Medicinal & Aromatic Plants
ISSN: 2167-0412

Thebo et al., Med Aromat Plants 2012, 1:6
DOI: 10.4172/2167-0412.1000108

Research Article Open Access

Therapeutic and Antioxidant Potential in the Shell Extract of *Prunus amygdalus* against Dermal Mycosis

Nasreen Thebo[1]*, Wazir Sheikh[1], M. Iqbal Bhangar[2], Pervaiz Iqbal[3], M. Hassan Nizamani[4]

[1]*Mycology Research laboratory Institute of Botany, University of Sindh, Jamshoro, Sindh, Pakistan*
[2]*Free Radical Research Laboratory, National Centre of Excellence in Analytical Chemistry, University of Sindh, Jamshoro, Pakistan*
[3]*University medical health and Sciences, Skin Unit 11 Liaqat, Jamshoro, Pakistan*
[4]*Al-Mafraq Hospital, Dubai*

Abstract

Mycosis is a common harmful problem for health throughout the world. Ringworm infection is one of the most dermatomycoses caused by species of pathogenic fungi such as Epidermatophyton, Trichophyton and Microsporum. The potential of biotechnology is to provide health protection through medicinal plants because it also facilitates the earlier detection of various ailments by new biotechnological techniques. Almond posses a variety of benefical properties affecting most notably cancer, high blood pressure, infectious disease and diabetes. In our study, we have used first time Almond hard shell extract for the evolution of antioxidant, antimicrobial potential and therapeutic efficacy against dermatomycosis pathogenic fungi. The extract was prepared by soxhlet method and TPC in the extract was determined by spectrophotometerically. The antioxidant activity of crude extract of almond shell was evaluated by using DPPH (2-2-diphenyl-1-picryhydrazyl) and radical scavenging system. The Minimum Inhibition Concentration (MIC) of extract was determined by well method against isolated pathogenic causative organisms for confirmation. The possibility of short term therapy was only 15 days. The total antioxidant activity varied from 93.32 to 94.40% and total phenolic content was found 3.422 mg/gm in almond shell extract. Hard shell extract of almond was found to be most effective as an antifungal and antibacterial agent against human pathogenic. Finally the results provide a therapeutic potential for microbial infections.

Keywords: Almond; Antimicrobial activity; Antioxidant activity; UV spectrophotometer; Dermal therapy

Introduction

The Almond, (*Prunus amygdalus*), apparently originated from lower mountain slopes of central south western, Mediterranean region and Asia from which about 28% of the world production is obtained [1]. There are two main types of almond as bitter and sweet, which differ from each other in the presence of amygdalin [2]. Almonds (*Prunus amygdalus* Miller D.A. Webb) are a rich source of phytochemicals and nutrients such as monounsaturated fatty acids, vitamin E, and polyunsaturated fatty acids [3]. *Prunus* species keeps biological properties such as anti-inflammatory sedative, anti-hyperlipidemia, antitumor and antioxidant activities [2]. The natural antioxidants that scavenge reactive oxygen species are known to be of great value in preventing from oxidative stress and its related diseases [3]. Cyanogenic compound and Prunasin, is found only in the vegetative parts of the almond plant [4]. Research also proved that out of nine phenolic compounds in almonds exhibit strong antioxidant potential. Frison reported that Methyl quercetin, Protocatechuic acid, flavonoids, Kaempferol, P- tlydroxybenzoic acid, Vanillin acid, and glycoside net in photo catechetical have powerful effects on health problems as antioxidants [5]. Epidemiological studies have shown flavonoid intake is inversely related to mortality from coronary heart disease, colon cancer antiproliferative activities during an incident of heart attack. Other workers also reported that almond nuts are a source of, phytochemicals, proteins, unsaturated fats, minerals, micronutrients, alpha-tocopherol, and dietary fiber [7-10].

With the development of modern isolation, purification and identification techniques, many bioactive natural products have been isolated from a number of dry fruits [7]. The nutritional value of almond is related to its kernel. Other parts of fruit such as shells and hulls were used as fuel, live stock feed and as waste material.

