

**Phytochemical analysis and evaluation of *Cucurbita maxima* seeds extract against
*Malassezia furfur***

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ABSTRACT

Medicinal herbs or plants have great potential to treat the ailments with less or no toxicity. *Cucurbita maxima* (Family: Cucurbitaceae) is a medicinal plant that has great therapeutic potential. Its seeds are used for antioxidant, antidiabetic, antimicrobial, antioxidant, and for anti-inflammatory activity but less is known about its antidandruff capabilities. The purpose of this research study to ascertain antifungal (anti-dandruff) activity, and to investigate the presence of phytochemicals in seeds of *Cucurbita maxima* using ethanol as an extracting solvent. For proximate analysis, the samples were analysed for crude protein, moisture, ash, total fat, crude fibre, total carbohydrate, and total phenolic content as per USP, 2015 method prescribed for botanic origin. Evaluation of phytochemicals (primary i.e., carbohydrates, lipids and proteins, and secondary i.e., alkaloids, tannins, glycosaponins and flavonoids) were also performed. FTIR was also performed for plant characterization and for identification of different organic and inorganic parts of plants and to identify various functional group like -CH, =CH and -OH and associated pharmacological activities. Atomic absorption spectroscopy was done to analyse mineral contents of *Cucurbita maxima* seeds powder. Maceration was used for extraction of dried seeds of *Cucurbita maxima* by using 70% ethanol as a solvent. Antidandruff activity was performed against *Malassezia furfur* (A dandruff causing fungus) by using well diffusion method and by taking ketoconazole as a standard. Zone of inhibition was measured to determine anti-dandruff activity. Proximate analysis values were well within the ranges given in USP analysis 2015 guidelines. The qualitative analysis showed the presence of various primary and secondary metabolites. *Cucurbita maxima* seeds extract undergoes

phytochemical analysis, which identifies the presence of proteins, carbohydrates, flavonoids, saponin and tannins. *Cucurbita maxima* seeds extract show significant anti-dandruff activity at 0.5% concentration, which is comparable with anti-dandruff activity of standard drug (ketoconazole). Seeds extract of *Cucurbita maxima* showed the presence of very useful phytochemicals that has many medicinal uses. Further, *Cucurbita maxima* have considerable effect against *Malassezia furfur* so can be used in a polyherbal mixture to treat dandruff.

Keywords: *Cucurbita maxima*, *Malassezia furfur*, Dandruff, FTIR, Atomic Absorption Spectroscopy, Phytochemicals

INTRODUCTION

About 70% of the world's population from developing countries are preferring the herbal medicinal products over synthetic drugs as a primary source of healthcare due to their less cost and more safety [1]. It is reported that \$83 billion US are derived from global market of products of plants, and it is continuously in growing phase. That is also reported about 25% of synthetic or modern drugs and 60% of antitumor drugs are obtained from plants [2]. According to the WHO, the ratio of population of developing countries is between 65% and 80%, currently using medicinal plants as remedies because of its various pharmacological properties. In response to the widespread use of medicinal plants, the WHO published the Monographs on Selected Medicinal Plants contain a list of species with documented medical benefits and the recommended methods to use them safely, and they are updated frequently [5].

Among the most varied domesticated species is *Cucurbita maxima* that is most widely cultivated due to its pharmacological potential [6]. *Cucurbita maxima* has a place in family Cucurbitaceae that includes cucumber and squash. It is most known as "Pitakusmandah" in Sanskrit; "Kaddu" or "Sitaphal" in Hindi and "Squash" or "Red protects pumpkin" in English, and in ancient literature, it demonstrates itself to be viable against numerous ailments. It may be a trailing yearly herb with some degree thorny or shaggy stem and axillary ringlets, blooms huge, yellow, unisexual, singular; natural products plump, circular or oval, brown; seeds ovoid or oval, compressed. It is broadly developed all through India and in most warm districts of the world, such as Indonesia, Malaysia, and Philippines for utilize as vegetable as well as medication. Both of its natural products and the ethereal parts are commonly used as vegetable [7], [8].

