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Research Article

IDENTIFICATION, FRACTIONATION, EVALUATION ANDPHYSICOCHEMICAL STANDARDIZATION OF*CITRULLUS LANATUS* SEEDS (FAMILY: CUCURBITACEAE)

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ABSTRACT

Plants are very useful in life and can be used for various purposes including the treatment of illnesses. Many valuable compounds like quinine, reserpine, morphine, alkaloid, saponins, tannins etc. have been obtained from plants, thus, nearly 30 % medicines are obtained from plants. In recent years, pharmacognostic study including standardization of the particular medicinal plant having therapeutic value has increased tremendously. Though the modern techniques are available, identification of plant and their active chemical constituents by such study are commonly used these days. *Citrulluslanatus* commonly known as watermelon seeds belonging to family Cucurbitaceaeis native to the Middle East and Asia and is widely used as traditional herbal medicine.

By looking the high traditional use of the plant *Citrulluslanatus*, the present investigation was undertaken for research with the purpose of drawing the pharmacopoeia standards for this species. The present study deals with pharmacognostic parameters for the seeds *of Citrulluslanatus* which mainly consists of macromorphology and microscopical characters, physicochemical characters and phytochemical screening. This information will be of used for further pharmacological and therapeutic evaluation of the species and will assist in determination of quality, purity and safety parameters.

Keywords: *Citrulluslanatus*, Pharmacognosticstudy, Phytochemical screening, Standardization.

INTRODUCTION

Medicinal plants are useful in many communities in the world and they are widely used for the treatment of various ailments. Ayurvedic system of medicine is dealing with the practice of such medicine under research[1]. Today, there is widespread use of herbal drugs. This is because herbal drugs are safe, inexpensive and have no side effects even if consumed in large quantities.Various people are using medicinal plants these days due to these reasons, so they are considered as an alternative to a modern system of medicine. About 1.42 billion people worldwide are dependent on traditional medicines for the treatment of various diseases. However, the main problem is that, due to which the acceptance of the alternative medicines in the developed countries has failed, is the lack of research over it and documentation of the research carried out. There is a need for proper documentation of research work carried out on these herbal medicines. With this background, it becomes extremely important

to make an effort towards standardization of the plant material to be used as a medicine. The process of standardization can be achieved by its pharmacognostic, phytochemical and physicochemical studies. These studies help in identification and standardization of the plant material. Precise identification and quality assurance of these plants is an essential step to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy and can also be tried over treatment of variety of diseases[2].

Cucurbitaceae plants are known to contain bioactive compounds such as cucurbitacin, triterpenes, sterols and alkaloids. In the Middle East and Asia, cucurbit plants were used actively as herbal remedies over the treatment of a variety of diseases. *Citrulluslanatus* (Water melon), is used widely in the traditional herbal system of medicine. The fruit is diureticand effective in the treatment of dropsy and renal stones. The root is laxative and in the large dose is said to be emetic. The rind of the fruit has also shown the beneficial effect in cases of diabetes and alcoholic poisoning. It is sometimes used in the treatment of bed wetting and urinary passage obstruction. The seed is demulcent, diuretic, pectoral and tonic. The seed is also a good vermifuge and has hypotensive action.The fatty oils in the seed, as well aqueous or alcoholic extracts, paralyze tapeworms and roundworms. In some countries, it is often used forthe treatment of burns, swelling and as laxative[3]. Studies have shown that fruits and vegetables contain vital vitamins, nutrients,phytochemicals, antioxidants fibers and it is also documented that consumption of at least 3-4 serving daily significantly reduce the risk of chronic diseases. It is beneficial to keep optimum good health[4]. It also contains carotenoids, steroids, flavonoids, terpenoids, tannins, alkaloids, and glycosides which act as antibiotics. These phytochemical have been reported to exhibit fungistatic, antifungal and anti-inflammatory activities[5]. Although leaves root, stem, flowers and whole plant were examined for useful phytochemical in many kind of research, few reports are available regarding the use of seeds, their phytoconstituents and their useful effects. The seeds contain high quantities of oil and proteins. The immature fruit is usually consumed as a vegetable, but the seed from the mature fruit is used in the form of roasted and salted food items. The kernel, extracted from the seed in the whole form, traditionally called ‘‘Magaz’’, is in great demand as an adjunct or condiment to many Indian confectionery items.The seed, which is 10 to 12% of the mass of the mature fruit, is estimated to contain 20 to 30% oil[6]. Consumptions of varieties of plant food including watermelon seeds in diabetes may provide additional health benefits. The current article is an attempt to describe some pharmacognostic, physicochemical and phytochemical characteristics of *Citrulluslanatus* seeds for its identification, evaluation, characterization, and standardization. The fruit and seeds are shown in figure 1.

