]) and many more are documented in the experimental conditions. Although several biological activities are reported for the fruit of SG, there is no report on the standardization criteria of it. Therefore, physico-chemical and phytochemical standardization of the fruit of SG is attempted as per WHO guidelines.

MATERIALS AND METHODS

Chemicals and reagents:
All the chemical and solvents used in this study are analytical grade**.**

Collection of plant material:
The fresh fruits of *Sesbania grandiflora*, family – leguminosae: papilionoideae was collected from garden of K. V. K., R.G.S.C., B.H.U. Uttar Pradesh state, India. Both the plants were identified and authenticated by Dr. K. Karthigeyan Scientist 'C', Central National Herbarium, Botanical Survey of India, Howrah- 711103, India (letter No – CNH/ 77/ 2012/ Tech. II/ 920). The fresh fruit of SG was washed with tap water and dried in shade for 15 days. The physico-chemical and phytochemical standardization of the fruit of SG was carried out based on the standard methods in WHO guidelines and Ayurvedic Pharmacopoeia.

Fluorescence analysis of powder
Fluorescence studies were performed after adding the powder of fruit of SG with various reagents like picric acid, sodium hydroxide, nitric acid etc and the fluorescence of individual product was observed under daylight, long UV at 365 nm and short UV at 254 nm [20].

Physico-chemical evaluation
The air-dried fruit of SG was used for the determination of various physicochemical constants. The parameters were determined as per WHO guidelines on quality control methods for medicinal plants material 2011**.** The procedure followed for the determination of physico-chemical parameters were as mentioned below [21].

Foreign matter
Accurately weighed 100 gm of the sample of the fruit of SG. The fruit of SG was spread in a white paper and unwanted materials were separated from the essential drug using magnifying lanes. The dust was removed by using sieve. Finally the plant drugs were weighed to determine the average percentage weight of foreign matter

Ash value: The total ash, acid-insoluble ash and water soluble ash are the parameter used to represent the ash value of medicinal plant.

Total ash determination: The air-dried coarse powder drug (2gm) of fruit of SG was placed in previously dried and tared silica crucibles. The powder sample was then spread evenly and ignited it to a constant weight with gradual increased temperature to 450^{o}C till the plant material was whitish, that represent the absence of organic matter. The crucibles was cooled in desiccators and finally weighed to determine the total ash. The data obtained was then calculated in terms of percentage w/w of the total ash and the result of the total ash was further used for the determination of acid insoluble and water soluble ash.

Acid insoluble ash determination: The total ash obtained from fruit of SG was placed in a crucible and 25 ml of hydrochloric acid (2N) was added and the crucible was then covered with a watch-glass and boil gently for 5 minutes. The watch glass was then washed with 10 ml of hot water and collected to the crucible. Final mixture was filtered on an ashless filter-paper and washed with hot water. The insoluble residue matter was transferred to the clean crucible along with the filter paper and ignited upto 450°C constant weight. The residue was allowed to cool in a suitable desiccator for 30 minutes and weighed. The final data of acid-insoluble matter was calculated in mg per gm of air-dried material.

Water-soluble ash determination: The total ash of both plant material was placed in separate crucible and 25 ml of distilled water was added to it. The mixture was boiled for 5 minutes and filtered on an ashless filter-paper. The filter papered was washed with hot water and finally insoluble matter was collected. It was ignited in a crucible for 30 minutes at a temperature about 450°C. Final weight was deducted from weight of ash used. Finally the percentage of water soluble ash was determined with respect to the air dried material.

Extractive value: The extractive value of medicinal plant material indicates the amount of the active constituents extracted with specific solvents from a given quantity of crude drug. The solvent selected for determination of extractive value are petroleum ether, alcohol, water. The weight of extractable matter was represented in terms of percentage petroleum ether, alcohol, water soluble extractable matter respectively in the table 2.

Determination of foaming index: It explains the saponin phytochemical content in a crude drug. In this method the coarsely air-dried powder drug of fruit of SG was weighted and boiled with water for 30 minutes. The decoction was used to determine the foaming index as per the WHO standard procedure in the specified test tubes. The result was represented in table 2.

