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**PRELIMINARY PHYTOCHEMICAL SCREENING AND EVALUATION OF THE
SELECTED PLANT PARTS**

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ABSTRACT

Our nature gifted us with several plants which constitute outstanding source for many bioactive constituents, which exhibit remarkable therapeutic potential and diverse pharmacological actions. Herb-herb combination (polyherbalism) is concept where two or more herbal drugs are mixed in certain proportion to assess their synergistic or antagonistic effects. The present study was carried out for the extraction, phytochemical screening and evaluation of plant parts of interest. Evaluation of herbal drugs is essential in order to assess the quality of drugs, and to detect presence of various constituents responsible for the pharmacological activity.

For the present study leaves of *Moringa concanensis* and seeds of *Sesbania grandiflora* were collected and processed for extraction. Phytochemical investigation was performed for both the plants. *M. concanensis* leaves and seeds of *Sesbania grandiflora* have the potential to act as a source of useful drugs because of presence of various phytochemical constituents such as Alkaloids, Flavonoids, Phenol, Terpenoids, Saponin and Carbohydrates. These phyto constituents seemed to have the potential to act as a source of useful drugs and also to improve

the health status of the consumers. The presence of these phyto constituents have vital role in good health, wellness and therapy. The aim of present study was to prepare ethanolic extracts of different parts of selected plants, perform preliminary phytochemical screening and their physico-chemical evaluation.

Keywords: *Moringa Concanensis*, *Sesbania grandiflora*, Herbal products, Specific gravity, Turbidity, Particle size

INTRODUCTION

Herbal plants are the essential part of human civilization to meet its basic needs of food, shelter and clothing. Besides that they are fulfilling the requirements for health care, healing and other comforts [1].

Herbal extracts, and powders are used in the preparations to enhance memory and increase the attractiveness of the person and to promote the public life as being “natural” and completely “safe” alternatives to conventional medicines [2].

Moringa Concanensis also known as *Moringa concanensis nimmo*, is one of the important medicinal plant belongs to Moringaceae. *M. concanensis* is an evergreen tree with a spreading crown, upto 8 feet. Leaves alternate, 2-3- pinnate, obovate, caducous. Flowers large, white, hermaphrodite, irregular in axillary panicles. Calyx thinly tomentose, long, segments white, oblong, reflexed. Petals yellow, veined with red, oblong. Stamens 5 fertile and 4-5 staminodes. Capsule straight, actively triquetrous, slightly constricted between the

seeds. Seeds white or pale yellow 3- angled [3-4].

Moringa concanensis is used for treating various human ailments like to reduce cholesterol and body weight, Eye care, **Thyroid Problems**, Fertility in women, Aphrodisiac, Tiredness, to reduce Blood Pressure, Abortion, Leucorrhoea, Menstrual pain, Splenomegaly, Jaundice, Bloat, Constipation, Intestinal worms, Skin Tumor, Diabetes, Head ache, Spinal Cord Pain [5-6].

The leaves are use as anti-diabetics because they help in insulin secretion and help in reduction of insulin resistance and utilization of glucose by the peripheral tissues and inhibition of gluconeogenesis by the liver.

The seeds of *Sesbania grandiflora* is a perennial branching tree growing upto a height of 15m with white, yellowish, rose, pink or red flowers with 15-22 mm long calyx. The leaves are 30cm long and are pinnately compound. A total of 20-50 leaflets are found in pairs which are oblong to elliptical in shape with 12.4 x 5.15 mm

dimensions [7]. The seeds and leaves are containing nutrients like protein, minerals and vitamins. *Sesbania grandiflora* are a good source of Vitamin A, Folate, Thiamin, Niacin and Vitamin C. Flowers also render a large amount of Magnesium, Phosphorus, Potassium and Selenium. The seeds comprise powerful chemoprotective agents like leucocyanidin and cyanidin. Besides these, seeds also contain Saponins and Sesbanimide which possesses strong antibacterial and antimicrobial properties and detoxifies the system. The aim of the present study includes preparation, phytochemical investigation and the evaluation of ethanolic and hydroalcoholic extracts of leaves and seeds obtained from the selected plant parts.

MATERIALS AND METHODS

Plant Material

The Leaves of *Moringa concanensis* and seeds of *Sesbania grandiflora* were collected from hill area of Tirupati, Andhra Pradesh in the month of december and authenticated by Dr. A. Vijaya Bhaskar Reddy, Botanist, Osmania University, Hyderabad. The selected fresh plant parts were thoroughly cleaned and subjected to shade drying. The drying process was performed under controlled condition to remove too many chemical changes in components during extraction process. Drying under direct

sunlight should be avoided as this may destroy heat sensitive phyto constituents, also little air circulation may lead to fungus formation. So, for effective drying well ventilated room and homogenous distribution of plant material should be ensured.

