

Clinical Study of the *Prunus dulcis* (Almond) Shell Extract on *Tinea capitis* Infection

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Abstract

Prunus dulcis (Almond) shell extract demonstrated for its biomedical applications. Shell extract prepared by soxhlet method and further characterized by UV-Visible spectrophotometer, Atomic Absorption Spectrophotometer (AAS) and MIC control method. In this study the antifungal activity of almond shell extract was observed against clinically isolated pathogenic fungus by strip method. The antioxidant potential of crude shell extract was also evaluated by using DPPH (2,2-diphenyl-1-picrylhydrazyl) and radical scavenging system. The total antioxidant activity varied from 94.38 to 95.49% and total phenolic content was found 4.455 mg/g in almond shell extract. Finally the results provide a great therapeutic potential against infection of scalp caused by *Tinea capitis* and result can be achieved in 20 days therapy. In this study of shell extract results provide a great therapeutic potential against infection of scalp caused by *Tinea capitis* and provides scientific evidence for clinical efficacy against dermatologic disorders. The present study is aimed to summarize the therapeutic importance of cheap and discarded coconut shell and the future prospects in order to develop a new generation of modern antimycotic drugs.

Keywords: *Prunus dulcis* (Almond); *Tinea capitis*; UV-Visible spectrophotometer

Introduction

The phyto-medicines derived from plants are still used in the developing countries and according to W.H.O it is estimated that 80% of the humans in the world mainly rely on traditional medicines for primary cure of health problems [1]. Nuts are considered to be one of the most nutritionally complete feed for human wellness due to their high nutritional contents such as carbohydrates, protein, unsaturated fatty acids, vitamins and essential minerals [2]. Nut consumption reduce the risk of cardiovascular disease (CHD), which may be partly explained by the cholesterol-lowering effect [3,4]. Several antimicrobial agentive roles were extracted and tested from the matrix and leaf of these species and many researchers are working to test in vitro effect of this work against bacteria, viruses, and fungi. Several nuts such as walnuts and peanuts are among this dietary work known to have significant antioxidant contents [5,6]. The stable radical species 1,1-diphenyl-2-picrylhydrazyl (DPPH), has been widely used for antioxidant capacity cover and estimation due to its clear chemical reaction mechanism, solvent compatibility and the technical simplicity which requires no special equipment [7]. The shells can be used for fuel and are a source of charcoal [8] and excite carbon manufacturing from coconut shell is considered super effective for the firing of impurities [9]. Shells are the rough exterior part of the nuts [10] and are not used for food but can be utilized in several ways, including creating enriched potting soil and buff floor at household.

Tinea capitis is superintendent ficial dermatophyte characterized to form crust on the scalp, which is primary cause of hair loss. The dermatophytes which are most common cause of the disease is *M. canis* genus; *M. gypseum*; *T. equinum*; *T. verrucosum*; *T. tonsurans*; *T. violaceum*; and *T. schoenleini* [11]. The disease is always chronic, difficult to be treated or requires long term medicine and susceptible to have super infection with bacterial agents. Sweet almond possess a variety of beneficial properties affecting most notably cancer, high blood pressure, infectious diseases and diabetes but I am looking for nut shells a biodegradable material which can be used as potential reservoir for prevention of skin diseases.

Material and Method

Shell Material

Prunus dulcis (Almond) sample was collected at its optimum commercial maturity from the local market of Hyderabad. The Voucher specimen was deposited in herbarium at the Institute of plant science Figure 1.



Figure 1: *Prunus dulcis* (Almond) sample.

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Preparation of the *Prunus dulcis* shell extract

The shell of almond powdered with the help of warring blender. 20 gm of dried powder was filled in thimble and macerated with 100 ml of analytical grade solvent methanol. Sample was used by soxhlet extraction method for the formation of crude extract till 16 hours and kept on a rotary shaker for 24 h. The sample was filtered through Whatman No. 1 filter paper to remove rough particles. The crude extract was stored at 18°C until analyzed.