Cucurbita maxima, *Cucurbita pepo*, *Cucurbita moschata*, *Cucurbita ficifolia*, and *Cucurbita turbaniformis* are only a few of the numerous pumpkin kinds. Pumpkin seed germination

requires a temperature greater than 16°C, and the ideal temperature range for this trim is between 20 and 35°C. Production of pumpkins, squash, and gourds was estimated to over 23 million tonnes in 2019 across an area of 1.54 million acre, with 2.7 lac tonnes in Pakistan on a 26515-hectare range [9]. *Cucurbita maxima* belongs to Family: Cucurbitaceae, Genus: Cucurbita, Order: Violales, Kingdom: Plantae, Division: Magnoliophyta, Species: maximum [6]. Pumpkins are good source of protein and have a protein level of 29–42%, a lipid content of 40–50%, and a -CHO content of 9–11%. All oil seeds from the Cucurbitaceae family are a good source of a few important amino acids, which aids in the digestion of protein [10]. Unsaturated fatty acids were present in significant concentrations in the extract of pumpkin seeds, with linoleic acid (27 to 80%), oleic acid (15 to 30%) palmitic acid (1.16-20.81%), and stearic acid (0.16-5.56%) being the most prevalent. Amino acid glutamic acid is abundant in these pumpkin varieties in their amino acid profiles, ranging from 33.03 to 34.76 g/100g protein, according to amino acid analyses [11]. *Cucurbita maxima* has the potential to produce beneficial pharmaceuticals for human usage due to the presence of tannins, phenolic, flavonoids, and saponins in its alcoholic extract, which makes the plant useful for treating a variety of diseases [12].

The very common scalp condition known as dandruff is characterised by the presence of clusters of corneocytes that take the form of flaky, white to yellowish scales and are itchy [13]. Fungus that plays an important role in causing dandruff belongs to *Malassezia* specie. *Malassezia* also cause many diseases in human skin besides causing dandruff such as psoriasis, atopic dermatitis, seborrheic dermatitis, and pityriasis versicolor [14]. Use of antifungal drugs such as Ketoconazole, Climbazole, Selenium sulphide, Zinc Pyrithione, Coal tar and salicylic acid to treat dandruff is excessive resulting in the adverse drug reactions and increased tolerance to drugs for pregnant women, children, and immunocompromised patients. There is also restriction in using anti-dandruff drugs in the case of pregnant women, to avoid complications [15]. To solve these problems, it is necessary to develop a plant having antifungal activity against *Malassezia furfur*.

MATERIAL AND METHODS

Chemicals and solvents

Analytical grade chemicals and solvents were used that include, Serum albumin bovine, Glucose, Aluminium nitrate, Sodium bicarbonate, Lactopherol cotton blue stain, KOH, NaOH,

Quercetin 3-rhamnoside, Anthrone reagent, Gallic acid, Folin and ciocalteu's reagent, Triton X 100, DMSO, Methanol, Ethanol, Sabouraud dextrose agar, Ketoconazole disc.

Plant collection and authentication

The *Cucurbita maxima* seeds were obtained from a local market in the city of Gujrat, and they were identified and authenticated by the GC University Herbarium Department of Botany in Lahore (Punjab), Pakistan. The herbarium department has issue a voucher specimen number (GC.Herb.Bot.3920) on file for the future use.

Apparatus

Pestle and mortar, China dish, Petri dishes, conical flask, measuring cylinder, spatula, iron stand, watch glass, tripod stand, test tubes, beakers, funnel, filter paper, flat bottom flask, pipette and glass rods, Sample vials 5 and 15ml, Petri dish plastic, Falcon tubes.

Instrument

Electronic weighing balance, water bath, Laboratory hot air oven, Autoclave, Incubator, Incinerator, Atomic absorption spectrophotometer, Ultrasonic mixture, Ultraviolet spectrophotometer, FTIR Spectrophotometer, Refrigerator, Oven.

Preparation of the plant extract

Cucurbita maxima (pumpkin) seeds were purchased from a local market in Gujarat. The total weight of the seeds was approximately 1 kg, or 1000 g. The seeds were properly rinsed with tap water after purchase to get rid of all the contaminants. *Cucurbita maxima* seeds were washed and then air dried for about 5 days to completely remove moisture. These *Cucurbita maxima* seeds undergo grinding after they had dried completely in order to create powder. To reduce the size of the seeds and turn them into powder, this grinding was accomplished through milling. *Cucurbita maxima* complete seed parts, including the kernel and shell, were ground into a powder, yielding about 1000 g of *Cucurbita maxima* seed powder. *Cucurbita maxima* seed powder was kept in an airtight container to prevent the possibility of contamination from airborne moisture, dust, or other environmental contaminants. Then brought this powder into the lab where it was extracted by the maceration in 70% ethanol to obtain an extract from *Cucurbita maxima* seed. 500 g of *Cucurbita maxima* powder and 1000 ml of 70% ethanol were added to a beaker, and then it was continually stirred to thoroughly combine the ingredients. After some time, aluminium foil was appropriately placed over the beaker that was utilised for