MATERIAL AND METHODS

Collection and authentication of *Citrulluslanatus*

Fresh *Citrulluslanatus* seeds were purchased from local markets of Pune and they were identified and authenticated by J. Jayanthi at Botanical Survey of India, Koregaon Road, Pune-01. (Voucher No. BSI/WRC/Tech/2012). The fresh seeds were used for the determination of macromorphological and microscopical characters; whereas the dried seeds coarse powder was used for determination of physicochemical evaluation. They were also extracted and fractions were separated to carry out their phytochemical screening.

Botanical and macromorphological description

The macroscopic studies of seeds including organolepticcharacteristics *viz*. color, taste, odor, appearance, fracture, shape, texture, etc. of the drug (seeds). These parameters areconsidered as quite useful in the further research of the crude drug and were evaluated as per standard WHO guidelines[7-9].

Microscopy of the seeds

Transverse section of seeds was taken by using a sharp razor and stained with Phloroglucinol: Iodine (1:1) in a watch glass. It was observed under a Motic microscope (Model.BA210, Serial 1100002500). The observed parameters were studied and reported [10, 11].

Physicochemical analysis

Analysis of Physicochemical constants of the powder seed has been done to evaluate the quality and purity of the drug. Various physicochemical parameters like Moisture contents, Ash values, extractive values, crude fiber, specific gravity, refractive index, the boiling point were determined as per WHO guidelines. The information collected from these test was useful forstandardization and obtaining the quality standards[8, 12 and 13].

Extraction and fractionation of *Citrulluslanatus* Seed

The crushed drug was weighed exactly about 650 gm and minced using mixer grinder for a fine powder. It was extracted with 80% methanol by Soxhlet apparatus for 7 days in dark under room temperature under supervision. After 7 days, the extract was collected and filtered using muslin cloth and then filter paper. Complete removal of solvent was done by evaporation. The percentage yield was determined after drying using formula. Percentage yield = weight of extract/weight of powder X 100. Later about 13.47 g portion of concentrated methanolic extract was measured separately into a 500 ml separating funnel and diluted in a 150 ml of water. As it was not completely soluble, it was filtered and the nonaqueous fraction was obtained. Then to the filtrate 200 ml of n-butanol was added. The solution was shaken vigorously; separating funnel was hanged for several minutes for separation to occur. Two portions were obtained, n-butanol and aqueous fraction. N-butanol fraction was separated. The aqueous fraction was then extracted with chloroform to get chloroform fraction[5, 14].

Phytochemical analysis of methanolic extract

The analysis for phytochemical constituents was performed using generally accepted laboratory techniques for quantitative determinations (AOAC, 1984). The constituents analyzed for were tannins, cyanogenic glycosides, saponins, flavonoids, oxalates, and alkaloids[15].

Preliminary phytochemical screening of all extracts

The methanolic extract and its fractions were subjected to qualitative chemical examinations for the presence of flavonoids, triterpenoids alkaloids, anthraquinones, carbohydrates, proteins, saponins, sterols, coumarins glycosides, and tannins according to standard methods[8].

Thin layer chromatographic studies

All fractions' thin-layer chromatography was carried out using TLC pre-coated plates by silica gel usinga one-way ascending technique. The plates were cut with scissors and marked with a pencil about 1cm from the bottom of the plate. Each sample was faintly dissolved in methanol and the sample was applied uniformly on plates using capillary tubes allowing plates to dry. The plates were kept in a chromatographic tank using solvent systems; hexane: ethylacetate (9:1; 8:2, 7:3) The plates were dried and observed under normal daylight, ultraviolet light (254 nm & 366 nm) and by spraying with 10% sulfuric acid followed by heating at 105^{o}C for 5-10minutes in an oven. The retention factor Rf for each active compound was calculated for each fraction using the following formula;

Rf = Distance moved by the solute or compound /Distanced moved by the solvent (solvent front)[16].

RESULT

Percentage yield

The percentage yields of all extract are shown in table 1.

Botanical and macromorphological description

Citrulluslanatus is prostrate or climbing annual with several herbaceous, rather firm and stout stems up to 3 m long; the young parts are densely wooly with yellowish to brownish hairs while the older parts become hairless. The leaves are herbaceous but rigid, becoming rough on both sides; 60–200 mm long and 40–150 mm broad, ± ovate in outline, sometimes unlobed and ± entire, but usually deeply 3-lobed with the segments again lobed or doubly lobed; the central lobe is much the largest. The leaf stalks are somewhat hairy and up to 150 mm long. The tendrils are rather robust and usually divided in the upper part. Male and female flowers occur on the same plant (monoecious) with the flower stalk up to 40 mm long and hairy. The receptacle is up to 4 mm long, broadly campanulate and hairy, the lobes are ± as long as the tube. The corolla is usually ± green or green-veined outside and white to pale or bright yellow inside and up to 30 mm in diameter. The seeds are obovate to elliptical, flattened, 0.5–1.5 cm × 0.5–1 cm, smooth, yellow to brown or black, rarely white[17]. The botanical features are given in table 2.

