Citrullus lanatus seed extract's phytochemical evaluation and investigation against *Malassezia furfur* (dandruff)

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ABSTRACT

A medicinal plant known as *Citrullus lanatus* (Family: Cucurbitaceae) has been used for a wide range of ailments throughout history. When the plant's phytochemistry was examined, it contained carbohydrates, alkaloids, steroids, saponins, glycosides, flavonoids, tannins and phenolic compounds. Additionally, fat, proteins, vitamins, and amino acids were found in the plant. Numerous scientists and researchers have conducted extensive research on the pharmacological properties and therapeutic applications of the plant, which include antimicrobial, antifungal, antiulcer, antioxidant, anti-inflammatory, gastroprotective, analgesic, laxative, hepatoprotective, and atherosclerosis. The purpose of the current study is to produce watermelon seed extract and identify its pharmacological and phytochemical properties. The samples were evaluated for proximate analysis like moisture, crude protein, ash, total fat, crude fiber, total carbohydrate, and total phenolic content. In the

maceration process the *Citrullus lanatus* seeds were extracted in ethanol for seven days. Phytochemical analysis of primary metabolites (proteins, lipids and carbohydrates) and secondary metabolites (alkaloids, tannins, flavonoids and glycosides) was performed according to standard protocols. FTIR was also carried out to identify functional groups. The antidandruff activity was performed by diffusion method and ketoconazole disc was used as standard. The results of proximate analysis were found to be within the suggested ranges (USP, 2015). The phytochemical analysis had revealed that the extract contained a number of different phytochemicals i.e. protein, carbohydrates, lipids, polyphenols, flavonoids and alkaloids. The presence of different functional groups, such as -OH, -CH, and =CH, was detected in the FTIR spectrum. At various dilutions, *Citrullus lanatus* ethanolic extract showed significant antifungal analysis, with a inhibitory zone of 0.2 mm at 0.1% dilution, increasing to 0.25 mm at 0.3% dilution, and 0.3 mm at 0.5% dilution. The results are comparable to ketoconazole which is used as standard drug and its zone of inhibition was found to be 0.5 mm. This study showed that *Citrullus lanatus* ethanolic seed extracts revealed the presence of significant phytochemicals. Studies on the effects of *Citrullus lanatus* ethanolic extracts against *Malassezia furf*ur showed considerable anti-dandruff activity.

Key words: *Citrullus lanatus*, Anti-dandruff, Ketoconazole, Minimal inhibitory concentration.

INTRODUCTION

Medicinal plants have been use as primary source of curative and preventative treatment regimens for humans and animals due to the presence of various phytochemicals. A medicinal plant known as *Citrullus lanatus* (Family: Cucurbitaceae) has been used for a wide range of ailments throughout history. The vernacular names of the plant are: Watermelon, Wild Watermelon, Local name: Tarbooz, English: watermelon Marathi: Watermelon, Kalingad Bengali: Tormuz, Malayalam: Thannimathan Canada: Kallagadi Assamese: Watermelon, Telugu: Pendulum. When the plant's phytochemistry was examined, it contained carbohydrates, alkaloids, steroids, saponins, glycosides, flavonoids, tannins and phenolic compounds [1]. Additionally, fat, proteins, vitamins, and amino acids were found in the plant. Therapeutic plants contain bioactive natural chemical compounds that are frequently referred to as phytochemicals. Common foods and vegetables contain common bioactive compounds. The seeds, rind, and skin of some common items contain more vitamins, fiber, minerals, and other vital nutrients than squash. Watermelon (*Citrullus lanatus*) has the highest water content of 92% [2]. Plants have been commonly used for centuries to treat a variety of fertility problems. It was a fundamental therapeutic plant within Ayurvedic pharmaceuticals. This herb is

known for its medicinal functions and supportive approaches such as antimicrobial, antifungal, antibacterial, antidiabetic [3], anti-ulcer, antioxidant, anti-inflammatory [4], digestive protector, pain relief, laxative, anti-giardia, hepatogenesis and protosporosis [5].

The watermelons were found to create inside the chill and dry climate. Their scattering and densities changed altogether over a long time. The melons were popular to use for a more conspicuous degree for the animals [6]. In warm or dry season the watermelons act as water source [7]. This species was among the preeminent created agrarian crops inside the mellow parts of the world. This species was nearby to Africa and its advancement dates back to outstandingly ancient times, in truth archeological remains of watermelons (seeds) of almost 5000 a long time back had been found inside the north-eastern districts of Africa [8].