The objective of the study focused on clinical and chemical analysis of shell extract of almond. The principle aim of this work involved study of the antimicrobial and antioxidant activity as well as therapeutic and clinical applications on some common pathogenic fungi and bacteria responsible for skin disease, urinary tract infection, pneumonia, food spoiling.

Material and Method

Materials

Almond (*Prunus amygdalus*) sample was collected at their optimum commercial maturity from the local market of Hyderabad. The Voucher specimen was deposited in herbarium of Institute of plant science. The identification of plant sample was confirmed by plant taxonomist, Prof. Dr. Tahir Rajput in the Institute of Plant Science, University of Sindh.

Methods

Preparation of the almond hard shell extract: The hard shell of almond powdered with the help of warring blender. 15 gm of dried powder was filled in thimble and macerated with 100 ml of analytical grade solvent methanol. Sampler 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 Whattman No. 1 filter paper to remove waste rough particles. The crude extract was stored at 18°C until analyzed.

***Corresponding author:** Dr. Nasreen Thebo, Mycology Research laboratory Institute of Plant Sciences, University of Sindh, Jamshoro, Sindh, Pakistan, Tel: 0923332723518; E-mail: nasreenthebo@gmail.com

Received March 08, 2011; **Accepted** July 12, 2012; **Published** July 16, 2012

Citation: Thebo N, Sheikh W, Bhangar MI, Iqbal P, Nizamani MH (2012) Therapeutic and Antioxidant Potential in the Shell Extract of *Prunus amygdalus* against Dermal Mycosis. Med Aromat Plants 1:108. doi:10.4172/2167-0412.1000108

Collection of dermatophytes: The pathogenic fungi and bacteria clinically isolated from different tinea infections of patients. These are namely bacterial strains *E. coli*, and *Staphylococcus aureus*, whereas fungal samples *Aspergillus niger*, *Candida albican, Trichophyton rubrum, Trichophyton mentagrophyte, Candida krusi* and Penicillium were examined. The crude extract was individually tested against eight microorganisms, two bacteria, five fungi and Penicillium (yeast). All fungal and bacterial cultures were tested for Minimum Inhibitory Concentration (MIC).

Antimicrobial screening: Sabouroud, the antimicrobial activity of almond shell extract was individually tested against bacteria namely *E. coli* and *Staphylococcus* in liquid nutrient media. The nutrient broth tubes contain antimicrobial tests were carried and by Micro well dilution method using 100 ml of suspension containing 10^8 CFU/m of bacteria, 10^6 CFU/m1 of yeast / and 10^4 spore/m1 of fungi spread on Nutrient Agar (NA), Sabourad Dextrose Agar (SDA) respectively. The wells (8 mm in diameter) were cut from the agar with a sterilized cork borer and different concentration of crude extract solution was delivered into them. The effect of shell extract on pathogenic fungi was determined by measuring the diameter of colony obtained after incubation on treated plates and comparing with control. Each experiment was done in triplicates and average reading was recorded. Extract treated fungal and bacterial plates were incubated at 37°C for 24 h for bacterial strains 48 h for yeast and 72 h for fungi.

Agar well dilution assays: In antimicrobial study, a considerable reduction in the growth of fungi and bacteria was observed when compared to control plates and is suggestive of antimicrobial effects of shell extract. For the Minimum Inhibition Concentration (MIC) total 192 plates were prepared for MIC values. The wells (6 mm in diameter) were then cut from agar and shell extract or drug was delivered to them. MIC of shell extract from stock solution concentration was carried out among the range of 10, 30, 50, 70, 80, and 100 Ug/ml. The results were read visually as recommended by the NCCL [11]. Minimum Inhibitory Concentration (MIC) values were determined for the fungal, yeast and bacterial strains which were sensitive to the crude extract of seed coat. The 192 well plates were prepared by dispensing into each well of nutrient and SDA of the inoculums to find the MIC of crude extract of seed coat of almond experiments were carried out by the different concentrations of crude extract from stock solution concentration range were 10, 30, 50, 70 80 and 100 µg/ml initially crude extract was first diluted to the highest condition (100 µg/mL) to be tested and then serial dilution was done in order to obtain a concentration range from 10 to 100 µg/m. Then 100 µg/m form this serial dilutions were transferred into six consecutive wells.