the maceration process. The aluminium foil had several holes drilled into it for the ethanol to evaporate. After one week of stirring, when the seed powder had completely exhausted in the ethanol, filtration of the extract was done. The filtration process takes roughly 4 to 5 hours to finish. The extract was then dried using a rotary evaporator that we had obtained during the filtration process. Complete extract drying and alcohol evaporation took 48 hours. The extract is semi-solid in texture and has a chocolate brown tint. To keep this extract in a refrigerator at 4 degrees Celsius for later usage, we utilise an extract vial.

Physicochemical evaluation

This is also called proximate analysis that check the purity of powder (crude drug) and help to identify plant shelf life to determine either our medicinal plant is safe to store for long period of time or not because plants with low moisture content are less prone to contaminate. Seeds of *Cucurbita maxima* was screened for physicochemical properties which was included Total Ash test, Water insoluble ash test, Acid soluble ash test, Sulphated ash test, PH test, Moisture content test, water-soluble and Alcohol extractive values. Following formulas were used to conduct proximate analysis through standard procedures.

- Total ash (%) = (ash weight/sample weight) x 100
- Acid insoluble ash (%) = (ash weight/sample weight) x 100
- Calculating the difference between total ash and acid insoluble ash allowed us to establish the percentage of acid soluble ash.
- Water insoluble ash (%) = (ash weight/sample weight) x 100
- (Weight of sulphated ash/Weight of Sample x 100) = Sulphated Ash (%)
- Dry matter = sample weight after oven drying minus sample weight after air drying.
- (Dry matter/Weight of Air-Dried Sample) x 100 = Moisture Content (%)
- (Weight of dried filtrate/Weight of air-dried sample) x 100 = Alcohol Soluble Extractives (%)
- (Weight of dried filtrate / weight of air-dried sample) x 100 equals the percentage of alcohol-soluble extractives.

Phytochemicals evaluation

The goal of the phytochemical investigation was to identify the presence of different active ingredients in the alcoholic extracts of *Cucurbita maxima* seeds. The seeds of *Cucurbita maxima* were evaluated for estimation of primary metabolites (total lipids, total protein, and total amount of carbs) and for estimation of secondary metabolites (total alkaloids, total glycosides, total sterols, total tannins, total flavonoids, total saponins, total anthraquinones and total phenolic content).

FTIR analysis

Analytical method like FTIR analysis was performed on seeds of *Cucurbita maxima* to investigate the purity of plant and at 4000-400 cm, FTIR spectra was recorded.

Atomic Absorption spectroscopy

Atomic absorption spectroscopy or mineral content analysis was also performed on seeds of *Cucurbita maxima* that revealed presence of many useful minerals in this plant.

Percentage yield of *Cucurbita maxima*

70% ethanol was used for extraction of seeds of *Cucurbita maxima* and percentage yield was determined.

Chemicals preparation for biological (antidandruff) activity of *Cucurbita maxima*

Preparation of Sabouraud Dextrose Agar (SDA):

SDA is a medium suitable for growth of *Malassezia furfur* and for this preparation we dissolve 65g of medium in 1 liter of distilled water. Following this, the material was heated while being stirred frequently until the medium was entirely dissolved. After that, the material was autoclaved for fifteen minutes at 121⁰ C. Temperature was then reduced to 45 to 50⁰ C before aseptically spreading it into petri dishes [16].

Isolation of dandruff causing agent:

The scalps of participants with dandruff were scraped to get samples of dandruff. On Sabouraud's agar that had olive oil added as a supplement, the isolates were inoculated, and petri dishes were then incubated for 7 days at 37 °C. (Leeming & Notman, 1987).

