Phytochemical Evaluation and Anti-Dandruff Effect of *Momordica charantia* Seeds Extract

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ABSTRACT

Momordica charantia, a member of the Cucurbitaceae family, is grown in tropical and subtropical climates. Since ancient times, *Momordica charantia* has been used as a treatment for a number of illnesses such as cholera, bronchitis, anaemia, blood diseases, ulcers, diarrhoea, dysentery, gonorrhoea, rheumatism, gout, worms, and colic. Triterpene, protein, steroid, alkaloid, inorganic, lipid, and phenolic compounds make up the majority of *Momordica charantia* phytochemicals and are responsible for the plant's biological and pharmacological effects. Hence, the present study is planned to perform phytochemical analysis and to evaluate biological activity of *Momordica charantia* against the dandruff causing agent *Malassezia furfur* as previously no work was documented. For proximate analysis performed according to USP 2016 guidelines. *Momordica charantia* ground seeds were extracted in ethanol using the maceration method for 7 days. According to accepted protocols, phytochemical analysis of primary (proteins, lipids, and carbohydrates) and secondary (alkaloids, tannins, flavonoids, and glycosides) metabolites was carried out. Metal and mineral contents were determined by using

atomic absorption spectroscopy. The anti-dandruff activity was carried out using the disc diffusion method, and ketoconazole was used as a standard drug. The extract contained a variety of phytochemicals, including protein, carbohydrates, lipids, polyphenols, flavonoids, and alkaloids. The presence of various important minerals i.e. Na, K, Ca etc. were also found in sample. *Momordica charantia* ethanolic extract demonstrated significant antifungal activity at different dilutions, with a zone of inhibition measuring 7.5 mm at 0.1% dilution, 9.2 mm at 0.3% dilution, and 13 mm at 0.5% dilution. This study has demonstrated a considerable effect of *Momordica charantia* ethanolic extracts against *Malassezia furfur*.

Keywords: Anti-dandruff, Ketoconazole, *Momordica charantia*, Pharmacological activity, Phytochemicals

INTRODUCTION

Herbal therapy is gaining popularity for disease cerement in the world again because of toxic reactions or chemical treatments. Due to their low side effects, herbal medicines are an effective substitute to synthetic pharmaceuticals [1]. The history of using medicinal plants to treat diseases is extensive. In different countries of the world, using therapeutic plants to cure illnesses is a regular practice [2]. The world's rural population, excluding those in western nations, uses conventional medicines for 75–90% of their healthcare desires [3].In addition to having a large supply of active chemicals, medicinal plants also have a number of pharmacological qualities, such as the ability to modulate immune system components [4].

Bitter guard *(Momordica charantia)* grown anciently as a medicinal vegetable crop [5]. Bitter gourd tea is also becoming important herbal remedies against many diseases [6]. *Momordica charantia* extracts have shown to reduce plasma corticosterone levels as well as monoamine levels in the hypothalamus, cortex and even in the hippocampus areas of the brain [7]. Many mechanisms may contribute to the antitumor effects of *M. charantia* extracts, including triggering apoptosis, suppression of cancer stem cells, cell cycle arrest, and autophagy [8], antimicrobial activities [9, 10], antimutagenic property [11], antidepressant and anti-obesity [12], anti-inflammatory properties and anti-viral properties [13]. Many phytochemicals including proteins, amino acids, phenolic acids, fatty acids, minerals, alkaloids, flavonoids, triterpenoids, vitamins, quinine, saponins, triterpene, glycosides, and other compounds, are present in all parts of *Momordica charantia*, but particularly the fruit and seeds [14]. According to recent scientific

research, *Momordica charantia* is one of the promising medicinal plants that can normalize sugar level in blood [11]. In type 2 diabetes, it has been shown that oleanolic acid glycosides improve glucose tolerance [15]. Alongside curing diseases, *Momordica charantia* is equally beneficial in treating some fungus which cause dandruff issue in human [16]. Based on medicinal importance, the present study is planned to perform phytochemical analysis and to evaluate biological activity of *Momordica charantia* against dandruff causing agent *Malassezia furfur* as previously no work was documented.

MATERIALS AND METHODS

Plant Collection and Authentication

Momordica charantia seeds were obtained from a local market and verified by Department of Botany, the Government College University Lahore, Punjab, Pakistan (GC. Herb. Bot.3921). For future reference, a specimen number was recorded in the herbarium department.

Preparation of the Plant Extract

Seeds were washed properly to ensure the eradication of any contaminant. Then seed were air dries up to five days until these were complete dry. Seeds were then ground with grinder to ensure its uniformity in powder form. Whole seed powder was used for further processing [17]. The fine ground seed powder was sealed in a container to store and transport to laboratory for analysis. Ethanol (1000 ml 70%) was added to the 500g prepared powder, stir it thoroughly and then kept in a beaker covered with almunium foil. The stirring was done for a week on regular basis, when powder was completely extracted in ethanol, it was filtered using No. 42 filter paper. The golden coloured filtrate obtained after filtration was then dried using rotary evaporator and stored it in an extract vial at 4°C.

