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Research Article

QUALITATIVE SCREENING AND STANDERDIZATION OF EVALUATION PARAMETERS OF *MORINGA OLEIFERA* LAM. AND *SYZYGIUM CUMINI* (L.) SKEELS

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ABSTRACT

The plant screening for phytochemical constituents seems to have the potential to act as a source of useful drugs and cures many infections as a result of the presence of various bioactive compounds that evident to have enormous activity against array human pathogens. The objective of the study was to undertake a qualitative phytochemical analysis of the seeds of *Moringa oleifera* Lam. (Sarjan) and *Syzygium cumini* (L.) Skeels (Jamun), a traditional herb used against several diseases. The total Flavonoid content in *Moringa oleifera* Lam. (Sarjan) and *Syzygium cumini* (L.) Skeels (Jamun) seeds were found 0.356 and 0.481 mg/100 mg of dried extract. The total phenolic content in hydroalcoholic extract of *Moringa oleifera* and *Syzygium cumini* seeds was found 0.660 and 0.600 mg/100 mg of dried extract respectively. The total alkaloid content in *Moringa oleifera* and *Syzygium cumini* seeds extract was found 0.351 and 0.361mg/100 mg of dried extract. The results revealed the presence of Alkaloids, Flavonoids, Diterpenes, Phenol, Proteins, Carbohydrate and Saponins in seeds extract of *Moringa oleifera* and *Syzygium cumini*. These findings suggested that *Moringa oleifera* and *Syzygium cumini* seeds extract could be a potential source of drugs which in future may serve for the production of synthetically improved therapeutic agents.

Keywords: Phytochemical Screening, Pharmacognostical Parameters, Extraction, *Moringa oleifera, Syzygium cumini*.

1. INTRODUCTION

In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects [1-3]. Many traditional medicines in use are derived from medicinal plants, minerals and organic matter. A number of medicinal plants, traditionally used for over 1000 years named *Rasayana* are present in herbal preparations of Indian traditional health care systems. In Indian systems of medicine most practitioners formulate and dispense their own recipes [4]. The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the world. Among these 2500 species are in India, out of which 150 species are used commercially on a fairly large scale. India is the largest producer of medicinal herbs and is called as botanical garden of the world [5-7]. The present work was aimed on development of standardization parameters of selected herbs used in the treatment of diabetes mellitus, a major crippling disease in the world leading to huge economic losses.

Moringa oleifera Lam., family. Moriangaceae is commonly cultivated throughout India up to 1500 m. All part of the plant is used medicinally. The leaves and fruits are rich in iron, proteins and vitamins. The plant possess analgesic, anti0inflamamtory activity and used extensively for the treatment of ulcer in traditional system of medicine. *Syzygium cumini* (L.) Skeels, Family: Myrtaceae is commonly cultivated throughout India up to 1800 m. Bark is used in nonspecific acute diarrhoea and in topical therapy for mild inflammation of the oral-pharyngeal mucosa; externally in mild, superficial inflammation of the skin. The seed is used in hyperglycaemia and polyuria. Seed extract exhibited potent anti-inflammatory action against both exudative and proliferative and chronic phases of inflammation, besides exhibiting significant anti-arthritic, antipyretic and analgesic activities [8-11].

2. MATERIAL AND METHODS

2.1. Selection, Collection and Authentication of the plant materials

The seeds of *Moringa oleifera* Lam. (Sarjan) and *Syzygium*

cumini (L.) Skeels (Jamun) were extensively used in the treatment of ulcer as mentioned in folklore; several tribal people of India are using these plants for the treatment of ulcer, therefore these plants were selected. The seeds of the plant were collected from local area of Bhopal in the month July-Sep.' 2019 and were authenticated by Dr. S. N. Dwivedi, Retd Professor & Head, Department of Botany, Janata PG College, Rewa, (M.P.) and was deposited in our laboratory, Voucher No. J/Bot/2019-117MOS & J/Bot/2019-118SCS were allotted to the selected plant on the date 13/09/2019.

2.2. Preparation of extract

The seeds of *Moringa oleifera* and *Syzygium cumini* were shade dried and reduced to coarse powder in a mechanical grinder and passed through sieve No. 40. The powdered bark was defatted with petroleum ether (40°-60C) for about 09 hrs and complete defatting was ensured by placing a drop from the thimble on a filter paper which did not exhibit any oily spot. The defatted material was removed from the soxhlet apparatus and air dried to remove last traces of petroleum ether. The defatted material was subjected to extraction by hydroalcohol as solvent. The extracts were collected in a tarred conical flask. The solvent was removed by distillation. Last traces of solvent was removed under vacuum. The extract obtained with each solvent was weighed to a constant weight and percentage w/w basis was calculated. The obtained crude extract was stored in dark glass bottles for further processing. The extracts thus obtained were subjected to phytochemical analysis.