Estimation of antioxidant potential:

- **DPPH radical scavenging activity:** ***Antioxidant activity:*** Free radical scavenging capacity of almond shell extract was determined according to the previous reported procedure using the stable 2,2-diphenyl-1- 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) assay. The DPPH assay was carried out following the same method as reported elsewhere [5]. The antioxidant properties of phenolic compound in almond contain effective health benefits. Antioxidant may play an important role in the chronic disease prevention by arresting oxidative damage caused by Relative Oxygen Species (ROS) to vital bimolecular such as DNA, lipids and proteins [7]. The absorbance of the resulting solution was read spetrophotometrically at 517 nm). A freshly prepared solution of DPPH (5.0 ml) was added to 1.0 m1 of shell extracts. 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 antioxidant activity scavenging from radicals AA (%) was calculated as follows:

A(%) = Ac - As100

Where, Ac is control absorbance, As is sample absorbance.

The remaining concentration of DPPH in the reaction mixture was calculated from a standard calibration curve.

The antioxidant potentials observed in this study were much stronger than those of crude extract of almond seed in different fractions showed values of only 0.24 and 0.09 µml [12].

Determination of total phenolic content: The total phenolic compounds were extracted from shell of almond and identified by spectrophotometrically using the Folin-Ciocalteu method described previously [13,14]. The reaction mixture contains 200 µl diluted shell extract, 800 µl of freshly prepared diluted Folin-ciocalteu reagent and 2 m1 of 7.5% sodium carbonate. The final mixture was diluted to 7 ml with demonized water. Mixtures were kept in dark at ambient conditions for 2 hrs to complex the reaction. Then absorbance at 765 nm was measured 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 compounds was calculated for two different experiments, and the values reported are the average of two replicates. Gallic acid was used as the standard for a calibration curve. The phenolic compound content was reported as Gallic acid equivalents.

Result and Discussion

Total phenolic contents were determined by modified Folin Ciocalteu reagent was used for the determination of the total phenolic compounds and results are expressed as Gallic acid equivalents. A Gallic acid calibration curve ranging from 0.002 to 0.008 mg\gm was prepared and the results determined from the regression equation of the calibration curve(y=1786x-0.0047, R=0.008 were expressed as mg Gallic Acid Equivalents (GAE). In order to determined total phenolic content of hard shell almond was 3.422 mg/g in methanol shell extract of almond.

The phenolic compounds may contribute directly to antioxidative action [1]. Significant differences have been reported for TPC among the verities. Jasna et al. [15] has found TPC in the range of 2.51-3.5 mg\g for bran extracts. Moreover Sathe et al. [2] has determined differences in TPC extracted in various solvents with highest antioxidant activity for the n-butanol extract of almond skin T. while the lowest antioxidant activity for methanol extract.

Results of Radical scavenging activity DPPH.A 100% scavenging activities of the 2-2-diphenyl1-picrylhydrazal (DPPH) radical was observed for shell extract at 100 and 200 ppm concentration respectively. Total results were seen at variation of time. Results of these assays showed highest values at the time 30 (min) 94.40 whereas maximum difference among the extract was observed at 0.5, 1.0 and 15 min with the values 93.67, 93.00 and 94.32. Similarly Krings et al. [16] observed the scavenging activity of extracts of almond seed and its byproducts ranged from 67 to 97% DPPH. A 100% scavenging activity of the 2-2-diphenyl1-picrylhydrazal (DPPH) radical was observed

for seed coat extract at 100 and 200 ppm concentration. Total results were obtained at different variation of time. Highest values 94.40% were observed at 30 minutes whereas maximum difference among the extract was observed at 0.5, 1.0 and 15 minutes with the values 93.67, 93.00 and 94.32% respectively (Figure 2a and 2b).