DPPH radical scavenging activity

Free radical scavenging capacity of almond shell extract was determined according to the previous reported procedure using the stable 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) assay. The same DPPH assay method was reported by [12]. The absorbance of the resulting solution was read spectrophotometrically at 517 nm). Fresh prepared solution of DPPH (5.0 ml) was added to 1.0 ml of shell extract. The decrease in absorbance measured at different intervals, i.e., 0.05, 1, 2, 5 and 10 min (up to 50%) and 515 nm was determined with a Hitachi UV- Vis Spectrophotometer. The percentage of 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) assay was calculated as follows:

$$\% \text{ DPPH scavenging} = [A_{\text{control}} - A_{\text{sample}}] / A_{\text{control}} \times 100.$$

Where A_{sample} is the absorbance of sample after the time necessary to reach the plateau (30 min) and A_{control} is the absorbance of DPPH. The remaining concentration of DPPH in the reaction mixture was calculated from a standard calibration curve.

Determination of total phenolic content

The total phenolic contents were extracted from shell of *P.dulcis* and determined by spectrophotometrically using the Folin-Ciocalteu method described previously (Moure A, et al 2007). The reaction mixture contains 200 μ l diluted shell extract, 800 μ l of freshly prepared and diluted Folin-ciocalteu reagent and 2 ml of 7.5% sodium carbonate. The final mixture was diluted to 7 ml with deionized water. Mixtures were kept in dark at ambient conditions for 2 hrs to complete the reaction and absorbance was measured at 767 nm on a Perkin – Elmer Lambda-2 spectrophotometer with a 1 cm cell. Gallic acid was used as standard and results were calculated as Gallic acid equivalents (100 g of shell). The experiments and the values reported are the average of two replicates of total phenolic contents. Gallic acid was used as the standard for a calibration curve and the phenolic content was reported as Gallic acid equivalents.

Determination of elements

Atomic absorption spectrophotometer and UV technique was used for the determination of elements. For this purpose, samples were digested with nitric acid and 30% hydrogen peroxide solution. Appropriate working standard solution of Calcium (Ca), Cadmium (Cd), Chromium (Cr), Copper (Cu), Iron (Fe), Potassium (K) Magnesium (Mg), Manganese (Mn), Nickel (Ni), Zinc (Zn) and Cobalt (Co) were prepared from stock standard solution (1000 ppm) in 2N nitric acid. Calibration curves were drawn for each element using atomic absorption spectrophotometer (Hitachi model 180-50®).

Collection of dermatophytes

The samples were collected by scraping *hair scalp* and all fungal cultures were observed for (MIC) using strip method. Patients were observed clinically at skin department Liaquat University of Medical and Health Sciences, Hyderabad.

Preparation of culture for dermatophytes

Sabouraud's glucose agar was used to culture the fungi. Following composition was used for this purpose: peptone 10 g, glucose 20 g, agar 20 g and distilled water 1000 ml with pH 5.4. All the contents were mixed and dissolved in distilled water. The solution was then autoclaved at 120°C, 15 lb/inch² pressure for 20 minutes.

Antimycotic screening

The antifungal activity of *Prunus dulcis* (almond) shell extract was individually observed against dermal mycosis namely *Aspergillus niger*, *Microsporum canis*, *Microsporum gypsum*, *Aspergillus flavus* by strip method. The effect of shell extract on dermal mycosis was detected by measuring the diameter of colony obtained after incubation on treated plates and were compared with control. Each experiment was done in triplicates and average reading was recorded. Extract treated fungal plates were incubated at 37°C for 72 hours for fungi.

Case Report

05 year old patient was suffering from *Tinea capitis* (diagnosed clinically) 6 months before starting the treatment patient suffered with different sign and symptoms such as itching, radish coloration of skin and border inflammation (Figures 2-7).



Figure 2: Showing colony on sabouraud agar x 40. *M.canis*.



Figure 3: Showing colony on sabouraud agar x 40. *M.gypsum*.

