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COMPARATIVE IN-VITRO ANTIOXIDANT AND ANTI-DERMATOPHYTES ACTIVITIES OF *MYRTUS COMMUNIS* LEAVES, FLOWER AND STEM BARK EXTRACTS

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Abstract

The current study aim was to find out the antioxidant and antifungal potential of leaf, flower and stem bark extracts (methanol, ethyl acetate, ethanol, aqueous and acetone) of Myrtus communis. The antioxidant activity was measured by standard In vitro antioxidant test systems i.e. 1, 1'-diphynyl- 2-picrylhydrazyl (DPPH) method using ascorbic acid as standard. Agar well diffusion method was used to check the antifungal activities. Methanol and ethyl acetate extract at 250 µg/mL observed antioxidant activity was 90±2% and 85±1% respectively, while vitamin C showed 88±02% scavenging activity. The result investigated that the inhibition effect of plant leaves at 100 mg/ml give more prominent zone of inhibition as compared to stem and flower extracts.

Keywords: *Myrtus communis, scavenging activity, skin disease, antifungal activity, aqueous extracts, organic extracts.*

Introduction

Reactive oxygen species (ROS) excessive synthesis disturb the scavenging protection system of the tissue have been shown to oxidize the biochemical compounds and stimulate injury to DNA, carbohydrate, proteins and cell membrane. Many pathological conditions such as heart attack, diabetes, hypercholesterolemia and hypertension are caused due to this oxidative stress [1]. Antioxidant agents defend man from destructive outcome of ROS and DNA/protein damages [2].

Herbs produce different types of anti-oxidant agents as phenolic substances; they prevent oxidative stress in plants. When these plants are used as food by humans, they defend humans from ailments [3]. To prevent the ROS damage by the use of artificial antioxidant molecules have raised the questions many side effects like carcinogenicity. These are the reasons to utilize the herbal antioxidants with strong ability to inhibit lipid per oxidation and scavenge free radicals [4].

Many skin ailments like tinea and ringworm are caused by dermatophyte which lives in hot and semi hot places of the world. These microbes dwell in the lifeless, upper layer of dermis tissues in moist locality, like under the breast, toes and between the groins. These fungi can go into the tissues and originate scaling, blistering and swelling [5]. Skin is considered the most flexible and strong part of the body that provide border to the external environment. Its chief role is to stop the attack of viruses and bacteria and as a result to sustain inner homeostasis [6].

Myrtus communis Linn (M. communis) belongs to Myrtaceae family, which grows instinctively all over the Mediterranean region. Its flowers, berries and leaves have been utilized for its therapeutic properties and as tonic [7]. The extracts and essential oil of M. communis have been utilized for thousands of years in pharmaceutical, alternative medicine, food preservation, food products, cosmetics and natural therapies [8].

The medicines used against skin infections exhibit limited efficacy and have side effects. As a result, there is a definite need for the invention of novel safer and more efficient antifungal drugs [9]. In the world utilization of plants for treating skin ailments is considered an aged medicine [10]. Medicinal plants utilization in folk medicine could assist to defeat the arising issues of antifungal drugs such as resistance, side effects and relative toxicity [9].

One of the main worries in healthcare nowadays is the non effectiveness of antibiotics. Therefore it is necessary to discover new antimicrobial agents. Keeping in view the above problems, current study was carried out to determine antioxidant and antifungal activity of M. communis leaf, flower and stem bark extracts.

Methods

Plant parts Collection

The healthy, fully matured leaves, flowers and stem bark of M. communis were collected from Medicinal Botanical Garden of Pakistan Council of Scientific and Industrial Research (PCSIR) Peshawar-Pakistan. These plant parts were washed with clean water to eliminate dust, then shade dried and ground into powder with the help of grinder. The ground materials were stored in a sterilized glass bottle for further utilization.

Extraction Process

Stem bark, leaves, and flower powder 50 grams were taken and soaked separately in 500 mL acetone, ethyl acetate, ethanol and methanol for forty eight hours. Each soaking material was poured turn by turn into 01 L flask and vacuum filtered through Whattman No. 1 filter paper in a Butchner flask. This practice was repeated 3 times for each solvent extract. For water extract 50 g material was soaked in 500 mL boiling water for five minutes. Concentrations of the soaking material were carried out under reduced pressure at temperature below 50°C in rotary evaporator (Buchii Japan) until the condensation of the solvent stopped dropping. The extracts were poured in a sterilized glass vial and kept on water bath at 50°C to obtain complete dried extract. The extracts were stored in clean glass vials at low temperature in cold incubator at 20 °C till further use.