Radical scavenging effects of extract was analyzed using the bleaching of the stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and found antioxidant activity of ethanol extract from Ground and roasted hazelnut and sweet almond wheat germ [16]. Miller et al. [14] evaluated the antioxidant potential from Almond hull at the 85.20% The methanolic extract of the plant showed a strong dose-response antioxidant activity [17]. Phenolic constituents had showed strong antioxidant activity against DPPH radical by spectrophotometric method [15], whereas, Milbury [18] detected total phenolic contents of the extract of pine sawdust and almond hulls but, despite this, phenols from almond hulls showed a higher antioxidant capacity (58 vs 34%) of inhibition. This antioxidant activity has been thoroughly studied and a wide variety of methods have been developed to evaluate it.

Reducing/antioxidant power (FRAP) 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay for the evaluation of reducing power (P_R) and correlate them with the total phenolic content and found variation of antioxidant activity due to different methods.

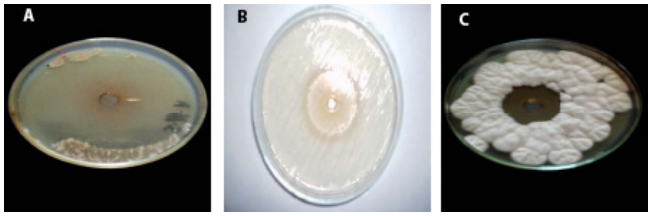

Figure 1: Inhibitory zones showing therapeutic effect on pathogenic organisms A) 99% inhibition of *Candida albican* in shell extract which is the highest activity among all fungal species. B) Second highest inhibition against *S. aureas*. C) Third moderate inhibition showed on penicillium by well diffusion method.

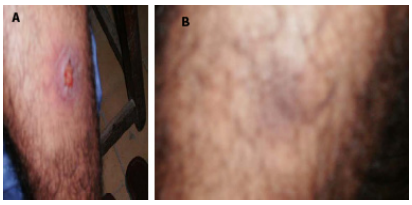

Figure 2. Radical scavenging activity of almond shell extract of almond. A) Infected region of patient before shell extract treatment. (B) Photograph showing clinically efficacy of shell extract on the same infection *Tinea corporus* after two time applications.

Anti microbial activity test

In vitro antibacterial and antifungal screening is generally performed by well dilution method. This method is qualitative test indicating sensitivity or resistance of micro organisms to the test material as well as becteriostatic or bactericidal and fungistatic or fungicidal activity of an almond hard shell antioxidant extract. The inhibitory effect of seed coat extract on the growth of test bacteria and fungi presented in Table 1. The antifungal activity of the seed coat extract were tested against 5 pathogenic fungi *Aspergillus niger, Aspergillus flavus, Candida albican, Penicillum, Fusarium* and *Trichophyton rubrum*. *Staphycocus aureus* and *E. coli* were used in this experiment for inhibitory growth of crude extract. At concentration of 100, 70, 80, µl/well, the extract has shown a remarkable antibacterial activities against the tested gram positive and gram negative bacteria. *E.coli* and *Candida albicans* showed highest values of zones of inhibition between the ranges of 2.12 to 2.145. Different phenolic compounds of almond skin showed similar activity against *S. aureus* being the most sensitive strain to these pure compounds. Naringenin showed the greatest inhibitory effect in the range of 250–500 µg ml^{-1}. Epicatechin and protocatechuic acid were the next most effective compounds, whereas isorhamnetin-3-O-glucoside catechin is only active against *L. monocytogenes* and *S. enterica* (Figure 1a, 1b and 1c).

Gulluce [17] observed the antimicrobial inhibitory effect of *Sautureja hortensis* oil from bacterial strains showed maximal inhibition zones. Antifungal activity showed positive healthy inhibitory effect on pathogenic fungi. Penicillium sensitive to shell extract were 1.75 mm. similar results were reported that, *C. albicans, C. krusei, M. smegmatis, S. pneumonia* and *C. perfringens* showed inhibitory effect between the range of 2.25 to 18 mg/ml methanol extracts of almond hull [2].

