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COMPARATIVE ANTIOXIDANT POTENTIAL OF MYRTUS COMMUNIS LINN LEAVES AQUEOUS AND ORGANIC EXTRACTS

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Abstract

The total phenol content, flavonoid, condensed tannins and free radical scavenging potential of aqueous, chloroform, n-hexane and methanol extracts of Myrtus communis (M. communis) leaves were evaluated. Folin-Ciocalteau method was applied to determine phenolic compound, colorimetric method was used to quantify flavonoids and Vanillin assay procedure was used for condensed tannin estimation. While antioxidant activity was measured by standards In Vitro antioxidant test systems i.e. H_2O_2 scavenging activity, Superoxide dismutase (SOD)-like activity and NO scavenging activity uses ascorbic acid as standard. The highest total phenolic content (gallic acid equivalents/g of extract), total flavonoids (rutin equivalents/g of extract) and condensed tannins (catechin equivalents in mg CE/g) were measured in methanol extract i.e. 120±3 mg, 6.5±1 mg and 14±1 mg respectively.

Methanol extract at 250µg/mL remarkably inhibited hydrogen peroxide scavenging activity (84±1%), superoxide dismutase-like activity (85±0.5%) and nitric oxide scavenging activity (75±1%). These results indicated that Myrtus communis leaves extracts have potent antioxidant activities that would have beneficial effects on human health and methanol extracts are superior with better antioxidant potential.

Keywords: *Reactive oxygen species, diseases, natural antioxidant, leaves extracts, phenols and flavonoids.*

Introduction:

Reactive species such as OH–, H2O2, O–2 radicals and singled 1O2 are biological metabolites synthesized by aerobic microorganisms as a product of the metabolism of oxygen. These Reactive oxygen species (ROS) are highly reactive and unstable. Too much production of such molecular components can cause damage to DNA, proteins, cell membranes and lipids through chain reactions [1]. Antioxidants, molecules with a radical-scavenging capacity, are considered to exercise a defensive consequence against free radical injury. These molecular components may add to prevent many chronic diseases like as cancer, hepatitis, asthma, atherosclerosis, arthritis, heart disease and diabetes [2]. Currently many synthetic antioxidants are also used. These antioxidants have been supposed to damage liver and cause cancer [3]. Due to side effects and toxicity to health, there utilization is restricted [4]. The consumption of plant based additives as antioxidant increases day by day [5]. The herbal based antioxidant demand increases in the market, as they are used in food, cosmetic and pharmaceutical industries to replace synthetic antioxidants. Several studies have shown that medicinal plants contain various natural products possessing antioxidant properties with useful health effects.

Among natural antioxidants, phenolic substances have been of special interest because they are found extensively in plants [6]. Natural products containing phenolic substances have antioxidant potential, mostly because of their redox ability, which enable them to behave as hydrogen donor, single oxygen quenchers and reducing agents as well as they have a potential to chelate metals [4]. The phenolic substances have taken part in the body cell protection from harm by H2O2, lipid peroxide, unsaturated fatty acid, neutralizing and absorbing free radicals[7].

Myrtus communis (M. communis) is an evergreen small tree. It is mostly distributed in America, Africa, Asia and Europe. A refreshing and aromatic smell is emitted by its leaves and its taste is strongly bitter, quite unpleasant and intensive [8]. This herb is conventionally utilized for the treatment of urinary tract infection, dry coughs, vaginal discharge, stomach problems, sinusitis and respiratory system. The leaves are balsamic, haemostatic, tonic and flavoring agent in dishes [9].

Currently the research interest is increased in herbal medicine because they have an excellent source of natural antioxidants and to utilize it in pharmaceutical and food industries to substitute artificial antioxidants. The current study was aimed to assess the phenol, flavonoids, condensed tannins and scavenging potential of n-hexane, methanol, aqueous and chloroform extracts of M. communis leaves.

Materials and Methods:

Fresh leaves of *M. communis* were collected from Medicinal Botanical Garden of Pakistan Council of Scientific and Industrial Research (PCSIR) Labs Complex, Peshawar-Pakistan. The collected leaves were dried in an Air Cabinet Dryer (England) at 35 °C for 3 days and were ground in a Waring® Commercial Laboratory Blender USA. Leaves powdered material was kept in airtight plastic bags at 4°C.

