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Research Article

PRELIMINARY SCREENING OF SOME OF THE EMERGING ANTIDIABETIC PLANTS FOR QUALITY CONTROL

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ABSTRACT

Annona squamosa, Morus alba, Nelumbo nucifera and *Psidium guajava* are emerging medicinal plants used in the treatment of diabetes. In the present investigation physicochemical evaluation, preliminary phytochemical screening and TLC studies were carried out for the development of standards for quality control for the leaves of above antidiabetic ayurvedic plants.

Keywords: Physicochemical evaluation, Phytochemical screening, Hydroalcoholic extract of leaves, TLC analysis.

INTRODUCTION

Traditional systems of medicines are being used by 80% of global population for their care and are most important source for the development of new drugs. In recent years there is a global shift towards the use of herbal medicines as short coming of modern medicines have started getting more apparent. As plants are liable to show variations in active principles due to ecoclimatic variations, age of plants, season and method of collection, processing, drying and shortage conditions etc, the quality control of crude drugs and their formulations are of paramount importance as justifying their acceptability for the therapeutic use. Realizing the need, various pharmacopeias like Indian Pharmacopeia, Ayurvedic Pharmacopeia of India, Russian Pharmacopeia, British Herbal Pharmacopeia and WHO Guidelines for Quality control of Plant Drug materials etc have described methodology as well as parameters for quality control of herbal drugs [1].

In the present study, preliminary study for the development of standards for quality control for the leaves of antidiabetic ayurvedic plants viz *Annona squamosa*, *Morus alba, Psidium guajava* and *Nelumbo nucifera* has been taken up as the drugs are not included in Indian Pharmacopeia and Ayurvedic Pharmacopeia of India.

Annona squamosa (leaves)

Annona squamosa is a small well branched tree or shrub belonging to family Annonaceae. Leaves occur singly, lanceolate or oblong lanceolate thin, dull green to dark green on top surface, and pale blue-green and covered with bloom on underside; apex short or long pointed; base short pointed or rounded; petioles 0.6-1.3 cm long, green, sparsely pubescent[2].

Morus alba (leaves)

Morus alba is woody tree or shrub belonging to the family Moraceae, that can reach 3-10 m in height and 0.5 m in diameter. The leaves up to 30 cm long, and deeply and intricately lobed, with the lobes rounded. On older trees, the leaves are generally 5–15 cm long, unlobed, cordate at the base and rounded to acuminate at the tip, and serrated on the margins. The leaves are usually deciduous in winter, but trees grown in tropical regions can be evergreen[3].

Psidium guajava (leaves)

It is a low evergreen tree or shrub 6 to 25 feet high of family Myrtaceae. Leaves of guava are simple, alternate, short-petiolate, exstipulate, gland dotted, aromatic, entire, apex ovate .They are 10-12 cm in length, 5-7 cm in width. They have a green colour and leathery texture. The lamina is green, simple with acute apex, entire margin and symmetric – asymmetric base. The venation is pinnate reticulate. The midrib is more prominent on the lower surface. The upper surface is slightly paler in colour than the lower surface. Both surfaces are pubescent. The petiole is short (0.3-0.4 in length and 0.2-0.3 in diameter), green in colour, showing a groove on the upper surface and hairy [4].

Nelumbo nucifera (leaves)

Nelumbo nucifera belongs to the family Nelumbonaceae. The leaves may be as large as 60 cm in diameter, while the showy flowers can be up to 20 cm in diameter [5].

MATERIALS AND METHODS

Selection, Collection and Authentification of Plant material

The leaves of *Annona squamosa, Morus alba, Psidium guajava* and *Nelumbo nucifera* collected locally in the month of July to October and were authentified from National Botanical Research Institute Lucknow India and a voucher specimen has been deposited in the museum of the institute. The collected plant material were washed, dried, coarsely powdered and stored in air tight container for further use.

Physicochemical Evaluation[6]

The powdered leaves of above plants were subjected to standard procedures for the determination of various physicochemical parameters as per the method described in Ayurvedic Pharmacopeia of India.

Foreign Matter

Drugs should be free from moulds, insects, animal fecal matter and other contaminations such as earth, stones and extraneous material. Weigh 100 –500 g of the drug sample to be examined or the minimum quantity prescribed in the monograph, 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). Separate and weigh it and percentage was calculated.

Ash value

The determination of ash value is meant for detecting low grade product, exhausted drugs and sandy or earthy matter. It can also be utilized as mean of detecting the chemical constituents by making use of water soluble ash and acid value.

Total ash value

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

Acid insoluble ash

The total ash obtained (in the determination of Total ash value), was boiled for 5 minute with 25 ml of dil HCl. The residue was collected

on ash less 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.

Water soluble ash

The ash obtained in total ash was boiled for 5 minute with 25 ml of water. The insoluble matter was collected on the ash less filter paper and 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 percentage of water soluble ash with reference to the air dried drug was calculated

Alcohol soluble Extractives

5 gm of coarsely powdered air dried drug was macerated with 100 ml of ethanol in cork fitted conical flask for 24 hrs shaking frequently for 6 hours and the allowed to stand for 18 hours. It was then filtered rapidly taking precautions against loss of alcohol.25 ml of filtrate was evaporated to dryness in tare flat bottom shallow dish and dried at 105°C and weighed. The percentage of alcohol soluble extractive was calculated with reference to air dried drug.