Table 1 shows crude shell extract were highly effective against both bacterial strains tested with the MIC ranging from 1.42 mm to 2.145 mm. The zone of inhibition increased on increasing the concentration of extract in well whereas lowest concentration also showed inhibitory effect at 10 µl, 30 µl and 70 µl. The efficacy of plant extracts was comparable with standard antibiotics. Among different fungi tested *Candida albicans* and *Fusarium* species were found to be more sensitive to the shell extract whereas Penicillin, *Aspergillus* and *Actinomycetes* also showed positive inhibitory effect at the values of the present work has shown that shell extract of almond is potentially a good source of antimicrobial agents which can be used in assisting primary health care in Pakistan.

Those bacterial strains showed maximal inhibition zones. Antifungal activity showed positive healthy inhibitory effect on pathogenic fungi.

Inhibition zone diameter (mm)								
S. No.	Microorganism	Concentration of Shell extract By Well's Method						
		10 µl	30 µl	50 µl	70 µl	80 µl	100 µl	Standard Itraconazole
1	*Escherichia coli* (Gram-negative)	0.325	0.625	0.75	1.175	1.190	2.145	2.22
2	*Staphylococcus aureus* (Gram-positive)	0.80	1.125	1.325	1.35	1.39	1.42	2.4-
3	*Aspergillus niger*	0.275	0.575	0.95	1.175	1.195	1.229	2.9
4	*Penicillium*	0.325	1.075	1.425	1.525	1.634	1.745	2.8
5	*Candida albicans*	0.345	2.15	1.734	1.974	2.012	2.146	2.7
6	*Candida krusi*	0.290	2.35	1.402	1.654	1.754	1.954	2.6
7	*Trichophyton mentagrophyte*	0.54	1.135	1.378	1.424	1.452	1.747	2.7
8	*Trichophyton rubrum*	0.395	1.125	1.295	1.348	1.487	1.854	2.5

Table 1: Antibacterial and antifungal activity of (Shell) extract of almond.

Penicillium sensitive to shell extract were 1.75 mm. Similar results were reported that, *C. albicans, C. krusei, M. smegmatis, A. lwoffii, S. pneumonia* and *C. perfringens* showed inhibitory effect between the range of 2.25 to 18 mg/ml methanol extracts of *Salvia cryptantha* [2].

Table 1 shows crude shell extract were highly effective against both bacterial strains tested with the MIC ranging from 1.42 mm to 2.145 mm. The zone of inhibition increased on increasing the concentration of extract in well whereas lowest concentration also showed inhibitory effect at the 10 µl, 30 µl and 70 µl. The efficacy of plant extracts was comparable with standard antibiotics. Among different fungi tested *Candida albicans* and *Candida krusei* species were found to be more sensitive to the shell extract whereas Penicillin, *Aspergillus* and *Trichophyton* species also showed positive inhibitory effect at the values of the present work has shown that shell extract of almond. Reoccurrences of the infection are frequent; especially tinea infections and their treatment can be quite serious. This shell extract has been proved antibiotic and antimycotic resistance as compared to pharmaceuticals. Almond shell extract remedies may tend to have no deleterious side effects than corresponding herbal remedies may tend to have less deleterious side effects than corresponding pharmaceuticals. After treatment on patients clinically sign symptoms has been completely removed till fifteen days therapy.

Almond is potentially a good source of antimicrobial agents which can be used in assisting primary health care in Pakistan.

Conclusion

In conclusion, the data and results obtained in this study demonstrated that hard shell of almond contain highest values of antioxidant activity due to presence of phenolic content in good quantity therefore the use of shell extract of almond as a nutraceuticals may reduce the risk of common environmental microbial infections, particularly in the, skin disease, cancer; intestinal and respiratory action provided by its phenolic compounds. The use of extracts is recommended to achieve health benefits and further works on the isolation of bioactive components and purification of individual compounds can reveal exact potency of seed shell of almond plant recommended to achieve health benefits due tract, mainly due to the protective shell of almond.