Solvent extraction:

100 gram leaves powder was extracted using one liter of methanol, n-hexane and chloroform separately at room temperature for seven days. While for water extraction preparation, dried sample 100 gram was extracted using one letter of hot water for twenty minutes on water bath. This process of extraction was repeated three times for each sample material. The liquid extracts prepared in different solvents were filtered through Watman No. 1 filter paper and the Buchi Heating Bath B-490, Buchi Rotavapor R-200, Switzerland rotary evaporator was used to remove solvents and a dry extract was obtained. For further processing, dried extracts were kept at 4 °C.

Phenolic content determination

Phenolic quantification in dry extract was estimated through Ultra violet Spectrophotometer (U-2900 Hitachi Tokyo Japan). 100 microgram extract/ml solvent of plant extract was prepared and from this sample took 0.5 ml solution, added 2.5 ml Folin-Ciocalteu reagent to it and then two milliliter of Na_2CO_3 (75 g/l) was added after 0.5 to 8 min interval. The mixture sample was kept in incubator at 50 °C for five minute. As a control, distilled water 0.5 ml was used. Absorbance was taken at 760 nm, when the solution becomes blue-colored. Gallic acid

dissolved in methanol and then measured reading to draw the standard curve. The average of three readings (±SD) was expressed as GAE (gallic acid equivalents) in mg/g dry myrtle extract [4].

Total flavonoid content

In a test tube add 1.25 ml of distilled water and mix individually with 0.25 ml rutin (15-250 mg/ml) and plant extract (0.625– 5 mg/ml) and after that five percent sodium nitrite of 75 µl was added. Subsequent to six minutes added 150 µl $AlCl_3$ (10%) and this blend was kept for five minutes to react, after that added 0.5 ml of sodium hydroxide solution (1M). Before mix well added distilled water to the mixture to prepared the mixture up to 2.5 ml. At 510 nm absorbance was measured by Ultra violet Spectrophotometer (U-2900 Hitachi Tokyo Japan). The mean standard deviation results for three consecutive measurements were expressed as RE (rutin equivalents) in mg/g of plant extract [4].

Determination of Condensed Tannins

The modified Vanillin assay was used to determine the condensed tannin in plant leaves extract. [10]. From the 10% sample obtained 250 µL of the sample was blended with 1.5 ml of 4% Vanillin solution, 750 µL of 12N HCl solution and 750 µL of H_2O. This reactant was reserved for twenty minutes in a dark cabinet and its absorbance was measured at 500 nm by UV Spectrophotometer (U-2900 Hitachi Tokyo Japan). For standard catechin was used and quantification was expressed as mg/g in terms of catechin

In Vitro Antioxidant Activities

Hydrogen peroxide scavenging activity

Two millimoles of H_2O_2 and each extract/standard at different concentrations (50-250 µg/mL) were mixed in ratio (1:0.6) and kept in incubator for 10 min at 25℃. A control solution using a phosphate buffer without H_2O_2 was prepared. At 230 nm with UV Spectrophotometer (U-2900 Hitachi Tokyo Japan) absorbance was taken and calculated by the following formula [11].

Scavenging Activity%=

$$\frac{\text{(Control Absorbance – Sample Absorbance)}}{\text{Control Absorbance}} \times 100$$

Superoxide dismutase (SOD)-like activity

The 0.2 mL of extracts/standard (50-250 µg/mL) was added to 0.2 mL of pyrogallol (7.2 mM), 2.6 mL (pH 8.5) of tris buffer (10 mM EDTA, 50 mM tris) and at 25oC for ten minutes kept in an incubator. Then 0.1mL HCl (1N) was added and at 420 nm absorbance wased using U-2900 Hitachi spectrophotometer (Tokyo Japan). The following equation was used to calculate SOD-like activity [12].

%SOD like activity=

[1– (Extract absorbance/ Control absorbance)] ×100.

NO Radical Scavenging Potential

Each standard and extracts (50-250 µg/mL) was prepared and from these diluted samples one milliliter solution was taken and blended with sodium nitroprusside one milliliter (10 mmol/L) reaction solution using 20 mmol/L phosphate buffer (pH=7.0). At 37 ℃ incubation of reaction mixture was carried for 1hrs and mixed 0.5 mL of Griess reagent with 0.5 mL of incubated mixture. At 540 nm absorbance was measured by U-2900 Hitachi UV Spectrophotometer (Tokyo Japan). NO radical scavenging potential was calculated as [13].