Antidandruff assay

First, three concentrations of the *Cucurbita maxima* seeds extracts (0.1%, 0.3%, and 0.5%) were prepared in sterile water, and using ketoconazole as a reference, the antidandruff activity

of the resulting extracts was evaluated using the well diffusion method. to check anti dandruff activity, we created 4 wells on an equal distance in a petri dish which contained sabouraud dextrose agar medium. Dandruff isolates were inoculated on Sabouraud's agar that had been supplemented with olive oil using the spread plate approach. At 37 °C, the plates were incubated for 7 days. The radius of the zone of inhibition was measured in millimetres in respect to the relevant concentration. In the experiment, each treatment was performed three times.

RESULTS AND DISCUSSIONS

Physicochemical analysis

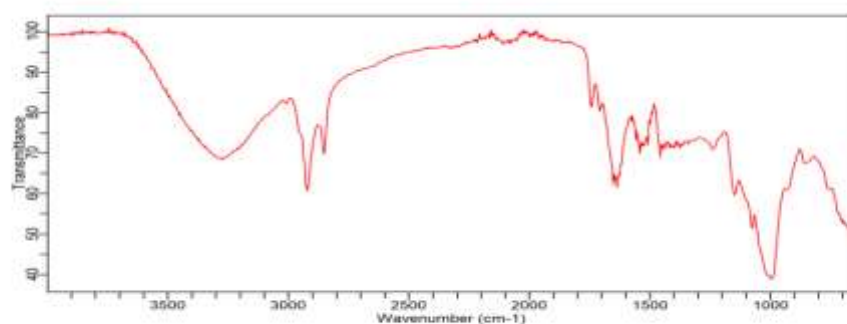
The results are tabulated below contains values of total ash, acid insoluble ash, and water-soluble ash. The ash value was calculated, which provides insight into the earthy or inorganic composition and other contaminants present in the medicine. According to the analytical findings, the observed values for total ash, acid insoluble ash, and water-soluble ash were 2.908, 0.287, and 0.766% W/W, respectively. The loss on drying in seeds at 105 °C drying was determined to be 7.09% W/W. Exhausted or contaminated drugs can be identified largely using the extractive values that were discovered. Extractive values that were alcohol- and water-soluble were also determined. The existence of polar elements such phenols, steroids, glycosides, and flavonoids were indicated by the alcohol soluble extractive value, while polar sugar, acid, and inorganic compounds were indicated by the water-soluble extractive value. *Cucurbita maxima* seeds had extractive values that were 16.2 and 8.9%, respectively, for substances that were soluble in water and alcohol.

Table 1. Depicts Results from physicochemical analysis.

Physicochemical Variables	Contents (Weight/weight %)
Ash total value	2.908
Insoluble ash value	0.287
Ash water-soluble value	0.766
LOD (loss on drying value)	7.09
Extractive value (water soluble)	16.2
Extractive value (alcohol value)	8.9

FTIR analysis

FTIR analysis of powder of *Cucurbita maxima* seeds was also done to identify the organic and inorganic compounds and to know the quality verification of *Cucurbita maxima* seeds powder and Results of FTIR analysis are represented in figure 1.



Peak Number	Wavenumber (cm ⁻¹)	Intensity
1	855.42403	0.46127
2	995.19920	0.00000
3	1075.33697	0.20310
4	1149.88372	0.33523
5	1239.33983	0.51624

Figure 1. FTIR of *Cucurbita maxima* seeds

Results for phytochemical analysis

Table 2 represents results of phytochemical analysis of *Cucurbita maxima* alcoholic seeds extract that contains phenolics, proteins, carbohydrates, proteins, tannins, flavonoids, and saponins, which may make it valuable for treating many illnesses and have the potential to produce beneficial medications for human use. Table 4 represents concentration results of primary metabolites and table 5 represents estimation of secondary metabolites presents in *Cucurbita maxima* seeds extract.

Table 2: Depicts presence of phytochemicals in *Cucurbita maxima* seeds extract.

Phytochemicals	Ethanol extract of seeds of <i>Cucurbita maxima</i>
Alkaloids	+
Flavonoids	+
Carbohydrates	+
Glycoside	-
Polyphenols	+
Tannins	+
Phenols	+
Saponins	+
Terpenoids	+
Proteins	+
Anthraquinones	-
Phytosterols	-

Table 3. Nutrient composition or primary metabolites concentration of *Cucurbita maxima* seeds.