Scavenging procedure of DPPH

Different concentrations of each extract/standard (50 – 250 µg/mL) were prepared in (95%) methanol. Mixed this solution with one milliliter of 0.004%

(DPPH) solution prepared in methanol (95%) Positive control and blank used were vitamin C and methanol (95%) and DPPH in 1:1 mL ratio respectively.

These reactants were reserved for twenty minutes and at 517 nm absorbance were taken with the help of UV Spectrophotometer (U-2900 Hitachi Tokyo Japan). The inhibition potential (I %) was calculated by applying the equation given as below (11).

$$\text{Antioxidant Activity \%} = \frac{(A0 - A1)}{A0} \times 100$$

A0 = Control Absorbance, A1= Extract absorbance

Tested Microorganism

The tested fungi (shown in Table 2) were procured from Environmental Research Section of PCSIR Laboratories Complex Peshawar, Khyber Pakhtunkhwa-Pakistan. The microorganisms were preserving on slant of Sabouraud Dextrose Agar (SDA) and kept in refrigerator and subcultures after every week.

Antifungal activity

On the surface of SDA plates, 1 mL of spore suspension (105spore/mL) was seeded. Well was made by the help of a six millimeter diameter sterile cock borer in a center of each cultured plate. Five hundred milligram each extract was dissolved in five milliliter dimethyl sulfoxide (100 mg/mL) and from this mixture fifty microliters extract was poured in the well.

For positive control Fluconazole was used and dimethyl sulfoxide (DMSO) used as negative control. The experimental plates were kept at ambient temperature for one to seven days in an incubator and diameters of the zones of inhibition were measured [12].

Statistical Analysis

Computer program SPSS version 14 was applied for statistical analyses.

Results

The DPPH scavenging activity of M. communis leaves, flower and stem bark extracts are shown in table 1. The DPPH activities of extracts were found to increase in a dose dependent manner.

Antifungal potential of M. communis leaves; flower and stem bark extracts against common fungi related to skin diseases is shown in table 2. This extract shows the sensitivity pattern of decreasing activity from methanol to acetone as well as from leaves to stem bark. Methanolic extract of leaves has the highest activity in all the studied fungi followed by flower and stem extract. The extracts activity increases in the order methanol>ethyl acetate>ethanol>aqueous>acetone, while the plants parts biologic activity showed increase in the sequence as, leaves>flowers>stem. According to the results obtained in the current findings, the myrtle extracts possess a good antioxidant and antifungal activity. Among the extracts, methanol and ethyl acetate have high activities. These findings confirmed the potential of this plant as a valuable source of natural bioactive molecules in pharmaceutical and nutraceutical industry. This plant has great potential of offering affordable, economical and acceptable medicines obtained from herbal sources for developing countries like Pakistan.

Discussion

Currently skin diseases are our serious health problems. These problems are rising due to fungi resistance of the anti-fungal medicines and side effects. In many parts of the world use of herbal medicine for treating skin diseases is an old phenomenon.[13] Herbs contain essential oils, alkaloids, glycosides, tannins, flavonoids, phenols and triterpenoids which have documented antibiotic potentials [13]. Phenols, tannins, flavonoids and terpenoids were present in the methanol extract of M. communis [14]. Methanol and acetone leaves extract of M. communis was found to have quercetin (2,289 mg/g) and gallic acid (2,424 mg/g) [15]. Myrtle leaves ethyl acetate extract have flavonoid (130.75 mg/g) and phenolic (435.37 mg/g) content in terms of gallic acid and quercetin respectively [1]. Ethyl acetate extract of M. communis leaves had the best antifungal activity

(mg/mL) against dermatophytes in the range from 0.187 to 0.375 [16]. M. communis leaves contain numerous polyphenolic compounds and due the presence of these polyphenolic compounds they have antimicrobial properties [17]. Phenolic compounds attack the cell membranes and cell walls, in turn affecting their porosity and discharge of intracellular components, as well as disturbing and interacting with membrane func¬tions such as nutrients uptake, enzyme effect and electron transport. Therefore, phenolic constituents might have numerous attacking points which ultimately stop microbial growth [18]. The antimicrobial activities of M. communis leaves were due to the phenolic compounds [19].