The dried parts of crude drugs were coarsely powdered, sieved, weighed and used for extraction.

Preparation of ethanolic extracts

Previous studies reported the anticonvulsant activity (MES- and PTZ- induced seizures) and anti-amnesic activity (celecoxib- induced amnesia) of ethanolic leaf and seed extracts of *M. concanensis* and *S. grandiflora*.

Preparation of ethanolic leaf extract of *Moringa concanensis*

About 200g *M. concanensis* leaf powder was weighed and taken in 2lt round bottom flask, subjected to cold maceration using Ethanol (80 % v/v), left 7 days at room temperature with occasional shaking for every hour in a closed vessel. Then the marc was removed by filtering the extract and then filtrate was then concentrated on water bath maintained at <50 C to get ethanolic extract EEMC [8].

Preparation of ethanolic seed extract of *Sesbania grandiflora*

About 200g powder of *S. grandiflora* seeds was weighed and taken in a round bottom flask (2000 ml) and subjected to maceration

with ethanol (80%v/v), sealed and kept aside 7 days at room temperature with occasional shaking for every hour in a closed vessel. Then the marc was removed by filtering the extract and then it was concentrated on a water bath maintained at $< 50^{\circ}\text{C}$ to get the ethanolic extract EESG [9].

Preparation of combined extract [10]

Herb-herb combinations are proved to exhibit better therapeutic efficacy and lesser toxicity compared to individual herbs. In view of this point, the dried ethanolic extracts of seeds of *S. grandiflora* and leaves of *M. concanensis* leaves were accurately weighed and mixed in 1: 1 ratio to get combined plant extract and named as *M.concanensis* and *S. grandiflora* combined extract (MESG-CE).

Preliminary Phytochemical Screening

A systematic study of a crude drug embraces through consideration of both primary and secondary metabolites derived as a result of metabolic processes that occur. The ethanolic (EEMC) extracts of *M. concanensis* (leaves) *Sesbania grandiflora* (seeds) and combined extract (MCSG-CE) were subjected to phytochemical screening for identifying presence of chemical constituents using the standard phytochemical analysis methods and results are shown in Table 1.

Test for total Flavonoids

To the test solution of plant, 5 ml of ethanol then few drops of concentrated. HCl acid are added, to this some magnesium turnings are added, if the solution gives pink color shade which indicate presence of flavonoids.

Test for total Phenolic compounds

To 1ml of test solution of plant, 4 drops 95% ethanol followed by 3 drops of 0.1% ferric chloride solution is added, red color indicate presence of phenolic compounds.

Test for total Alkaloids

The test solution of plant was treated with few drops of dilute HCl and filtered, then to this filtrate Drangendroff's reagent was added, the formation of orange-brown precipitation confirms the presence of alkaloids.

Test for total glycosides

Killer-killani test:- The small amount test solution, 2-3 ml of cold Acetic acid is added followed by few drops of FeCl_3 and concentrated H_2SO_4 , if rosy shade appear between to junction then it confirms the presences of glycosides.

Test for total carbohydrates

Fehling's test : Take one ml of Fehling's solution A and add one ml of Fehling's solution B and then add 2 ml of plant extract and boil for few minutes, if red colour ppt observe it confirm the presences of reducing sugar.

Test for total Saponins

Take the small quantity of sample in the test tube and add little amount of distilled water to it and shake the test tube, foam formation is seen which confirms the presence of saponins.

Test for total tannins

To the test solution of plant add 2-3 ml of water followed by few drops of ferric chloride solution, green to blue-green shade is observed.

Test for total Steroids

Liebermann- Burchard test: To the test solution of plant add few drops of acetic anhydride and then few drops of H_2SO_4 in inclined position, the green color ring is formed between two junction which confirms the presence of steroids.

Evaluation of prepared extracts

The parameters evaluated were as follows:

- a) Organoleptic parameters such as colour, appearance, odour, taste.
- b) Physico-chemical parameters such as pH, specific gravity or density, redispersibility and viscosity. Viscosity of MCSG-CE dissolved in Tween 80 was determined by Brookfield viscometer type III, using spindle # 2 at 250 rpm. Redispersibility is measured by placing extract solution in 100 ml graduated cylinder. After storage and sedimentation, the cylinder was rotated through 360° at 20 rpm. The end

point is taken when the base of the cylinder is clear of sediment including the uniformity of dispersed particles.

a) Color examination:

The extracts were taken into watch glass and placed against white background in white tube light. They were observed for their color by naked eye.

b) Odor examination:

The extracts were smelled individually. The time interval among two smelling was kept 2 minutes to nullify the effect of previous smelling.

c) Taste examination:

Small pinch of final extracts were taken and examined for their taste on taste buds of the tongue.

d) Determination of pH:

An accurately measured amount of combined extract was taken in 100 ml volumetric flask, made up the volume up to 100 ml with distilled water. The solution was sonicated for about 10 minutes. pH was measured with the help of digital pH meter.