Parameters	Content %
Lipids value	35%
Protein value	33.98%
Protein (total soluble)	16.9
Sugar value	1.03
Starch value	2.14

Fibre (crude) 2.98

Table 4. Represent results of phenolic and tannins content of *Cucurbita maxima* seeds powder.

Sample of plant	Phenolic content in mg GAE/g	Tannins content in mg TAE/g
c. maxima seeds extract	4.01 ± 0.1	12.9 ± 0.42

Cucurbita maxima contains Phenolic compounds that also known as free radical terminators. In our study, total phenol content (TPC) of *Cucurbita maxima* seed extracts was analysed by using Folin- Ciocalteu colorimetric method and represented in terms of gallic acid equivalent (GAE). Tannins is another secondary metabolite that play important role because of its stringent properties, that exhibit wound healing action and inflamed mucous membranes.

Minerals content of *Cucurbita maxima* seeds

Atomic absorption spectroscopy was performed to investigate various mineral contents in *Cucurbita maxima* seeds and it is determined and analysed that this plant has many useful and important minerals.

Table 5. List of mineral contents presents in *Cucurbita maxima* seeds

Element	Amount (ppm)
Na (Sodium)	270.12
K (Potassium)	307.66
Ca (Calcium)	260.21
Mg (Magnesium)	339
Ferus	291
Cu (copper)	71

Zn (Zinc)	42
Mn (Manganese)	19
Phosphorus	2271

Anti-dandruff activity

Malassezia furfur (dandruff) hydrolyse human sebum triglycerides into free fatty acids due to organisms' lipophilic nature, which speeds up the scalp's cell turnover and contributes to hair loss [17]. That's why the isolates of dandruff flourished on the Sabouraud's agar medium that had been supplemented with olive oil for quick growth of dandruff. On Sabouraud's media, *Malassezia furfur* developed as a white to cream-colored colony. On *Malassezia furfur*, all plant extracts demonstrated a respectable inhibitory zone. The standard deviation was determined since the studies were carried out in three duplicates. The following table lists the Minimum Fungicidal Concentration and the zone of inhibition for various plant extract dilutions of *Cucurbita maxima* seeds. Results showed that ethanolic extract of *Cucurbita maxima* seeds has considerable antifungal efficacy against *Malassezia furfur*.

Table 6. Results of zone of inhibition obtained through well diffusion method

Minimum concentration for fungicidal action (mg/ml)	Zone of inhibition (mm)
<i>Cucurbita maxima</i> 0.1%	0.2
<i>Cucurbita maxima</i> 0.3 %	0.25
<i>Cucurbita maxima</i> 0.5 %	0.3
Ketoconazole 10mcg disc	0.6

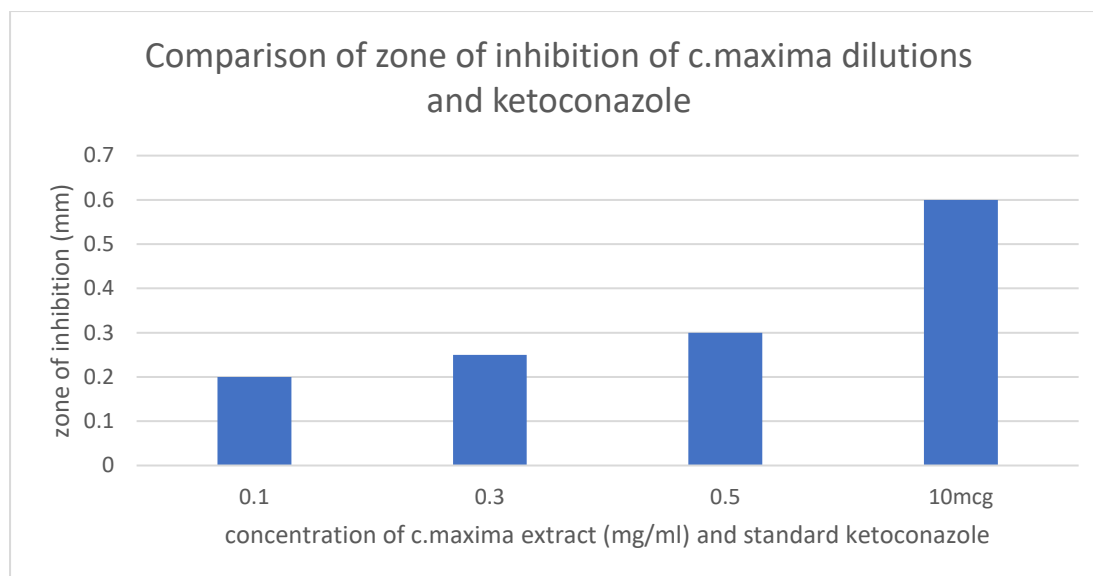


Figure 2. Graph represents the comparison of zone of inhibition of *Cucurbita maxima* dilutions and Ketoconazole