References

1. Amel B, Seddik K, Mussa AZ, Shtaywy A, Abderahmane B, Noureddine C. 2015. Phytochemical analysis, hypotensive effect and antioxidant properties of Myrtus communis L. growing in Algeria. Asian Pac J Trop Biomed 5(1): 19-28.

2. Javid A and Bashir A. 2015. Evaluation of antioxidant activities of Hippophae rhamnoides Linn leaves extracts. Journal of Coastal Life Medicine. 3(5): 387-389.

3. Niciforovic N, Mikhailovic V, Maskovic P, Solujic S, Stojkovic A, Pavlovic MD. 2010. Antioxidant activity of selected plant species; potential new sources of natural antioxidants. Food Chem Toxicol. 48: 3125-3130.

4. Gonçalves S, Gomes D, Costa P, Romano A. 2013. The phenolic content and antioxidant activity of infusions from Mediterranean medicinal plants. Ind Crops Prod. 43: 465-471.

5. Ping HC, Chi WL, Jia YC, Murugan M, Bor JS, Hueih MC. 2007. Anti-fungal activity of crude extracts and essential oil of Moringa oleifera Lam. Bioresource Technology. 98: 232–236.

6. Harshit V, Mandeep S, Rajesh C, Akanksha P. 2013. Assessment of antimycotic activity of seabuckthorn (Hippophae rhamnoides) leaf exacts against common fungi associated with skin dermatitis. Vet World. 6(4): 205-208.

7. Sara C., Paola M., Melania R., Marina C., Maurizio S., Bruno M., Stefania Z. 2013. Antimycotic activity of Myrtus communis L. towards Candida spp. from clinical isolates. J Infect Dev Ctries. 7(3):295-298.

8. Vahid R, Sharareh N and Ameneh T. 2012. Essentail oil composition changes affected by leaf ontogeny stages of myrtle (Myrthus communis L.). Int J Med Arom Plants. 2(1): 114-117.

9. Atiqur R., Sharif MAR., Shah AS., Taehyun C., Sun CK. 2014. Antifungal Potential of essential Oils and Ethanol Extarcts of Lonicera japonica thumb against Dermatophytes. EXCLI Journal. 13:427-436.

10. Irobi ON, Darambola SO. 1993. Antifungal activities of crude extract of Mitracarpus villosus (Rubiaceae). J Ethnopharmacol. 40:137-40.

11. Jong, HJ., Ji, WL., Kyoung, SK., Ju-Sung, K., Sang, NH., Chang, YY., Ju, KL., Yong, SK., Myong, JK. 2010. Antioxidant and antimicrobial activities of extracts from a medicinal plant, Sea Buckthorn. J Korean Soc Appl Biol Chem. 53(1): 33-38.

12. Bashir A, Javid A. 2013. Physiochemical, minerals, phytochemical contents, antimicrobial activities evaluation and fourier transform infrared (FTIR) analysis of Hippophae rhamnoides L. leaves extracts. Afr J Pharm Pharacol. 7(7): 375-388.

13. Balakumar S., Rajan S., Thirunalascundari T., Jeeva S. 2011. Antifungal activity of Aegle marmelos (L.) Correa (Rutaceae) leaf extract on dermatophytes. Asian Pac J Trop Biomed. 1(4):309-312.

14. Hossein M, Fatemeh E, Fariba S, Iraj S, Ebrahim SD. 2015. Antileishmanial and Cytotoxic Effects of Essential Oil and Methanolic Extract of Myrtus communis L. Korean J Parasitol. 53(1): 21-27.

15. Elif AE., Gulden G., Ayse E. 2014. Anti-candidal activities of some Myrtus Communis L. extracts obtained using accelerated solvent extraction (ASE). Journal of Applied Biology & Biotechnology.2 (05); 12-14.

16. Mitra M, Abdolhassan K, Seyyed AAM, Mehdi R, Hossein A, Akram N. 2013. Evaluation of Antifungal Activities of Myrtus communis L. by Bioautography Method. Jundishapur J Microbiol. 6(8): e8316.