Loss on drying (% w/w): The loss on drying test is designed to determine the moisture and volatile substances present in the sample. A glass bottle with stopper was taken and dried for 30 minutes at 105 ^{0}C, allowed to cool it in a desiccator and weighted. Two gram coarsely air-dried powder drug of fruit of SG was placed in the dry bottle and weighted. Again the bottle was dried in oven at 105^{0}C to a constant weight, cooled and weighted. The weight difference of the sample is loss on drying and the percentage w/w loss on drying was determined.

Crude fiber content: The "crude fibre" is the non digestible substances of vegetable origin obtained as the residue after specifically defined digestion process. Two gram of coarsely air-dried powder drug of fruit of SG defatted with petroleum ether. Then the material was treated with 200 ml of (0.255 ±0.005N) sulphuric acid for 30 min with bumping chips. The digested material was filtered and washed with distilled water. Again the residue was boiled with 200 ml of sodium hydroxide solution (0.313 ±0.005N), for 30 min followed by filtration. The residue washed with 25 ml of boiling 1.25% sulfuric acid, followed by sufficient water and 25mL alcohol. The residue transferred to a pre weighted crucible (w_1), dried at 120^0 C for 2hours and weighted (w_2).

%Crude fiber content = (($w_2 - w_1$) / Wt. of powder of fruit of SG) x 100

Phytochemical standardization

Extraction methodology: Extraction of the plant materials from the fruits of SG was done by soxhlation method. The air dried coarse powdered (2kg) fruit of SG was defatted with petroleum ether. Further the defatted crude drug was extracted by soxhlation process with methanol (80% v/v) for 72 hours at 65°C. The extract obtained from the fruit of SG was filtered and concentrated using rotary evaporator (Perfit, India Pvt. Ltd, Ambala) at 70 °C for 30 minutes [22]. The dried methanolic extract were weighted to determine the % yield and kept in desiccator for further study. Half of the methanolic fruit extract of SG was fractionated by four water immiscible solvents on the basis of polarity after reconstituting the methanolic extract in water. The solvents used to fractionate the extract were petroleum ether, chloroform, ethyl acetate and n-butanol [23]. Further each fraction were concentrated to semisolid mass and used for phytochemical analysis.

Preliminary phytochemical screening

The preliminary phytochemical screening of the whole methanolic extract of the fruit SG (MSG) and its successive petroleum ether, chloroform, ethyl acetate and n- butanol fractionated extracts were carried as per the standard procedure described by Khandelwal [24], Trease and Evans, [25] and Kokate [26]. The plant crude extract / successive fractions obtained were screened for the presence of various classes of phytoconstituents like alkaloids,

glycosides, phenolic, tannins, flavonoids, saponins, coumarins, steroids, carbohydrates and proteins. The specific colour reaction or formation of identifying precipitate was noted and reported in table 3.

Tests for alkaloids:
The preparation of whole methanolic plant extract and successive fractions extract of fruit SG was dissolved in 2N HCl and filtered. The filtrate was treated with following reagent to detect presence of alkaloids with Mayer's reagent (Potassium Mercuric Iodide), Wagner's reagent (Iodine in potassium iodide), Dragendroff's reagent (solution of potassium bismuth iodide).

Test for glycosides:
The preparation of whole methanolic plant extract and successive fractions extract of fruit SG was dissolved in 2N HCl and filtered. The preparation was treated with 0.5 ml sodium nitroprusside in pyridine, followed by methanolic KOH (Legal's Test) and the preparation was made alkaline with 10% NaOH, blue colour fluorescence was shown on long UV light.

Test for the presence of phytosterol
The preparation of whole methanolic plant extract and successive fractions extract of fruit SG was mixed with 5 ml $CHCl_3$ and filtered .To the preparation 4 ml of acetic anhydride and 2 drops of conc. H_2SO_4 were added from the side of the test tube (Liebermann-Burchard test).

Test for tannins
The preparation of whole methanolic plant extract and successive fractions extract of fruit SG was treated with methanolic lead acetate solution (10%; Lead acetate test).