Citrullus lanatus of the ground family, Cucurbitaceae, was known for its edible natural production. In addition to nutritional benefits, traditional Pakistani family doctors used these seeds to treat digestive, respiratory, and urinary disorders [9]. These seeds were regularly used as a laxative. Its roots were highly cleansing and stimulating. Its seeds were used to treat nocturia and urinary tract obstruction. Fatty oils, liquids, and alcoholic extracts in seeds paralyze tapeworms and roundworms [5]. The seeds act as a suppressant, chest and tonic. It is a common diuretic that has helped treat ascites and kidney stones. Studies have shown that consumption of watermelon may have antihypertensive effects [1]. The plant is used for burns, swelling, congestion, rheumatism and as a purgative [10] [11]. This investigation aims to assess the phytochemical content and anti-dandruff activity of *Citrullus lanatus* seed extract.

MATERIAL AND METHODS

Chemicals

Serum albumin bovine, Aluminium nitrate, Glucose, Sodium bicarbonate, NaOH, KOH, Lactopherol cotton blue stain, Quercetin 3-rhamnoside, Gallic acid, Anthrone reagent Olin and ciocalteu's reagent, Triton X 100, DMSO, Ethanol, Methanol.

Plant collection and authentication

Citrullus lanatus seeds was collected from a market and identified verified from botany herbarium dept. at the GC University Lahore Punjab, Pakistan, using the herbarium number (GC. Herb.Bot. 3922). In the herbarium section, each specimen was assigned a number for future use.

Plant extraction

Brought the seeds of Citrullus *Lanatus (*watermelon) from market. The weight of seeds was about 1kg. After purchasing, the seeds were washed properly with tap water to remove all the impurities from them. After washing the seeds of *Citrullus lanatus*, the seeds were air dried for about 5 days for complete removal of moisture. After complete dryness the seeds of *Citrullus lanatus* were subjected to grinding process to convert them into powder form. This grinding was done through milling to reduce the size of seeds and convert them into powder [12-14]. The powder of seeds of *C.Lanatus* was stored into airtight container so to reduce the chances of its contamination from moisture into air or through any dust or contamination agent from environment. After then the powder is transferred into lab for extraction purpose. The process of maceration was done to get an extract of seeds of *Citrullus lanatus*. For this purpose, the use of 70% ethanol as a solvent [12]. The stirring was continued for 1 week and after 1 week when powder of seeds was completely dissolved into ethanol, then the process of filtration of seeds was done by using a filtration stand and a filtration flask. It took about 4 to 5 hours to complete the process of filtration. Next step was to dry the extract by rotary evaporator that we obtained after filtration process [15, 16]

Storage of extract

We use extract vial to store this extract into refrigerator at 4 degrees Celsius for further use.

Proximal analysis

Total ash, acid soluble ash, water insoluble ash, sulphated ash, moisture content and alcohol and water soluble extractive values were determined according to standard procedures [17].

Phytochemicals evaluation

Citrullus lanatus seeds powder and extract were evaluated for the levels of metabolites (primary and secondary metabolites). These were estimated for the standard methods described as : total proteins, total ;lipids , carbohydrates , total phenols, total flavonoids, total polysacchrides and total glycosaponins [18] [19].

FTIR studies

The seed powder was subjected to FTIR analysis. FTIR spectrum was obtained at 4000-400 cm [20].

Atomic absorption spectroscopy: metal and mineral contents analysis:

Atomic absorption spectroscopy analysis of seed powder has shown the presence of various minerals.

Percentage yield of different extract of *Citrullus lanatus*:

Citrullus lanatus seed powder was extracted by the two different solvents which include; Ethanol, Water.

Preparation of chemicals for biological activity

Preparation of Sabouraud Dextrose Agar

For preparation of SDA, 65g of medium in 1litter of purified water. After this, heat was applied with frequent stirring and the material was boiled until the medium was completely dissolved. After this autoclaved the material at 121 ^{o}C for 15 minutes. Warm from 45 to 50 ^{0}C and pour into petri dishes aseptically [21].

Isolation of dandruff agent

Samples were collected by scraping the scalp of people suffering from dandruff. Isolates were inoculated onto agar supplemented with Sabouraud olive oil. Plates were incubated at 37˚C for 7 days.

Anti dandruff activity:

First, made 4 wells on equal distance in a petri dish that contains sabouraud dextrose agar medium and then 3 concentrations of the extracts of *Citrullus lanatus* (0.1%, 0.3%, 0.5 %) were arranged in distilled water and the final extracts was utilized to decide its anti-dandruff action through well diffusion method by utilizing ketoconazole as a standard [22]. Dandruff isolates were inoculated using the spread plate method on Sabouraud's agar swell with olive oil. For seven days, the dish was exposed to 37 °C. The radius of the ablation zone, in millimeters, was measured and recorded along with the corresponding concentration. Three samples of each treatment were used in the tests [23, 24]**.**

RESULTS AND DISCUSSION

Results of Physicochemical analysis

The material was analyzed for its physicochemical characteristics and results are presented in table 1.