Water soluble Extractives

5 gm of coarsely powdered air dried drug was macerated with 100 ml of chloroform water in closed flask for 24 hours, shaking frequently for 6 hours and allowed to stand for 18 hours. It was then filtered rapidly taking precaution against loss of chloroform water.25 ml of filterate was evaporated to dryness in tared flat bottomed dish and dried at 105°C and weighed. The percentage of water soluble extractives was calculated with references to air dried drug. The obtained values of all physicochemical parameters of various plants were shown in table 1.

Table 1: Physicochemical Evaluation

Parameters	*Nelumbo nucifera*	*Psidium guajava*	*Morus alba*	*Annona squamosa*
Foreign organic matter	1.94±.21	1.76±.31	1.82±.22	1.66±.54
Total ash value	9.03±.02	8.01±.50	13.83±.67	8.92±0.53
Acid insoluble ash value	1.28±0.23	1.67±0.52	1.12±0.37	1.21±0.04
Alcohol extractive value	2.12±.04	6.05±0.05	5.09±0.03	19.08± 0.87
Water soluble extractive value	16.45±.54	12.67±0.32	14.84±0.45	20.94±0.25

Each value represents an average of five readings obtained from samples collected from different localities.

Extraction, Phytochemical screening and TLC profiling [7-10]

Extraction

The dried powder of each plant material was extracted separately with hydro alcoholic solvent (40:60) in soxhlet apparatus. Solvent was removed by evaporation and dried under reduced pressure and resulting semisolid mass was dried under vaccum. Percentage yield of crude extract is presented in table 2.

Preliminary Phytochemical Screening[11-12]

Crude extract of each plant material was subjected to preliminary phytochemical screening to determine the presence of phytoconstituents. The results are given in table 3.

Thin Layer Chromatography of Hydro alcoholic extract [13]

TLC study of each extract was performed using different mobile phase and detecting agents. The results are presented in table 4.

Table 2: Percentage of hydroalcoholic extract obtained from Plant drugs

Plant Drug	Weight Plant drug (gm)	Volume of Solvent	Crude extract Obtained	% yield
Psidium guajava	100gm	800ml	30.58gm	30.58%
Annona squamosa	100gm	800ml	11.60gm	11.6 %
Morus alba	100gm	800ml	25 gm	25%
Nelumbo nucifera	100gm	800ml	7.32g	7.32%

Table 3: Preliminary Phytochemical screening of different plant extracts

Chemical Test	*Morus alba*	*Psidium guajava*	*Nelumbo nucifera*	*Annona squamosa*
Alkaloids	+	-	+	+
Glycosides	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Carbohydrates	+	+	+	+
Phenolic compounds and tannins	+	+	_	+
Steroids	+	+	-	+

+ = Positive and − = Negative

Table 4: TLC analysis of hydroalcoholic extracts

Hydroalcoholic extract	Solvent system	No. of spots appeared and detecting reagent	R_f value
Morus alba	Ethylacetate:CH_3OH:H_2O(7:2:1)	2(green and yellow green)in day light	0.6 0.9
	$CHCl_3$:Ethylacetate:CH_3OH(5:3:2)	3 spots light yellow and green(after spraying with 5% conc.H_2SO_4)	0.25,0.8,0.9
	$CHCl_3$:Glacial acetic acid:CH_3OH:H_2O (7.5:4:1.5:1)	1(Red) at 2.9 cm under U.V light (354nm)	0.71
Psidium guajava	Ethylacetate:CH_3OH:H_2O(7:2:1)	2(yellow green and blue)after spraying with 5% conc. H_2SO_4	0.7and 0.4
	$CHCl_3$:Ethylacetate:CH_3OH(5:3:2)	2(yellow)in day light	0.38,0.34
Annona squamosa	Ethylacetate:CH_3OH:H_2O(7:2:1)	1 (green)in day light	0.7
	$CHCl_3$:Ethylacetate:CH_3OH(5:3:2)	2(yellow)in day light	0.6,0.63
Nelumbo nucifera	Ethylacetate:CH_3OH:H_2O(7:2:1)	3(green, yellow green, dark green)in day light	0.37,0.75,0.93
	$CHCl_3$:Ethylacetate:CH_3OH(5:3:2)	3(yellow, light yellow, green)in day light	0.12,0.56,0.90

CONCLUSION

Different emerging Antidiabetic plants were subjected to physicochemical evaluation i.e Ash value, Alcohol extractive value, water extractive value were determined and are presented in table 1.Preliminary phytochemical screening of hydroalcoholic extract were carried out to determine the active phytochemicals present in the plants. It was found that glycosides, flavonoids, saponins and carbohydrates are present in all the hydroalcoholic plant extracts. Screening of all the plant extract shows the presence of alkaloids except *Psidium guajva*, which gave negative result in the alkaloids test, similary *Nelumbo nucifera* shows the absence of Phenolic compounds, Tannins and Steroids.

TLC analysis of all the hydro alcoholic plant extract were carried out to separate the phytoconsituents and to determine the nature of phytochemicals present and several mobile phase were tried to get the better separation and it was found that Ethylacetate:CH_3OH:H_2O(7:2:1) and Ethylacetate:CH_3OH:H_2O(7:2:1) were effective mobile phase for all the hydro alcoholic plant extracts. Continuing research is necessary regarding the isolation and characterization of compounds present in plant extract. Further studies on isolation and characterization of the specific constituent (s) are needed to validate our results. The study thus can be further utilized to formulate the natural herbal formulation which can be used antidiabetic polyherbal formulation.

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