Test for flavonoid
To the 5ml alcoholic solution of the preparation of whole methanolic plant extract and successive fractions extract of fruit SG, 0.5 gm of magnesium ribbon and few drops of concentrated HCl were added (Shinoda test).

Test for Saponin
The test for the presence of saponin was done by shaking vigoriously the preparation of whole methanolic plant extract and successive fractions extract of fruit SG with water (foam test).

Detection of phenols
The preparation of whole methanolic plant extract and successive fractions extract of fruit SG was treated with a few drops of ferric chloride solution (5%) (Ferric chloride test).

Test for the presence of carbohydrates
The preparation of whole methanolic plant extract and successive fractions extract of fruit SG was dissolved in 5 ml distilled water and filtered. The filtrate was treated with 2 drops of alcoholic α-naphthol solution in a test tube. Then 2 ml. of conc. sulphuric acid was added carefully along the sides of the test tube (Molisch's test).

Test for the presence of Proteins:
The preparation of whole methanolic plant extract and successive fractions extract of fruit SG was treated with 2ml 0.25% ninhydrin reagent and boiled for a few minutes (Ninhydrin test).

Thin layer chromatography study
Thin layer chromatography has been frequently used for the separation, identification, purification and the semi quantitative analysis of phytoconstituents. TLC also enables for evaluation of phytochemical constituents of herbal drugs. TLC monograph of individual drug can be use as reference standard for identification of phytoconstituents [27].

Method: Preparation of extracts, thin layer plate and development of chromatogram was performed as per the methods described by Wagner and Bladt [28]. For the development of chromatogram suitable solvent system was selected on the basis of polarity of extract. The sample was prepared by dissolving it in suitable solvent and applied on TLC plate by capillary tube. The plate was then developed by the mobile phase in a chromatographic chamber up to the specified height. Depending on the polarity interaction of the phytoconstituents of the extract with mobile and

stationary phase, different phytoconstituents traveled different distances on the plate. The distance traveled by the phytoconstituents were measured and represented as R_f. This R_f, represent the distance traveled by individual phytoconstituents from origin line upon the distance traveled by the solvent front from the origin line. It was visualized in long UV (366 nm) and/or interacting the extracts with various spraying reagents. Spraying reagent used for identification were dragendroff reagent for alkaloids, Liebermann-Burchard reagent for steroids, mixture of sulphuric acid and anisaldehyde for steroids, 5% ferric chloride for phenolics, 10% alcoholic KOH solution for coumarins, shinoda reagent for flavonoids and iodine vapour etc. The TLC study of the whole methanolic extract of fruit of SG and its respective fractions were carried in suitable solvent system as mobile phase and silica gel as statoionary phase reported in table 4.

RESULTS

Powder fluorescence analysis of fruits of *Sesbania grandiflora*

The powder study of fresh fruit of SG shows that grayish-white, fibrous, odorless powder with slight astringent taste in nature.

Table –1: Fluorescence analysis data of SG fruits powder

Sl. No.	Drug	Day light	UV 254 nm	UV 365nm
1	Powder as such	Grayish white	No fluorescence	No fluorescence
2	Powder + KOH(5%)	brown	No fluorescence	Dull green
3	Powder + NH_3 (25%)	Pale yellow	Dark yellow	Dark green
4	Powder + $FeCl_3$ (5%)	Yellowish green	No fluorescence	No fluorescence
5	Powder + NaOH(1N) in methanol	Brownish yellow	No fluorescence	Light green
6	Powder + H_2SO_4 (Conc)	Light orange	No fluorescence	Light brown
7	Powder +Acetic acid	Light yellow	Dark yellow	Orange
8	Powder + Picric acid	Dark yellow	Dark yellow	Dark green
9	Powder + Iodine(5%) solution	Blackish brown	No fluorescence	green
10	Powder + HCL (1N) in H_2O	Light brown	Dark yellow	Light yellow

Physiochemical parameters study result

The coarse powder of fruits of *Sesbania grandiflora* was subjected to various physiochemical study like determination of foreign matter, moisture content, ash value, extractive value and foaming index etc. The observed results were shown in the Table 2.