Result and Discussion

The modified Folin Ciocalteu reagent was used for the determination of total phenolic compounds and final result are expressed as Gallic acid eq. A Gallic acid standardization curvature ranging from 0.002 to 0.008 atomic number 12\gm was prepared and the results determined from the regression equation of the calibration curve($y=1786x-0.0047$, curve($y=1786x-0.0047$, $R=0.008$ were expressed as mg Gallic Acid Eq (GAE). In order to determine total phenolic content of hard shell *Prunus dulcis* 4.455 mg/g was dissolved in methanol. The phenolic compounds may contribute directly to antioxidative action [13]. The significant difference has been found and reported for TPC among the verities in the range of 2.51-3.5 mg\g for bran selection [14]. Deshpande [15] has determined differences in TPC extract in various solvents with highest antioxidant potential for the n-butanol extract of almond skin while the lowest antioxidant activity for methanol extract. 100% radical scavenging activity with assay method 2-2-diphenyl-1-picrylhydrazal (DPPH) was observed for shell selection at 100 and 200 ppm concentration respectively. Amount resolution was seen at variation of time and results of these assays showed highest economic value at the time 30 (min) 94.40 whereas maximum divergence among the extract was observed at 0.5, 1.0 and fifteen min with the values 94.38 to 95.49 Graph 1. Similarly [16] observed the scavenging activity of extracts of almond skin and its by-product ranged from 67 to 97% DPPH. Highest values 90.40% were observed at 30 minutes whereas maximum difference among the seed extract was observed at 0.5, 1.0 and 15 minutes with the values 91.67, 92.00 and 93.32% respectively [17].

Radical scavenging burden of selection was analyzed using the bleaching of the stalls 1,1-diphenyl-deuce-picrylhydrazyl radical (DPPH) and found antioxidant natural process of ethyl alcohol extract from ground and roasted hazelnut and sugariness sweet almond pale yellow microbe [18]. Pinelo [19] evaluated the antioxidant potential from almond hull at the 85.20%. The methanolic extract of the plant showed a strong dose-response antioxidant activity. Phenolic constituents had showed strong antioxidant activity against DPPH radical by spectrophotometric method whereas, Pinelo [19] detected

S. No.	Microorganism	Inhibition zone diameter (mm)						
		Concentration of Shell extract By Strip Method						
		10 µl	30 µl	50 µl	70 µl	80 µl	100 µl	Standard Myconazole
1	<i>Aspergillus niger</i>	0.320	0.612	0.85	1.17	1.127	2.132	2.44
2	<i>Aspergillus flavus</i>	0.84	1.122	1.14	1.71	1.89	1.80	2.3
3	<i>Microsporum Canis</i>	0.245	0.524	0.749	1.141	1.172	2.630	2.6
4	<i>Microsporum gypsum</i>	0.315	1.056	1.442	1.585	1.836	2.590	2.4

Table 1: Antifungal activity of (Almond) shell extract of *P.dulcis*

S/No.	Name Of Elements	Formula	Amount mg/ Kg
1.	Calcium	Ca	11.75 ± 25.56
2.	Cadmium	Cd	0.938 ± 0.002
3.	Chromium	Cr	293.5 ± 32.45
4.	Copper	Cu	21.6 ± 0.05
5.	Iron	Fe	308.6 ± 21.6
6.	Potassium	K	705.2 ± 20.4
7.	Magnesium	Mg	264.1 ± 0.04
8.	Manganese	Mn	21.2 ± 0.06
9.	Nickel	Ni	6.22 ± 0.35
10.	Zinc	Zn	26.9 ± 0.25
11.	Cobalt	Co	6.33 ± 0.02

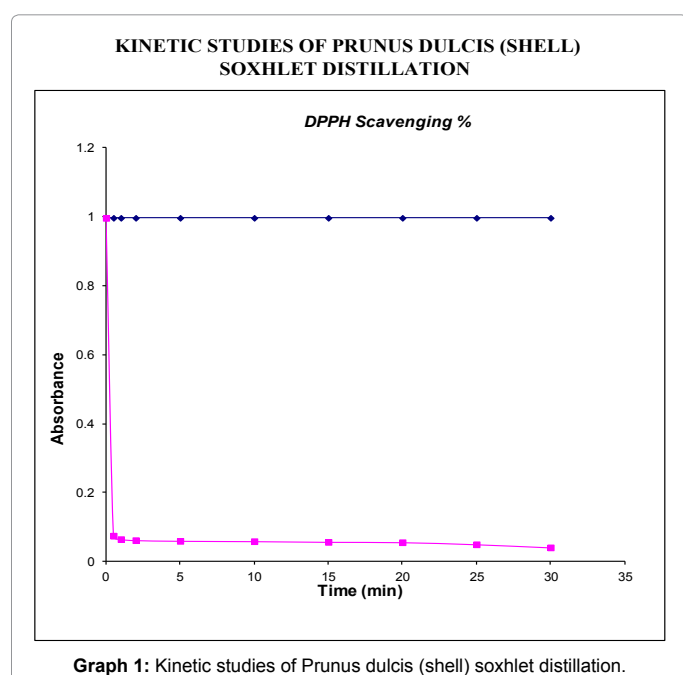
Table 2: Elementology of *Prunus dulcis* (Almond).