Macro-morphological study

Various organoleptic characters of seeds was studied and shown in table 3.

Microscopy of seeds

Transverse section of seed shows (Figure. 2) embryo, cuticle (seed coat), endosperm surrounding the embryo, starch grains as internal ring structure, epidermal cell producing extracellular proteinaceousmucilage and a volcano-shaped secondary cell wall containing stone cells.

Physicochemical evaluation of seed powder

Various physicochemical parameters like Moisture contents, Ash values, extractive values, crude fiber, specific gravity, refractive index, the boiling point were determined as per WHO guidelines. (Table. 4)

Preliminary photochemical screening

The methanolic extract and its fractions phytochemical study was carried out and the presence of various phytochemicals in each fraction are shown in table 5.

Phytochemical analysis of methanolic extract

The phytochemical analysis of methanolic extract was carried out and shown in table 6.

Thin layer chromatography study

The TLC study was carried out taking n-hexane and ethylacetate as solvent system in three ratios. The Rf values were calculated for each fraction. (Table 7)

DISCUSSION

Several concerns regarding safety, efficacy and quality of plant have been observed with their tremendous use. Plant materials are used throughout the world as a home remedy in many diseases and as raw material for the pharmaceutical industry. Certain herbs have become popular over the years, but the general public, medical practitionersand even botanist still do not know the safe and effective use of thesedrugs. So they are used indiscriminately and illegally. Hence, it becomes necessary to check their standardized qualities and safeties to ensure the supply of plants of good quality. The quality of herbal drug is those all factors which are directly or indirectly related with acceptability of drug. With the advancement of chemical knowledge of the drug, various methods such as phytochemical, physical, microbiological, microscopical, biological, spectrophotometric are used for identification of chemical constituents of the drug. After proper botanical identification, WHO guidelines are followed for processing, storage, evaluation, detoxification to improve its shelf life. In the present study, the water melonseeds are rich sources of valuable bioactive compounds and used as nutraceuticals. The seeds provideopportunities to develop as medicines, cosmetics, value addedproducts and dietary supplements.The morphology, microscopy and physicochemical characters of Citrulluslanatus seeds have been studied to establish the quality, identity and purity of the drug. The results of the phytochemical screening of *Citrulluslanatus* seeds carried to determine the presence of various chemical constituents and it revealed the presenceof flavonoids, saponins, terpenoids and steroids [18-19].Thin layer chromatography was also carried out taking n-hexane and ethylacetate as solvent system. The variation in Rf value was observed due to difference in polarity of solvent. This provides an idea about selection of appropriate solvent system for separation of pure compound in different fraction by using column chromatography. Mixture of solvents with variable polarity in different ratio can be useful for separation of pure constituent from plant extract. This can be carried out by determining the Rf values of constituent in different solvent system. Non aqueous and aqueous fractions seem to have complex spots as there was no separation in the solvent systems. Hence, there is still a need to find another suitable mobile phase (solvent system) where the spots migration would be clearly visible[20, 21].

CONCLUSION

Standardization is considered the basic and important method for checking quality,purity and safety of the drug. In this study, macro morphology,physicochemical evaluationof *Citrulluslanatus* seeds and phytochemical analysis weresuccessfully carried out to identify the chemical composition of the drug and to determine quality and purity of plant. The pharmacognostic information collected in this study will be useful for further pharmacological and therapeutical evaluation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in the publication of this paper.

Table 1: Percentage yield

S. No	Extract/ Fraction	Color	Weight of extract (g)	Percentage yield (%)
1	Methanolic Extract	Brown	27.55	4.23
2	Fraction A (Non-aqueous)	Brown	4.3	31.92
3	Fraction B (N-butanol)	Colorless	3.9	24.92
4	Fraction C (Aqueous)	Yellowish	4.4	31.42
5	Fraction D (Chloroform)	White	2.68	33.5

Table 2: Botanical features

Botanical name	*Citrulluslanatus*(Thunb.)	**Genus**	Citrullus
Class	Equisetopsida	**Kingdom**	Plantae
Common name	Watermelon, Wild Watermelon	**Local name**	Tarbooz
English name	Watermelon	**Order**	Cucurbitales
Family	Cucurbitaceae	**Part used**	Seeds and Fruit
Sindhi name	Hindaro, Chhaen	**subclass**	Magnoliidae
Superorder	Rosanae	**Synonyms**	Colocynthuscitrullus (L.), Cucurbitacitrullus L
Tribus:	Benincaseae	**Subtribus:**	Benincasinae