Table 2. Physiochemical study of fruits of *Sesbania grandiflora*

Sl. No.	Parameters (% w/w)	Value (%)
1	Total ash value	4.21 ± 0.20
2	Acid insoluble ash value	2.23 ± 0.03
3	Water soluble ash value	0.985 ± 0.13
4	Foreign organic matter	0.44 ± 0.05
5	Loss on drying	11.18 ± 0.41
6	Pet. ether soluble extractive value	4.16 ± 0.48
7	Alcohol soluble extractive value	7.25 ± 0.64
8	Water soluble extractive value	18.6 ± 0.29
9	Crude fiber content	7.18 ± 0.32
10	Foaming index	142.85

Extraction of Phytoconstituents

The extract of the fruit of SG was prepared by soxhlation method. The solvent used for extraction process was methanol (80% v/v). The extracts were concentrated in rotary vacuum evaporator and finally dried by water bath to get the solid mass. The yield value of the extract was 5.6% w/w for fruits of SG with respect to the air dried plant material. Half of the methanolic extract was successively fractionated in four water immiscible solvent. The percentage yield of the extract after fraction of the MSG was 8.3% in petroleum ether, 10.4% in chloroform, 11.8% in ethyl acetate and 14.1% in n-butanol.

Phytochemical screening of the extract:

The preliminary phytochemical screening showed both the extract and their solvent fractions contain various phytoconstituents. The result of the screening represented in the table 3

Table No. 3: Phytochemicals screening of MSG and various solvent fractions of MSG

Sl No.	Phytoconstituents	Whole extract	Petroleum ether fraction	Chloroform fraction	Ethyl acetate fraction	n-butanol fraction
1	Alkaloids	+	-	+	-	-
2	Glycosides	+	-	-	+	-
3	Coumarins	+	-	-	+	-
4	Phytosterols	+	+	+	+	-
5	Tannins	+	+	+	-	-
6	Flavanoids	+	-	+	+	+
7	Saponins	+	-	-	+	+
8	Phenolics	+	+	+	+	+
9	Carbohydrates	+	-	-	+	+
10	Proteins	+	-	-	+	+

Note: *+ sign indicate presence of phytochemicals and – sign indicate absent of phytochemicals.*

T. L. C. study of extract:

T. L. C. study of the methanolic extract of fruit of SG and its various fraction showed that the plant material contain various phytoconstituents. TLC of whole extract of SG and its fractions were performed in various solvent system but specific solvent system for particular extract showed good result. The data were in table no-4.

Table -4: The TLC study of whole methanolic extract of SG and its fractions

Sl. No.	Extract	Stationary phase	Mobile phase	Visualizing agent	No. of sport/ Rf value
1	whole methanolic	silica gel G	toluene: ethyl acetate (9:1)	U. V and iodine vapour	4 sports i. e. 0.24, 0.45, 0.65, 0.74
2	Petroleum ether fraction	silica gel G	hexane: acetone(8:2)	U.V. and iodine vapour	4 sports i. e. 0.4, 0.52,0.72, 0.87
3	chloroform fraction	silica gel G	chloroform: methanol(9: 1)	U. V and iodine vapour	5 sports 0.5, 0.68, 0.78, 0.82, 0.92
4	ethyl acetate fraction	silica gel G	butanol: acetic acid: water (2: 0.5: 2.5)	U.V. and Iodine vapour	Two sports 0.61 and 0.80
5	n – butanol fraction	silica gel G	chloroform: methanol(9:2)	U.V. and iodine vapour	4 sports 0.29, 0.5, ``0.73 and 0.76

DISCUSSION

The present study revealed that the physico-chemical parameters such as ash value, extractive value, foreign matter, loss on drying, foaming index and crude fiber content determination are the suitable parameters in the standardization of the fruit of SG. The fluorescence analysis of the powder drug further authenticated its validation. The preliminary phytochemical screening of MSG and its fractions revealed that the plant part contained alkaloids, glycosides, saponin, tannins, flavonoids, carbohydrates, proteins and sterols. Thus, it can be assumed that these are the standard parameters in the standardization of fruit of SG.