2.3. Preliminary phytochemical screening of extracts

The various extract obtained after extraction were subjected for phytochemical screening to determine the presence of various phytochemical present in the extracts. The standard procedures were adopted to perform the study [5].

2.4. Quantitative studies of phytoconstituents

2.4.1. Estimation of total phenol content

The total phenol content of the extract was determined by the modified folin-ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was used for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer [12].

2.4.2. Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filtered. Three ml (1mg/ml) of this extract was used for the estimation of flavonoids. 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm [13].

2.4.3. Estimation of total alkaloids content

The plant extracts (1mg) was dissolved in methanol, added 1ml of 2 N HCl and filtered. The solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100mg of extract [14].

2.5. Pharmacognostical Evaluation [3-5]

2.5.1. Macroscopic studies

The macroscopy of different parts of the plant such as color, odor, size, shape, taste, surface characters and fractures were carried out.

2.5.2. Physicochemical Evaluation

The dried parts were subjected to standard procedure for the determination of various physicochemical parameters.

2.5.3. Determination of foreign organic matter (FOM)

Accurately weighed 100 g of the drug sample and spread it out in a thin layer. The foreign matter should be detected by inspection with the unaided eye or by the use

of a lens (6X). Separated, weighed and the percentage present was calculate.

2.5.4. Determination of moisture content (LOD)

About 10 g of drug (without preliminary drying) was placed after accurately weighing in a tared evaporating dish and kept in oven at 105°Cfor 5 hours and weighed. The percentage loss on drying with reference to the air dried drug was calculated.

2.5.5. Determination of ash value

The determination of ash values is meant for detecting low-grade products, exhausted drugs and sandy or earthy matter. It can also be utilized as a mean of detecting the chemical constituents by making use of water-soluble ash and acid insoluble ash [15].

2.5.6. Total ash

Accurately about 3 gms of air dried powder was weighed in a tared silica crucible and incinerated at a temperature not exceeding 450°Cuntil free from carbon, cooled and weighed and then the percentage of total ash with reference to the air dried powdered drug was calculated. The percentage of total ash with reference to the air-dried drug was calculated [16].

2.5.7. Acid insoluble ash

The ash obtained in the above method was boiled for 5 minutes with 25ml of dilute HCl. The residue was collected on ashless filter paper and washed with hot water, ignited and weighed. The percentage of acid insoluble ash was calculated with reference to the air dried drug [17].

2.5.8. Water soluble ash

The ash obtained in total ash was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited to constant weight at a low temperature. The weight of insoluble matter was subtracted from the weight of the ash. The difference in weights represents the water soluble ash. The percentage of water soluble ash with reference to the air dried drug was calculated [18].

2.5.9. Determination of swelling index

Swelling index is determined for the presence of mucilage in the seeds. Accurately weighed 1 g of the seed and placed in 150 ml measuring cylinder, added 50 ml of distilled water and kept aside for 24 hours with occasional shaking. The volume occupied by the seeds after 24 hours of wetting was measured [19].

2.5.10. Determination of extractive value

This method determines the amount of active constituents extracted with solvents from a given amount of medicinal plant material. It is employed for materials for which as yet no suitable chemical or biological assay exists [20].

2.5.11. Cold maceration

Placed about 4.0g of coarsely powdered air-dried material, accurately weighed, in a glass-stoppered conical flask. Macerated with 100ml of the solvent specified for the plant material concerned for 6 hours, shaken frequently, allowed to stand for 18 hours. Filtered rapidly taking care not to lose any solvent, transferred 25 ml of the filtrate to a tared flat-bottomed dish and evaporated to dryness on a water bath. Dried at 105°C for 6 hours, cooled in a desiccator for 30 minutes and weighed without delay. Calculated the content of extractable matter in mg per g of air dried material. For ethanol-soluble extractable matter, use the concentration of solvent specified in the test procedure for the plant material concerned; for water-soluble extractable matter, use water as the solvent.

2.6. Statistical Analysis

The results of the experiment were analyzed statistically with student's T-test and ANOVA. [21]

3. RESULTS & DISCUSSION

The macroscopical and organoleptic standards which include the visual and sensory characters provided the simplest and quickest indication of the identity and quality of *Moringa oleifera* and *Syzygium cumini*. The seeds showed marked variations in their organoleptic properties. Seeds of *Moringa oleifera* and *Syzygium cumini* were extracted using hydroalcoholic solvent. Extractive values are mainly used to determine whether a substance is exhausted or adulterated, and they are a valuable method for determining the drug's consistency and difference in chemical constituents. The extractive values are measures of the overall soluble component in the solvent. Results of extractive value are shown in table 3.