References

1. Awika JM, Rooney LW, Wu X, Prior RL, Cisneros-Zevallos L (2003) Screening methods to measure antioxidant activity of sorghum (*Sorghum bicolor*) and sorghum products. J Agric Food Chem 51: 6657-6662.
2. Sathe SK, Wolf WJ, Roux KH, Teuber SS, Venkatachalam M, et al. (2002) Biochemical characterization of amandin, the major storage protein in almond (*Prunus dulcis* L.). J Agric Food Chem 50: 4333-4341.
3. Tepe B, Donmeza E, Unlu M, Candan F, Daferera D, et al. (2004) Antimicrobial and antioxidative activities of the essential oils and methanol extracts of *Salvia cryptantha* (Montbret et Aucher ex Benth.) and *Salvia multicaulis* (Vahl). Food Chem 84: 519-525.
4. Stévigny C, Rolle L, Valentini N, Zeppa G (2007) Optimization of extraction of phenolic content from hazelnut shell using response surface methodology. J Sci Food Agric 87: 2817 – 2822.
5. Challice JS, Westwood MN (1972) Phenolic compounds of the genus *Pyrus*. Phytochemistry 11: 37-44.
6. Cosentino S, Tuberoso CI, Pisano B, Satta M, Mascia V, et al. (1999) In vitro antimicrobial activity and chemical composition of Sardinian Thymus essential oils. Lett Appl Microbiol 29: 130-135.
7. Sathe SK, Wolf WJ, Roux KH, Teuber SS, Venkatachalam M, et al. (2002) Biochemical characterization of amandin, the major storage protein in almond (*Prunus dulcis* L.). J Agric Food Chem 50: 4333-4341.
8. Lovejoy JC, Most MM, Lefevre M, Greenway FL, Rood JC (2002) Effect of diets enriched in almonds on insulin action and serum lipids in adults with normal glucose tolerance or type 2 diabetes. Am J Clin Nutr 76: 1000-1006.
9. Spiller GA, Jenkins DA, Bosello O, Gates JE, Cragen LN, et al. (1998) Nuts and plasma lipids: an almond-based diet lowers LDL-C while preserving HDL-C. J Am Coll Nutr 17: 285–290.
10. Gulluce M, Sokmen M, Daferera D, Ağar G, Ozkan H, et al. (2003) In vitro antibacterial, antifungal, and antioxidant activities of the essential oil and methanol extracts of herbal parts and callus cultures of *Satureja hortensis* L. J Agric Food Chem 51: 3958-3965.
11. NCCLS (National Committee for Clinical Laboratory Standards) (1999) Performance standards for antimicrobial susceptibility testing. Ninth International Supplement M100-S9.
12. Amarowicz R, Troszyńska A, Shahidi F (2005) Antioxidant activity of almond seed extract and its fractions. J Food Lipids 12: 344-358.
13. McDonald S, Prenzler PD, Antolovich M, Robards K (2001) Phenolic content and antioxidant activity of olive extracts. Food Chem 73: 73-84.
14. Miller NJ, Rice-Evans CA (1997) The relative contributions of ascorbic acid and phenolic antioxidants to the total antioxidant activity of orange and apple juices and blackcurrant drink. Food Chem 60: 331-337.
15. Čanadanović-Brunet JM, Djilas SM, Ćetković GS, Tumbas VT, Mandić AI, et al. (2006) Antioxidant activities of different *Teucrium montanum* L. Extracts. Int J Food Sci Tech 41: 667–673.
16. Krings U, Berger RG (2000) Antioxidant activity of some roasted foods. Food Chem 72: 223-229.
17. Pacifico S, D'Abrosca B, Golino A, Mastellone C, Piccolella S, et al. (2007) Antioxidant evaluation of polyhydroxylated nerolidols from redroot pigweed (*Amaranthus retroflexus*) leaves. LWT - Food Sci Tech 41: 1665-1671.
18. Milbury PE, Chen CY, Dolnikowski GG, Blumberg JB (2006) Determination of flavonoids and phenolics and their distribution in almonds. J Agric Food Chem 54: 5027-5033.