Table 1. Results of physicochemical analysis

Physicochemical property	Contents (w/w%)
Total ash	2.876
Acid soluble ash	0.37
Water soluble ash	0.834
Loss on drying	6.4
Value of water soluble extract	12.7
Value of alcohol soluble extract	7.8

Results of FTIR

The results of FTIR analysis are presented in figure 1.

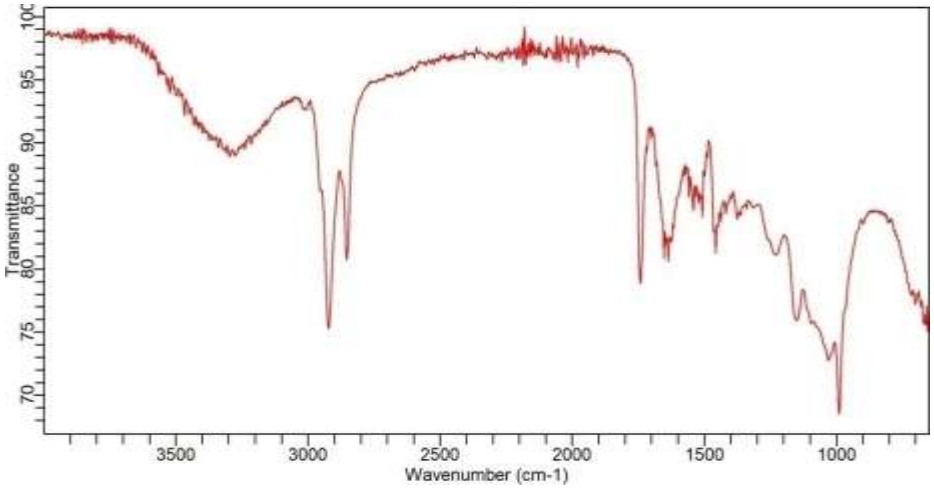

Figure 1. FTIR analysis of the *Citrullus lanatus* seed powder

The FTIR spectrum of the water extract shows a broad spectrum between 3500-3200 cm (OH stretching and H bonding) which shows the indication of alcohol and phenol. The peak between 3000-2850 cm (C-H stretch) indicates the presence of alkanes. The peak at 1555.8 cm shows a C-C stretch in the ring indicating the presence of an aromatic group.

Results of Atomic absorption spectroscopy: Metal and Mineral content analysis

The results of metal and mineral content analysis are summarized in table 2.

Table 2. Metal and Mineral content analysis

Determination metal and minerals	plant seeds value in ppm
Fe	2.5
Cu	104.0
Cr	50.0
Zn	68.0
Ni	0.001
Pb	1.21
Mg	0.71

Mn	365.0
P	220.0
Na	1730.0
K	4045.0
Ca	7.19

This shows that plants are an enriched source of minerals [25]. Seed powder samples contain the highest amount of sodium, potassium, phosphorus, zinc, copper, calcium and iron [26]. Calcium is a ubiquitous macronutrient found in plants. Calcium plays an important role in blood clotting and wound healing. Phosphorus helps to lower high blood pressure. Magnesium is an important component of plant chlorophyll, and iron is important to produce hemoglobin. The presence of zinc indicates that the plant can be useful in the treatment of diabetes caused by insulin deficiency [27] [28] [29].

Primary metabolites estimation

Primary metabolites estimation was included of total proteins, total lipids and carbohydrates (Table 3).

Table 3. Primary metabolites estimation

Parameters	Content (w/w%)
Total protein	34.86%
Total lipid	33.6%
Total soluble protein	114.6
Sugar	1.81
Starch content	2.23
Crude fibre	3.0

Analysis of primary metabolites appears that the seed powder varied number of proteins, lipid, sugar, starch, and crude fiber. Findings proposed that lipid are display in considerable sum. Presences of essential metabolites have appeared that plant has significant values [18].

Estimation of secondary metabolites

Findings of secondary metabolites [30] (total phenols, total flavonoids, total Saponins etc) were estimated and results are summarized in table 4.

Table 4. Secondary metabolites estimation

Extract	Table Polyphenols (mg/g)	Table Flavonoids (mg/g)	Table Glycosaponins (mg/g)	Table Polysacchrides (mg/g)
Ethanol	101.76±0.02	94.52±0.02	37.5±0.02	137.49±0.02
Water	117.83±0.03	73.72±0.02	24.75±0.02	134.9±0.01

Ethanol extract has maximum value for the overall flavonoids than the water. Ethanol extract has greatest value of glycosaponins than the water. Ethanol extract has most noteworthy esteem of polysaccharides than the water.