- e) **Specific gravity at $25^\circ C$:**- A thoroughly clean and dry Pycnometer was selected and calibrated by filling it with recently boiled and cooled water at $25^\circ C$ and weighing the contents. Assuming that the weight of 1 ml of water at $25^\circ C$ when weighed in air of density 0.0012 g/ml was 0.99602 g. The capacity of

the Pycnometer was calculated. Adjusting the temperature of MCSG-CE solution to 20°C, the Pycnometer was filled with it. Then the temperature of the filled Pycnometer was adjusted to 25°C, any excess on walls was removed and weight was taken. The tare weight of the Pycnometer was subtracted from the filled weight. The weight per milliliter was determined by dividing the weight in air, expressed in g, of quantity of solution which fill the Pycnometer at the specified temperature, by the capacity expressed in ml, of the Pycnometer at the same temperature. Specific gravity of the preparation was obtained by dividing weight of dispersed solution present in Pycnometer by weight of water contained, both determined at 25°C.

Stability Testing

The stability of MCSG-CE was evaluated by keeping the dispersed solution at different conditions. The stability testing was carried out under 25°C ± 2°C temperature and 60 ± 5% relative humidity and accelerated stability was carried out under temperature 40°C ± 2°C and relative humidity 75 ± 5%. The MCSG-CE dissolved in tween 80 was tested for the physico-chemical parameters, homogeneity and turbidity at the interval of 24 hr, 48hr and 72 hr to observe any change. This test was carried out to assess stability of dissolved MCSG-CE samples, so that they can be used for the study.

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

Preliminary phytochemical screening of the ethanolic extracts of *Moringa concanensis* (leaves), *Sesbania grandiflora* (seeds) and combined extract MCSG-CE was performed and the results were shown in Table 1.

Evaluation of combined extract

Physico-chemical parameters of combined alcoholic MCSG-CE were evaluated such as physical appearance (colour, odour, taste), pH and Specific gravity(g/ml) and obtained results were shown in Table 2.

Stability Testing

Immediately after preparation, the ethanolic combined extract was evaluated for all tested parameters along with turbidity/homogeneity which was compared with the changes in accelerated stability testing. The pH of final preparation was found to be 6.65 and density 1.02 g/ml (Table 2). The results of stability study of the prepared solution had revealed that no changes were noticed in all the tested physico-chemical parameters as well as turbidity/homogeneity during 24 hr, 48 hr and 72 hr. Thus it can be concluded that the extract dissolved may be used to test as liquid dosage form and can be stable at accelerated temperature and humidity conditions. Further studies need to be carried out to convert this extract into formulation as stable dosage form. The results were shown in Table 2.

Table 1: Preliminary phytochemical screening of ethanolic and hydroalcoholic combined (MCSG-CE) extracts

Chemical Constituents	<i>Moringa concanensis</i> ethanolic extract	<i>Sesbania grandiflora</i> ethanolic extract	MCSG-CE combined extract
Carbohydrates	-	+	+
Glycosides	-	+	+
Alkaloids	+	+	+
Flavonoids	+	+	+
Phenols	+	+	+
Tanins	+	+	+
Steroids	-	-	-
Saponins	+	+	+
Proteins & free amino acids	-	+	+
Terpenoids	+	+	+
Oils and Resins	-	-	-

(+) Present; (-) Absent

Table 2: Organoleptic and Physicochemical evaluation of Combined extract (MCSG-CE)

S.No	Physicochemical Parameters	Observation
1	Color	Light Brown
2	Odour	Pleasant odour
3	Taste	Bitter
4	pH	6.65
5	Specific Gravity	1.02g/ml

CONCLUSION

The present study demonstrated the presence of alkaloids, phenols, flavanoids, tannins, saponins, carbohydrates, glycosides, proteins and free amino acids in the ethanolic extracts suggesting their therapeutic potential.

The prepared extracts were evaluated immediately after preparation and all the extracts showed reliable results. The prepared solution was homogenous with no substantial change noticed in accelerated stability testing. The percentage yield of EEMC, EESG and MCSG - CE was found to be 12.60% w/w, 10.89 % w/w and 11.46% w/w respectively. The solubility of extract was checked and the extracts are dissolved in tween 80 prior to use i.e., administration through peroral (p.o) route. HPLC-based quantification needed for

quantitative analysis of bioactive compounds responsible for several therapeutic properties in near future.

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