The physicochemical and phytochemical screening is undertaken to establish the profile of crude drug with respect to its identification, adulteration and substitution. The assessment of fluorescence analysis of the powder drug in day light, long U.V. and short U. V. light after treating with specific chemical reagent, as preliminary quality control tool for crude drug. The study revealed that the powder drug showed special fluorescence at particular wavelength which can be useful for the characterization of crude drug analysis. It indicates that identification and differentiation of the drug from their substituent can be achieved by comparing with the fluorescence data of genuine drug [29].

The physico-chemical parameters like foreign matter, loss on drying, ash values, extractive value, foaming index and crude fiber content were determined for the fruits of SG. Physico-chemical studies revealed that loss on drying in the sample can be attributed to loss of water and volatile matter [30]. The loss on drying value for the fruit crude drug was high. Loss on drying data gives information about possibility of enzymatic destruction of active constituents and spoilage of crude drug due to growth of molds and bacteria. Proper drying, proper packing and storage of the crude drug are highly essential to prevent the growth of microbes [31].

Foreign matter determination is highly essential for the standardization plant material. Optimum care should be taken to remove poisonous and harmful foreign matters (mould or insects and other animal contamination, including animal excreta) from crude drug [32]. The collection of SG was made very carefully and considering all the aspects

to prevent any contamination with foreign matter, due to which the quantity of foreign matter is less than 1% in crude drug of fruit of SG.

The ash value represents the inorganic salts occurring naturally (physiological) or due to adulteration purpose (non-physiological) in the crude drug [33]. A high ash value is representation of contamination, substitution, adulteration or carelessness in collecting the crude drug [34]. The ash value of fruit SG was low, it indicates that the crude drug of SG contain low amount of inorganic material. Further, the water soluble ash value was low comparable to acid insoluble ash value for the crude SG. It indicates presence of more water soluble inorganic salt of chloride, carbonate, oxalate of sodium, potassium content etc. These data will be useful in prevention of improper handling and adulteration of crude drug with spurious, exhausted drugs [35].

Extractive value indicates the solubility of various classes of compounds in a particular solvent. It also explore that the plant is reach with which type of [36]. The extractive values of SG in petroleum ether, alcohol and water represented that the crude drug rich with more water soluble phytoconstituents than alcohol and petroleum ether soluble phytoconstituents. This study indicates polar solvents will give more yield than nonpolar solvents in the extraction of crude drug of SG.

The study of foaming index revealed the foaming ability of herbal drugs and their extracts in aqueous medium. Foaming index represents saponin content present in crude drug and their extract [37]. The foaming index of the SG fruit is low, it indicates that the plant part contain less amount of saponin phytoconstituents.

Quantitative estimation of crude fiber was carried by gravimetrically analysis after chemical digestion and solubilization of other materials present in the plant part. Mostly crude fiber is the fraction of carbohydrate that remains after treatment with acid and alkali [38]. Estimation of crude fiber contents of the plant part can also be utilized as tools for the standardization of the medicinal plant and differentiation of genuine drug from the substituent. A good percentage of crude fiber content was found in SG plant part. Further, the biological activity of medicinal plant is related to active phytoconstituents present in it. So standardization of medicinal plants based on phytochemical aspect is highly essential to justify their therapeutic activity experimentally [39]. In the present investigation the plant part was extracted with 80% methanol by soxhlation process to yield maximum phytoconstituents. The preliminary phytochemical screening and T.L.C. study of the whole extract showed positive test towards major phytoconstituents like steriods, alkaloids, glycosides, tannins, flavonoids, carbohydrates, proteins and coumarins. Further the whole extract was dissolved in water and fractionated with different water immiscible organic solvents. The phytochemical study of the different fractions of MSG reflects that the specific class of phytoconstituents was soluble in the specific solvent according their polarities.

CONCLUSION

The study of physico-chemical parameters like ash values, extractive value, foreign matter, loss on drying, foaming index and crude fiber content determination are helpful in the standardization of plant material. The fluorescence analysis of the powder drug further strengthens the identification of genuine plant part. The preliminary phytochemical screening of MSG and its fractions revealed that the plant part contain alkaloids, glycosides, saponin, tannins, flavonoids, carbohydrates, proteins and sterols. The benefit of the study would be to the group of researchers who will be working in the identification and use of the fruit of SG.

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