Phytochemical constituents such as phenol, flavonoids, alkaloids or secondary metabolites possess nutritive and pharmacological activities. Thus the preliminary screening test may be useful in the detection of the bioactive principles and subsequently may lead to the

drug discovery and development. The results of qualitative phytochemical analysis of *Moringa oleifera* and *Syzygium cumini* extracts were presented in table 4. The results revealed the presence of flavonoids, phenol, Diterpenes, Proteins, Carbohydrate and Saponins in seeds extract. There was little presence of alkaloids and glycosides in seeds of *Moringa oleifera* and *Syzygium cumini*. The results revealed the presence of Alkaloids, Flavonoids, Diterpenes, Phenol, Proteins, Carbohydrate and Saponins in seeds extract of *Moringa oleifera* and *Syzygium cumini*.

Total phenolic compounds (TPC) was expressed as mg/100 mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: $Y = 0.011X+0.011$, $R^2= 0.998$, where X is the gallic acid equivalent (GAE) and Y is the absorbance. Total flavonoids content was calculated as quercetin equivalent (mg/100 mg) using the equation based on the calibration curve: $Y=0.032X + 0.018$, $R^2=0.998$, where X is the quercetin equivalent (QE) and Y is the absorbance. Total alkaloid content was calculated as atropine equivalent mg/100 mg using the equation based on the calibration curve: $Y=0.007X+ 0.024$, $R^2=0.995$, where X is the Atropine equivalent (AE) and Y is the absorbance. The results of Total phenols, flavonoid and alkaloids were presented in table 4 and 5. The total phenolic content in hydroalcoholic extract of *Moringa oleifera* and *Syzygium cumini* seeds was found 0.660 and 0.600 mg/100 mg of dried extract respectively. The total Flavonoid content in *Moringa oleifera* and *Syzygium cumini* seeds was found 0.356 and 0.481 mg/100 mg of dried extract. The total alkaloid content in *Moringa oleifera* and *Syzygium cumini* seeds extract was found 0.351 and 0.361mg/ 100 mg of dried extract. The acid insoluble ash value on the other hand indicates contamination with siliceous materials e.g. earth and sand. The result showed that the seeds gave acid insoluble ash values of 1.06 %, 2.34 %, respectively. The water soluble ash is part of the total ash content which is soluble in water; this is a good indicator of either previous extraction of the water soluble salts in the drug or incorrect preparation. It is an important indication of the presence of exhausted materials substituted for the genuine article.

The hydro-alcoholic extract obtained after extraction were subjected for phytochemical screening to determine the presence of various phytochemical present in the extracts. The standard procedure was adopted to perform the study. The results were as presented in table 4.

Table 1: Macroscopic Characters of *Moringa oleifera* Lam. and *Syzygium cumini* (L.) Skeels

S. No.	Parameters	MOS	SCS
1.	Color	White	Deep purple
2.	Odor	Characteristics	Sweet
3.	Taste	Sweet	Sweet
4.	Shape	Oval & triangular	Oval
5.	Size	Variable	Variable
6.	Surface character	Smooth	Rough
7.	Fractures	Absent	Absent

MOS= Moringa oleifera Lam. (seeds); SCS= Syzygium cumini (L.) Skeels (Seeds)

Table 2: Physicochemical Evaluation of *Moringa oleifera* Lam. and *Syzygium cumini* (L.) Skeels

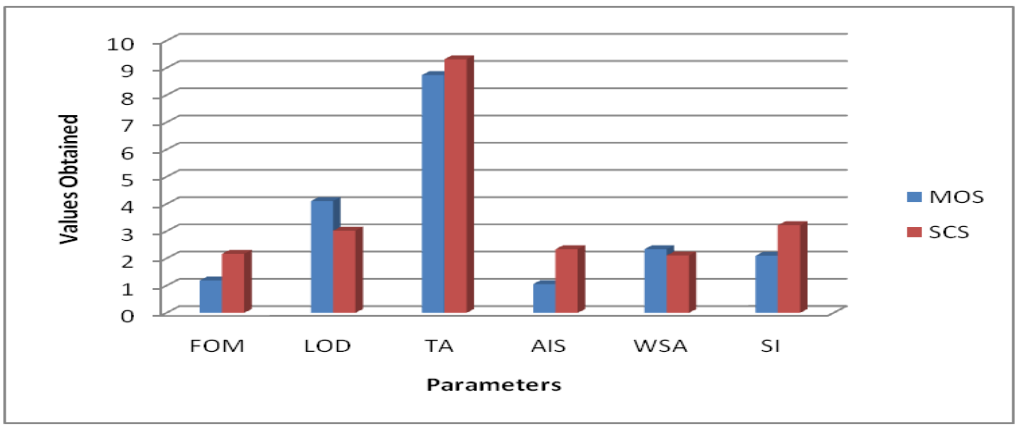
S. No.	Parameters	MOS	SCS
1.	Foreign organic matter	1.19±0.02	2.17±0.28
2.	Loss on drying	4.11±0.25	3.02±0.02
3.	Total ash	8.74±0.28	9.32±0.02
4.	Acid insoluble ash	1.06±0.02	2.34±0.92
5.	Water soluble ash	2.34±0.81	2.11±0.06
6.	Alcohol soluble extractive value	2.10±0.17	3.22±0.18
7.	Water soluble extractive value	18.32±1.18	19.98±1.02
8	Ether soluble extractive values	6.11±1.02	12.34±1.27