total phenolic contents of the extract of pine sawdust and almond hulls but despite this, phenols from almond hulls showed a higher antioxidant capacitance (58 vs 34%) of inhibition. This antioxidant activity has been thoroughly studied and a wide variety of methods have been developed to evaluate it. From the study, it can be concluded first time that *P. dulcis* shell has a higher level of antioxidant potential.

We determined trace elements from *P. dulcis* shell which are namely: Calcium 11.75 mg/kg, Cadmium 0.938 mg/kg, Chromium 293.5 mg/kg, Copper 21.6 mg/kg, Iron 308.6 mg/kg, Potassium 705.2 mg/kg, Magnesium 264.1 mg/kg, Manganese 21.2 mg/kg, Nickel 6.22 mg/kg, Zinc 26.9 mg/kg, Cobalt 6.33 mg/kg Table 2. Fungal infections remain a leading cause of chronic skin disease. Plants and animals depend on some metals as micro food. Metal 7 senses of element such as Na, K, Ca, Mg, Fe, Cu, Zn and Mn, are necessary nutrients for human growth and some metals keep high level of exposure to some toxic metals, especially cadmium, chromium, atomic number 27, nickel and lead [20]. The detection of these trace metals in the vegetable oil color and seed requires specific analytical procedures such as emission and atomic absorption spectrophotometric proficiency as well as electroanalytical techniques [21]. The high chromium value of the shell and low level of anti-cadmium indicates its potentials usefulness in fungal infection, whereas nickel value 6.22 showed suppression of fungal mycelium so it can be responsible for the control of *Tinea capitis* infection.

Antimycotic assays

Antifungal assays are generally performed by strip method. This method is qualitative test indicating resistance of micro organisms to the test material for fungicidal activity of *P.dulcis* shell antioxidant extract. The inhibitory effect of shell extract on the growth of dermal mycosis presented in Table 1. The antifungal activity of the *P.dulcis* shell extract was tested against 4 pathogenic fungi *Aspergillus niger*, *Aspergillus flavus*, *M.canis*, *M.gypsum* used in this experiment for



inhibitory growth of extract. At concentration of 100, 70, 80, $\mu\text{l}/\text{well}$, the extract has shown a remarkable antimycotic activities against the tested fungal species showed highest values of zones of inhibition between the ranges of 1.172-2.630 for *M. canis*, similarly *M.gypsum* inhibited at highest values between the ranges of 1.836-2.590. Different phenolic compounds of almond extract showed similar activity against *trichophyton spp.* being the most sensitive strain to these pure compounds. Naringenin showed the greatest inhibitory effect in the range of 250-500 $\mu\text{g ml}^{-1}$. Gulluce observed the antimicrobial inhibitory effect of *C. krusi* from bacterial strains showed maximal inhibition zones. Antifungal effect showed positive healthy inhibitory effect on pathogenic fungi sensitive to shell extract were 1.75 mm. Similar results were reported that *A. niger* showed lowest inhibition from 10 micro liter to 50 whereas it was suppressed at highest concentration 2.132. Inhibition of *A. flavus* was showed 1.80 with the high antioxidant potential.

Table 1 shows inhibitory effect on dermal mycosis with the therapeutic potential of *P. dulcis* shell extract were highly effective against all pathogenic fungi tested with the MIC ranging from initial 0.320 mm to final 2.590 mm. The zone of inhibition increased on increasing the concentration of extract *P. dulcis* whereas lowest concentration also showed inhibitory effect at 10 μl , 30 μl and 70 μl . The efficacy of shell extracts was comparable with standard antimycotic drugs. Among different fungi tested *M. canis* and *M. gypsum* were found to be more sensitive to shell extract and present work has shown that Hard shell extract of almond was found to be most effective as an antifungal and antibacterial agent against human pathogenic fungi and results provide a therapeutic potential for microbial infections no doubt which can be used in assisting primary health care in Pakistan. The main object of this study is to provide scientific valid information by developing and



Figure 4: After Treatment; Inhibitory zones showing therapeutic effect on *M.canis*.