Anti-dandruff activity

Citrullus lanatus ethanolic extracts have been tested for their ability to reduce dandruff. *Citrullus lanatus* extracts in various dilutions exhibit significant anti-dandruff action against *Malassezia furfur* (Table 5 and Figure 2).

Table 5. Anti-dandruff activities

Fungicidal concentrations (mg/ml)	Zone of inhibition(mm)
Citrallus lanatus 0.1%	0.15mm
Citrallus lanatus 0.3%	0.20mm
Citrallus lanatus 0.5%	0.25mm
Ketoconazole disc	0.50mm

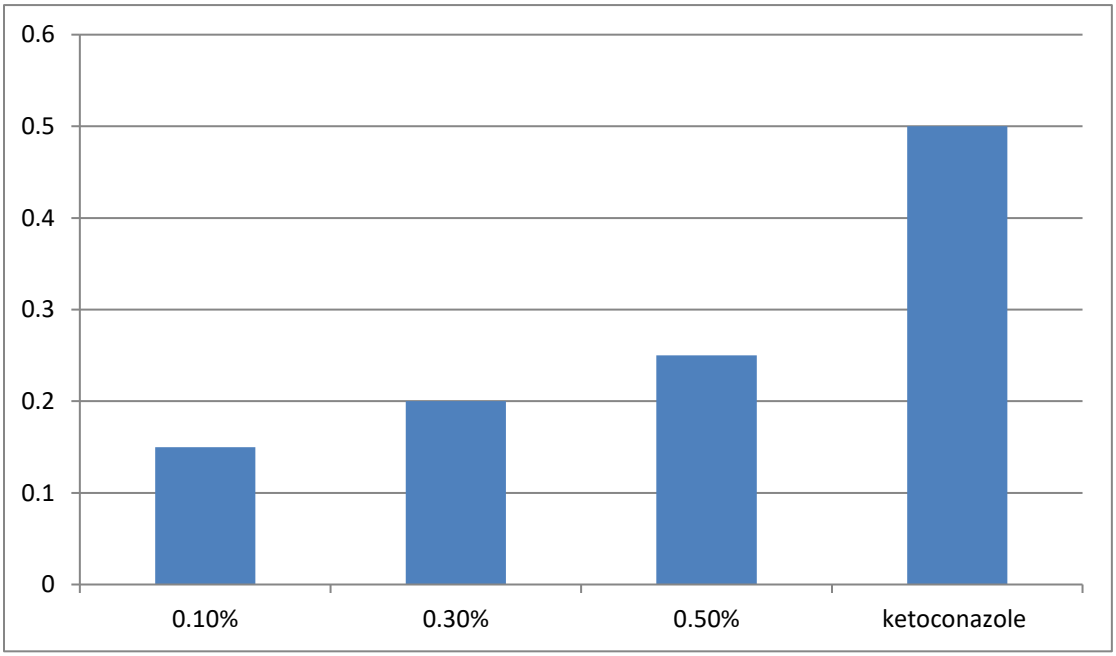

Figure 2. Graph representing zone of inhibition

Malassezia *furfur* is a common pathogen that causes dandruff. The lipophilic nature of these organisms causes the hydrolysis of human sebum triglycerides into free fatty acids, which accelerates the regeneration of scalp cells and thus results in hair loss. The seeds extract shows antidandruff activity against *Malassezia furfuri* [31]. As 0.1% dilution of *Citrullus lanatus* seeds extract has zone of inhibition 0. 2mm that is

increased if we increase the concentration of seeds extract like at 0.3%, zone of inhibition against *Malassezia furfur* is 0.25 mm and at 0.5%, zone of inhibition against *Malassezia furfur* is 0.3mm. .Evaluation of Minimum Inhibitory Concentrations (MICs) of a standard available drug, ketoconazole in the market against *Malassezia furfur* and their comparison with botanicals was done using different dilutions of this extract [21] [32] [33] [34].

CONCLUSION

The watermelon, or *Citrullus lanatus*, is more than just a pleasant fruit.

Its biological activities include a wide range of health advantages, including cardiovas cular protection, kidney function support, digestive health enhancement, and putative anti- cancer qualities. *Citrullus lanatus* seed extract has shown anti-dandruff activity and is traditionally used to stimulate hair growth. Phytochemical and analytical studies useful for standardization of *Citrullus lanatus* seed powder and extract. The plant under investigation was found to be rich in numerous primary and secondary metabolites. *Citrullus lanatus* exhibits many medicinal properties with antifungal activity against *Malassezia furfur*.

Conflict of interest: None

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