All values are expressed as Mean±SEM, n=3

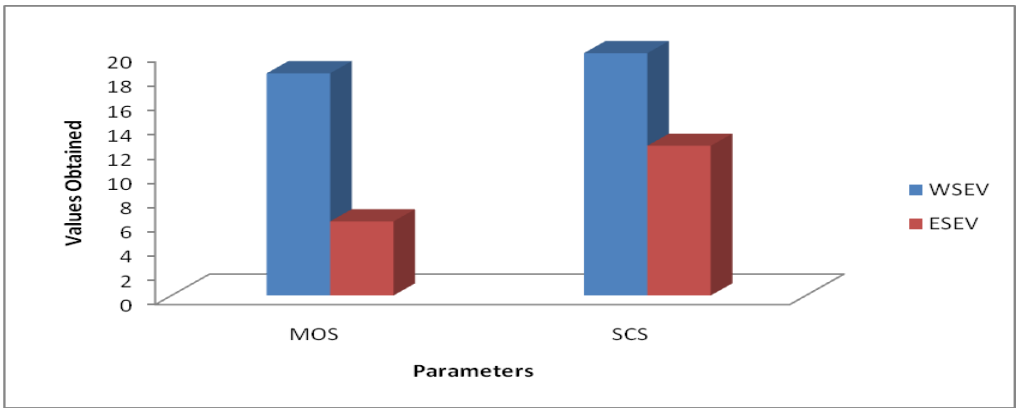
MOS= Moringa oleifera Lam. (seeds); SCS= Syzygium cumini (L.) Skeels (Seeds)

Table 3: Estimation of % Yield of Hydroalcoholic Extract of *Moringa oleifera* Lam. and *Syzygium cumini* (L.) Skeels

S. No.	Extract	Parameters			
		Nature of Extract	Color	pH	% Yield
1.	HAEMOS	Semi Solid	Cream	7.01	12.43
2.	HAESCS	Semi solid	Light Purple	7.02	18.61



Graph 1: Physico-chemical evaluation of *Moringa oleifera* Lam. and *Syzygium cumini* (L.) Skeels



Graph 2: Extractive values of *Moringa oleifera* Lam. and *Syzygium cumini* (L.) Skeels

Table 4: Preliminary Phytochemical Screening of *Moringa oleifera* Lam. and *Syzygium cumini* (L.) Skeels

S. No.	Constituents	Seed Extract	
		HAEMOS	HAESCS
1.	Carbohydrates	+	+
2.	Glycosides	-	+
3.	Alkaloids	-	-
4.	Protein & Amino acid	+	-
5.	Tannins & Phenolic compounds	-	+
6.	Flavonoids	-	+
7.	Fixed oil and Fats	+	-
8.	Steriods & Triterpenoids	+	+
9.	Waxes	-	-
10.	Mucilage & Gums	-	-

+ = Present; - = Absent

Table 5: Estimation of total phenol, flavonoids and alkaloid content of *Moringa oleifera* Lam.

S. No.	Hydroalcoholic Extract	Total phenol content	Total flavonoids content	Total alkaloid content
		(mg/ 100 mg of dried extract)		
1	Seeds	0.660	0.356	0.351

Table 6: Estimation of total phenol, flavonoids and alkaloid content of *Syzygium cumini* (L.) Skeels

S. No.	Hydroalcoholic Extract	Total phenol content	Total flavonoids content	Total alkaloid content
		(mg/ 100 mg of dried extract)		
1	Seed	0.660	0.481	0.361

4. CONCLUSION

Qualitative and quantitative phytochemical analysis of plant parts extracts is important as it indicate the nature of phytochemicals that are possessed by such medicinal plants. The results of the current study suggest more similarities in the phytochemical compositions of the different parts of *Moringa oleifera* Lam. and *Syzygium cumini* (L.) Skeels which is likely to contribute to some similarities in their biological activities.

Conflict of interest

None declared

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