Figure 5: After Treatment; Inhibitory zones showing therapeutic effect on *M.gypsum*.



Figure 6: After Treatment; Inhibitory zones showing therapeutic effect on *A.niger*.

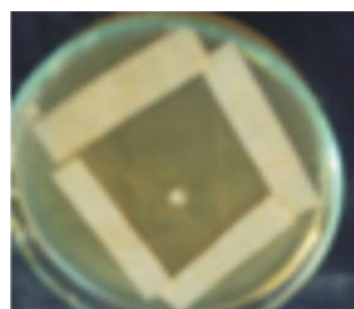


Figure 7: After Treatment; Inhibitory zones showing therapeutic effect on *A.flavus* MIC was recorded by strip method.

validating therapeutic and preventive approaches using the scientific method for all undeveloped hot and humid countries.

Treatment

The patient contacts a private dermatologist and received an antifungal compounds but no affect was noticed even after completing the course. Figure 8a- 8c showed the *Tinea capitis* infection of the patient with mycological confirmation due to presence of fungal spores. Shell extract (about 1gm) of the *P.dulcis* was applied in the form of 03 drops thrice a day on the affected area of the scalp. The patient baby showed recovery with complete removal of infected sign and symptoms of scalp. Reduction of itching, radish and inflammation of affected region showed potential inhibitory activity against *M. canis* and *M. gypsum*. Patient was completely recovered with removal of infection after 3 weeks without any side effects (Figures 8d). Patient has completely recovered with hair growth after a month of starting the treatment. Ring worm infection caused by dermatophytes is chronic with low recovery rate. Dermal mycosis is long term treatment and expensive even after disappearance of the fungal spores may germinate again and cause relapses [22]. According to the information collected from the patient she may suffer with insufficient healthy environment and malnutrition which would leads to the reappearance of the *Tinea capitis* infection. Hot and humid climate play a vital role in spreading the infection in Sindh, Pakistan. Successful treatment of *Tinea capitis* was observed by using *P. dulcis* and this indicates good affective antifungal activity of this cheapest waste material. Patient showed rapid recovery rate when the shell extract was applied thrice a day for three weeks, this result suggests the appropriate way of treatment (thrice/day for at least one month). We recommend the shell extract for patient's treatment with no side effects and no reoccurrence in future.

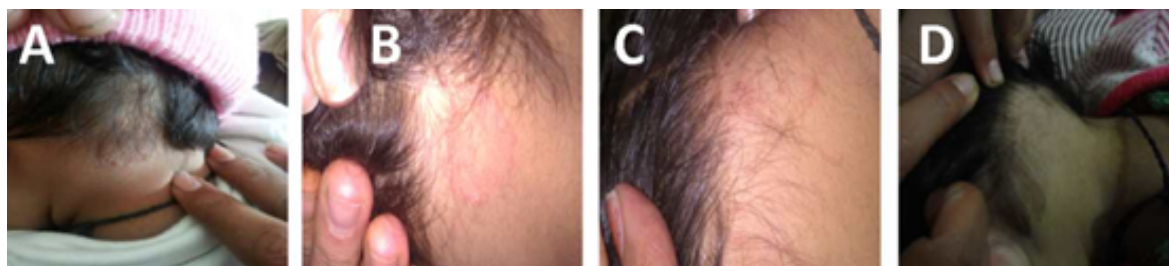


Figure 8a-8d: Clinically diagnosis *Tinea capitis* infection.

Conclusion

In conclusion, the data and results obtained during this study indicates that outer shell of *P.dulcis* contain highest values of antioxidant potential due to presence of phenolic content in good quantity, therefore the use of shell extract of almond as a antimycotic drug may reduce the risk of common environmental microbial infections particularly Ring worm or *Tinea* infections. The level of antifungal activities and antioxidant activities observed that the cheapest and discarded shell is a potential source of bioactive compounds and could be used in antimycotic drugs formulation. Finally suggests that *this waste material* is highly effective and safe antifungal drug and no doubt this shell extract of *P.dulcis* provide a therapeutic potential for microbial infections.

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