DISCUSSION

The results show that the seeds were not altered or contaminated with, according to physicochemical criteria such moisture content, total ash value, acids insoluble ash, and extractive value. Standard assays and methodologies were used to evaluate plant for the presence of various chemical elements. Carbohydrates, alkaloids, saponins, proteins, and flavonoids are all present, according to the research findings. It has been demonstrated that pumpkin seed oil contains high levels of the antioxidant vitamins tocopherol and carotenoid with adequate oxidative stability. The significant amount of tocopherol found in pumpkin seeds may act as a shield against harmful chemicals and free radicals [18]. Pumpkin seed phytoestrogens may potentially contribute to a decreased hormone-dependent tumour. Pumpkin seeds are high in vitamin E, particularly α - and γ -tocopherol, which is a developing anti-aging, antioxidant, and free radical scavenger [19]. Secondary metabolites or phytochemical components of plants, including tannins, phenols, alkaloids, and many other aromatic chemicals, act as a defence mechanism against herbivores, insects, and various microorganisms. Regarding their capacity to serve as radical scavengers, numerous classes of chemicals have drawn a lot of attention as potential natural antioxidants [20]. A class of antioxidants known as phenolic chemicals function as free radical terminators. Crude protein is present in large amounts in the pumpkin seeds. The storage protein in pumpkin seeds is 11s type globulin, which accounts for more than 80% of the dry seed's protein composition. According to reports, the mature seeds' dry weight contains a sizable amount of protein.

Because they are an excellent source of zinc, other vitamins, and protein, *Cucurbita maxima* seeds have several health advantages. They are also said to decrease cholesterol. Essential fatty acids found in *Cucurbita maxima* seed oil support the health of the tissues, neurons, and blood vessels. For their pharmacological effects, such as their anticancer and anti-aging capabilities, flavonoids have free radical scavenging and anti-oxidant properties [21]. Flavonoids are moderate vasodilator, and interactions with cytochrome p-450 that are beneficial for the treatment of cardiac disease. *Cucurbita maxima* seeds have a higher concentration of amino acids and are considered to be among the best organic sources of tyrosine. There have been claims that tyrosine help in breakdown of environmental toxin and aid our body to fight with depression [22, 23].

Because *Cucurbita maxima* dry powdered seed contains antifungal proteins and a large amount of tannins, which are responsible for both its antioxidant and antifungal action, it can be inferred from our findings that the ethanolic extracts from these seeds have antifungal activity [24]. During nutrients content analysis, through atomic absorption spectroscopy we identify many useful nutrients in pumpkin seeds, previous studies upon it also has some similar results about its nutritional contents values [25].

Well diffusion method for measurement of zone of inhibition by three dilutions of ethanolic extract of *Cucurbita maxima* and we find that if we increase the amount of *Cucurbita maxima* seeds extract then anti-fungal activity of *Cucurbita maxima* seeds extract is increased against *Malassezia furfur*. As 0.1% dilution of *Cucurbita maxima* 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 0.3 mm. Several dilutions of this extract were used to determine the Minimum Inhibitory Concentrations (MICs) of the commonly prescribed medicine ketoconazole against *Malassezia furfur* and compare them to natural remedies. So, from this study, it is demonstrated that seeds of *Cucurbita maxima* can be used for formation of herbal product against dandruff because this will be cost effective as compared to ketoconazole or other drugs that are available in market [26-29].

CONCLUSION

Cucurbita maxima seeds contain important compounds that may be used medicinally to treat dandruff. *Malassezia furfur* was significantly inhibited by extracts from *Cucurbita maxima* seed. So, our findings are encouraging and motivate us to conduct additional research on

Cucurbita maxima seeds that will be useful in creating a polyherbal remedy to treat dandruff and stop its complications, such as baldness and alopecia that can cause depression for people.

Conflict of interest: There is no conflict of interest of this study

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