Physicochemical Evaluation

Total ash test, acid soluble, water insoluble, Sulphated ash and moisture content were determined in physciochemical analysis. Physicochemical analysis was performed according to the United States Pharmacopoeia (USP, 2016).

Determination of Extractive Value

Extractive values are useful to evaluate the nature of constituents present in the crude drug. According to established protocols by USP, 2016 extractive values for both alcohol and water were computed.

Water soluble extracts

Water-soluble extractive was determined using the formula:

$$Water\ soluble\ extract\ (\%) = \frac{weight\ of\ dried\ filtrate}{weight\ of\ air\ dried\ sample} \times 100$$

Alcohol soluble extracts

Alcohol soluble extract determined by using formula

$$Acid\ soluble\ extract\ (\%) = \frac{weight\ of\ dried\ filtrate}{weight\ of\ air\ dried\ sample} \times 100$$

Qualitative Estimation of Phytochemicals

Test for proteins

Biuret test

Biuret method was used to detect proteins in sample. To 2 ml of filtrate, one drop of a 2% copper sulphate solution is added. Following the addition of extra potassium hydroxide pellets, 1 ml of 95% ethanol is added. The presence of proteins is shown by the ethanolic layer's pink colour.

Ninhydrin test

The presence of amino acids can be determined by adding two drops of ninhydrin solution to 2 ml of the filter, which results in a purple colour.

Test for alkaloids

One little extract was taken in 5 ml of 1.5 % hydrochloric acid (v/v) and filtered through Whatman's filter paper No. 1. The filtrate was used for testing alkaloids.

Dragendorff's test

If alkaloids are present in the sample, a few drops of Dragendroff's reagent added to the extract results in a red precipitate.

Wagner's test

A reddish brown precipitate shows the presence of alkaloids when a small amount of plant extract is mixed with Wagner's reagent.

Test for glycosides

Benedict's reagent test

Benedict's test result for reducing sugars was filtered, and then 1 ml of diluted hydrochloric acid was added to it to hydrolyze the glycosides. It was combined with an equivalent volume of

Benedict's solution, warmed in a water bath, and then brought to a boil. The appearance of the brownish precipitate indicated the existence of glycosides.

Fehling's reagent test

The result from Fehling's test was used to conduct this test. The clear solution was heated for 5 minutes with a few drops of mild hydrochloric acid to hydrolyze glycosides. Fehling's reagent was again used to look for any further reduction, which indicates the presence of glycosides.

Test for sterols

Salkowski reaction

In a test tube, 0.5 g of the extract was dissolved in Two ml of pure chloroform. Next 2ml of concentrated sulfuric acid was slowly and drop by drop introduced to it via the test tube's side wall. Sterols may be present in extracts as evidenced by the development of a red colour in the chloroform layer and a greenish florescence in the bottom section of the solution.

Test for tannins

With 5 ml of methanol, 1 gram of the extract was heated before being filtered. The filtrate was split into 2 halves and examined using the subsequent reagents. The methanolic extract was diluted with a few drops of a lead acetate solution. Precipitate formation suggests tannins are present.

Gelatin test for tannins

The plant extract is mixed with a few drops of a 1% gelatin solution that contains sodium chloride. The presence of tannins is shown by the production of white precipitate.

Test for flavonoids

Alkaline reagent test

Just few drops of sodium hydroxide solution were added to the extract. When diluted acid is added, a strong yellow colour forms that eventually turns colourless, indicating the presence of flavonoids.

Lead acetate test

A solution of lead acetate was added to the extract. The existence of flavonoids is shown by the precipitation of a yellow substance.

Test for saponins

5ml of sodium bicarbonate, five millilitres of water, and one gramme of the extract were added to a test tube, and the mixture was vigorously stirred. The emergence of aconsistent foam suggests the presence of saponins.

Test for anthraquinones

Bontrager's test

In a test tube, 0.5g of the extract was heated for one to two minutes with five ml of 10% sulfuric acid before being immediately sifted. After cooling, benzene was mixed into the filtrate. After isolating the benzene layer, it was shaken with 10% smelling salts in half the volume. The ammoniacal layer's improved pink ring indicates the presence of anthraquinones.

Quantitative Estimation of Phytochemicals

These were estimated by the standard methods: total proteins [18], total lipids [19], carbohydrates [20], total polyphenols [21], total flavonoids [22], total polysaccharides and total glycosaponins [23].

Metal and Mineral Contents Analysis

The presence of several minerals in seed powder has been demonstrated by atomic absorption spectroscopy research.

Preparation of Sabouraud Dextrose Agar

We dissolve 65 gram of medium in One litter of distilled water to make SDA. Following that, the item was heated while being stirred frequently until the medium had completely dissolved it. The material was then autoclaved for 15 minutes at 121C. Aseptically poured into petri dishes after cooling.

Isolation of Dandruff Causing Agent

Samples were taken by scratching the dandruff-prone participants' scalp tissue. On Sabouraud's agar that also included extra virgin olive oil, the segregates were infected. The plates were kept for 7 days at 37 °C.