Table 3: Macro-morphological description

S. No	Characters	Observation
Organoleptic (physical) characters		
1	Color	White with covered black shell, Yellow
2	Odor	Characteristic
3	Taste	Pleasant
4	Shape	Obovate or elliptical
5	Texture	Smooth
6	appearance	Small and shiny
Quantitative macromorphology		
7	Length	0.5–1.5 cm × 0.5–1 cm
8	Thickness	2 to 5 mm

Table 4: Physicochemical evaluation of seed powder

S. No	Parameter	Values (% w/w)	Mean % (w/w)
1.	Moisture content	5	5.33
		6.2	
		4.8	
2.	Total ash value	7	7
		7	
		6.8	
3.	Sulphated ash value	1.3	1.46
		1.9	
		1.2	
4.	Acid insoluble ash value	0.12	0.12
		0.12	
		0.11	
5.	Water soluble ash	0.62	0.61
		0.58	
		0.65	
6.	Crude fiber	33	33
		32	
		33	
7.	Alcohol soluble extractive value	13	12.95
		12.90	
		12.95	
8.	Water soluble extractive value	18.78	19.09
		19.20	
		19.29	
9.	Successive extractive value	Petroleum Ether	19.2
		Benzene	2.18
		Chloroform	1.02
		Alcohol	10.21
		Water	6.20
10.	Determination of lipid content		52.61
11.	Specific gravity		0.92
12.	Refractive index		1.36
13.	Boiling point		250-280^0 C

Table 5: Preliminary phytochemical screening of all fractions

Chemical constituents/Tests	Methanolic	Non-aqueous	N-butanol	Aqueous	Chloroform
Carbohydrate	+	-	+	+	-
Protein	+	+	+	+	-
Amino Acids	+	+	+	+	-
Steroid	+	+	+	+	-
Tannins and polyphenols	+	+	-	+	-
Phytosterols	+	+	+	+	-
Triterpenoids	+	+	-	+	-
Glycoside	+	-	-	+	-
Saponins	-	-	-	-	-
Alkaloids	-	+	+	+	+
Flavonoids	+	-	+	+	-
Anthraquinone	-	-	-	-	-
Coumarin	-	-	-	-	-

+ Present, - absent

Table 6: Phytochemical analysis of methanolic extract

Chemical constituents	%	Chemical constituents	%
Tannin	0.047	Oxalate	0.0530
Saponin	0.570	Flavonoid	0.99
Alkaloid	1.59	Cyanogenic Glycoside	0.00483

Table 7: Rf values of TLC solvent systems for different fractions of *Citrulluslanatus*

S. No	Fractions	Solvent system (n hexane:ethyl acetate)		
		Ratio	No of spots	Rf value
1	Methanolic extract	9:1	1	0.65
		8:2	2	0.84, 0.63
		7:3	1	0.85
2	Non aqueous	9:1	1	0.56
		8:2	-	-
		7:3	-	-
3	N-butanol	9:1	2	0.45, 0.75
		8:2	1	0.55
		7:3	-	-
4	Aqueous	9:1	-	-
		8:2	-	-
		7:3	-	-
5	Chloroform	9:1	1	0.54
		8:2	1	0.46
		7:3	-	-

Fig. 1: Watermelon fruit and seeds

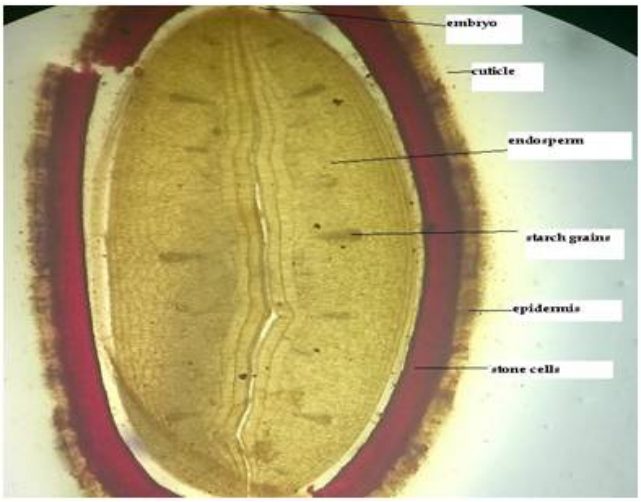

Fig. 2: Transverse section of *Citrulluslanatus* seed

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