17. Shaza AAL, Frdoos MAF. 2014. Antibacterial Activity of Various Plants Extracts against Antibiotic-resistant Aeromonas hydrophila. Jundishapur J Microbiol. 7(7): e11370-e11376.

18. Amensour M, Bouhdid S, Fernandez-Lopez J, Idaomar M, Senhaji NS, Abrini J. 2010. Antibacterial Activity of Extracts of Myrtus commu¬nis Against Food-Borne Pathogenic and Spoilage Bacteria. Int J Food. 13 (6):1215–24.

19. Ali T., Amir S., Samira J., Fatemeh N. 2013. Antibacterial Effect of Myrtus Communis Hydro-Alcoholic Extract on Pathogenic Bacteria. Zahedan J Res Med Sci. 15(6): 19-24

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Table 1 DPPH Scavenging (%) Activity of *M. communis* Extracts.

Extracts/ Standard	Plant Parts	Concentrations (µg/mL)				
		50	**100**	**150**	**200**	**250**
Methanol	L	45±1.3	55±1.0	70±0.5	80±0.5	90±2
	F	40±1.2	50±0.2	60±01	70±01	80±01
	S	30±1.0	40±0.5	50±00	60±1.8	70±01
Ethyl acetate	L	40±1.0	50±2.1	60±0.5	75±0.5	85±01
	F	35±0.5	45±0.4	55±1.5	70±1.1	80±1.5
	S	30±1.4	35±1.7	50±1.2	65±0.3	70±0.5
Ethanol	L	35±1.5	45±1.0	50±0.5	68±0.5	72±01
	F	30±0.2	40±0.3	45±1.2	60±0.4	70±1.5
	S	25±0.3	35±0.5	40±00	50±0.1	60±00
Aqueous	L	38±1.0	42±1.0	50±01	65±0.5	68±01
	F	35±1.6	40±0.1	45±1.1	60±0.8	65±00
	S	30±1.4	35±00	40±1.5	50±0.1	60±1.5
Acetone	L	25±1.0	30±0.5	40±0.5	50±0.5	65±01
	F	22±00	25±00	30±00	40±0.9	60±01
	S	20±00	22±0.8	24±00	30±0.5	50±1.5
Vitamin C	-	60±2.0	65±1.1	75±0.6	80±01	88±02

L=Leaves, F=Flower, S= Stem, Data are mean ± SD (n=3).

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Table 2 Antifungal Activity of *M. communis* Extract

Organisms	*	Zone of Inhibition (mm)						DMSO
		Extracts						
		CH_3OH	C_4H_8O	C_2H_6O	H_2O	C_3H_6O		
Microsporum canis	L	17±0.2	16±01	15±0.3	14±00	12±0	20±00	NZI
	F	16±0.8	15±0.5	14±0.7	13±00	NZI		
	S	14±0.5	14±0.1	12±0.9	09±00	NZI		
Trichophyton mentagrophytes	L	19±0.9	19±0.8	17±1.5	16±1.2	14±0	18±01	NZI
	F	18±01	17±0.2	16±01	14±0.1	NZI		
	S	15±0.6	15±00	16±0.1	NZI	NZI		
Trichophyton rubrum	L	18±01	17±01	16±0.8	13±1.4	12±0	23±0.5	NZI
	F	17±00	15±0.2	15±0.4	14±00	11±0		
	S	14±0.1	13±0.1	10±0.5	NZI	NZI		
Microsporum gypseum	L	14±0.6	14±0.6	13±0.7	11±0.4	08±0	22±1.5	NZI
	F	11±00	10±00	09±0.1	NZI	05±0		
	S	09±0.4	07±00	06±00	05±00	NZI		
Epidermophyton floccosum	L	16±0.6	13±0.7	12±0.2	09±00	07±0	19±0.7	NZI
	F	15±0.3	09±0.4	07±00	NZI	NZI		
	S	08±0.7	08±0.3	NZI	NZI	NZI		

*L=Leaves, F=Flower, S= Stem, Each value represents mean ± SD (n = 3), NZI= No Zone of Inhibition, F= Fluconazole (positive control), DMSO= Negative control.

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