Antidandruff Assay

First, we formed four equal-sized wells in a petri dish containing sabouraud dextrose agar medium. Next, we prepared *Momordica charantia* extracts in three different concentrations (0.1%, 0.3%, and 0.5%) in sterile water. We then used the extracts to test their anti-dandruff activity using the well diffusion method, using the drug ketoconazole as a reference. By using

the spread plate approach, dandruff isolates were inoculated on Sabouraud's agar enriched with olive oil. The plates were kept for 7 days at 37 °C. In millimetres, the radius of the zone of obstruction was measured and recorded with the contrasting concentration. Three duplicates of each treatment were used in the tests [24].

RESULTS AND DISCUSSION

Physicochemical Analysis

Data for physicochemical analysis is provided in table 1. All values were found to be in recommended limit that ensure quality of the plant material. Further, plant sample has lower moisture due to which there are minimum chances of fungal growth [25] [26].

Table 1. Physicochemical analysis of *Momordica charantia* seeds extract

Physicochemical property	Contents (w/w%)
Total ash	4.3
Acid insoluble ash	0.3
Water soluble ash	0.69
Loss on drying	6.7
Water soluble extractive values	17.8
Alcohol soluble extractive values	6.2

Qualitative Analysis of Phytochemicals

According to the early phytochemical data, certain phytochemicals were present in the extracts and others weren't. The plant's alcoholic extract includes phenolics, proteins, tannins, flavonoids, and saponins, which may make it valuable for treating a variety of illnesses and have the potential to provide beneficial pharmaceuticals for human usage. The results are summarized in table 2.

Table 2. Qualitative analysis of phytochemicals of *Momordica charantia* seeds extract

Phytochemicals	**Ethanol extract of seeds of *Momordica charantia***
Alkaloids	+++++
Flavonoids	++
Carbohydrates	+

Glycoside	-
Polyphenols	++
Tannins	+
Phenols	+++
Saponins	++
Terpenoids	+
Proteins	++
Anthraquinones	-
Phytosterols	-

- = not present

+ = present

Quantitative Estimation of Phytochemicals

The values recorded for primary metabolites are given in table 3.

Table 3. Primary metabolites in *Momordica charantia* seeds extract

Plant content	**Content (w/w%)**
Total protein	31.4
Total lipid	37.3
Sugar	3.73
Starch content	2.12
Crude fibre	4.51

Data for secondary metabolites is presented in table 4.

Table 4. Estimation of secondary metabolites of *Momordica charantia* seeds extract

Extract	Table Polyphenols (mg/g)	Table Flavonoids (mg/g)	Table Glycosaponins (mg/g)	Table Polysaccharides (mg/g)
Ethanol	109.85±0.03	93.82±0.03	38.4±0.02	123.59±0.03

Triterpenoids have appeared to have antifungal movement and were found to be display in as it were the seeds and mash. Tannins and saponins are other classes of phytochemicals known to have, anti-fungal. Tannins and saponins concentration was found higher in current research. The presence of polar substances such as tannins, phenols, glycosides, etc. was suggested by high alcohol and water dissolvable values [26] [27] [28].

Metal and Mineral Content Analysis

Table 5 lists the outcomes of the atomic absorption spectroscopy. The findings of the investigation demonstrated the existence of numerous significant metals and minerals required to preserve human health [26] [29] [30] [31].

Table 5. Metal and mineral content analysis

Determination metal and minerals	Plant seeds
Fe (%)	2.66%
Cu (ppm)	96.0
Cr (ppm)	45.0
Zn(ppm)	73.0
Ni(ppm)	-
Pb(ppm)	2.0
Mg(%)	1.9
Mn (ppm)	165.0
P(ppm)	223.0
Na(ppm)	1776.0
K(ppm)	3976.0
Ca(%)	6.29

Anti-Dandruff Activity

Momordica charantia ethanolic extracts have been tested for their antidandruff effects. *Momordica charantia* extracts in various dilutions have notable anti-dandruff action against *Malassezia furfur*. *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 turnover of scalp cells and thus results in hair loss. As a result, the isolates thrived on Sabouraud's agar medium that had been supplemented with olive oil. On Sabouraud's medium, *Malassezia furfur* developed as a white to cream-colored colony. The seeds extract shows antidandruff activity against *Malassezia furfur* as 0.1% dilution of *Momordica charantia* seeds extract has zone of inhibition 7.5mm which was increased to 13.0mm with increasing extract concentration to 0.5% (Table 6) [32] [33] .

Table 6. Anti-fungal activity of *Momordica charantia* seeds extract

Fungicidal concentrations(mg/ml)	**Zone of inhibition(mm)**
Momordica charantia 0.1%	0.09
Momordica charantia 0.3%	0.10
Momordica charantia 0.5%	0.19
Ketoconazole disc	0.20

CONCLUSION

The herb has long been used to promote hair growth. Extracts from *Momordica charantia* seeds shown a notable antidandruff activity. There are several beneficial phytochemicals present, which may be responsible for of this activity.

Conflict of interest: None

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