Scavenging Activity % =

$$\frac{\text{(Control Absorbance – Sample Absorbance)}}{\text{Control Absorbance}} \times 100$$

Analysis of Statistics:

All the measurements were noted in triplicate form and results were expressed in average ± SD. A significant value considered as $p<0.05$.

Results and Discussion

The bioactive compounds in M. communis leaves extracts are shown in table 1. Methanol extract showed the highest active components as compared to the other extracts.

It is considerd that H2O2 is not poisonous, but it is dangerous because of its capacity to make the hydroxyl ions, in this manner call attention to the significance of its removal. The H2O2 antioxidant activity of M. communis leaves extracts shows in table 2. The dissimilarities of hydrogen peroxide scavenging potential between the extract may be because of structural properties of its energetic

locations, which decide the donating potential of its electron, solvent extraction procedures and percent values of extractions.

To find out whether the M. communis extracts have superoxide-scavenging factors, we measured the ability of leaves to scavenge superoxide radicals. The M. communis leaves extract SOD-like activity is shown in table 3. The SOD-like activity results was found very good, which increased slowly with a rise in concentration (50–250 µg/mL) as compared to standard. At 250µg/mL extract the methanol and vitamin C were found the same SOD-like activity (85%). These studies support that the myrtle leaves extracts have active compounds scavenging superoxide radicals, dominantly present in the extract prepared in methanol.

Nitric oxide scavenging activity of various extracts of M. communis L. is illustrated in table 4. Increase in the leaves concentration shows increase in antioxidant activities. The results showed that 250 µg/mL vitamin C and methanol extract had the highest scavenging activity of 75±1.

Hydrogen peroxide has a potential to directly deactivate some enzymes, although H_2O_2 is a weak oxidizing molecule [14]. Hydrogen peroxide is converted into •OH which have poisonous effects, due to the occurrence of redox active iron and copper active transition metals [11]. So it is necessary to monitor the H_2O_2 amount accumulated in the tissue. M. communis scavenged H_2O_2 at a considerable rate in comparison to vitamin C, because myrtle leaves contained high amount of flavonoids.

A powerful intermediary in many physiological phenomena like regulation of cell mediated toxicity, inhibition of platelet aggregation, neuronal signaling and smooth muscle relaxation is nitric oxide. Like a diffusible reactive oxygen species NO have many activities, like an effectors agent in numerous life processes including antitumor, antimicrobial, vasodilatation and neuronal messenger activities [15].

Antioxidant defense mechanisms in cells against oxidative damage include various enzymatic and non-enzymatic antioxidants. SOD is a primary enzyme in enzymatic antioxidant defenses. Removal of superoxide anion radicals is considered important because they serve as precursors of other activated oxygen including H_2O_2 and •OH [12]. Therefore, the presence of factors having strong SOD-like activity in M. communis could effectively eliminate the toxic effects of ROS. Due to the reactivity of phenol moiety, phenol compounds act as antioxidants [16]. Dissimilarities in the total phenol content observed in extracts of different solvents appear to be due to the difference in solubility of the phenolic compounds [17]. It was previously reported that antioxidant activities, nutritional, color, sensory and color qualities of foods have been related with phenolic acids [18].

Conclusion

The present study concluded that leaves of M. communis extract have high polyphenol concentration and have good antioxidant properties. Their curative potential against a large number of diseases can be used effectively, especially those related with oxidative stress. The data obtained through this study revealed that all the leaves extracts are likely to have the effect of scavenging free radicals and can be used in pharmaceuticals, food and cosmetics for healthy skin and/or anti-ageing products.

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Conflict in Interests

We (authors) have no competitive interest.

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Table 1. Table 1 Bioactive Components in M. communis Leaves Extracts.

Extracts	Total phenolic content	Total flavonoids	Condensed Tannins
n-Hexane	85±02	5.5±0.7	12±0.5
Methanol	120±03	6.5±01	14±01
Aqueous	95±02	5.2±0.5	10±0.5
Chloroform	80±0.5	4.5±0.4	07±0.3

Results are average of three replicates ± SD. Phenolic quantity (gallic acid (mg) equivalents/g of sample). Flavonoids quantification (Rutin (mg) equivalents/g of sample), Condensed tannin contents (as catechin equivalents in mg CE/g)

Table 2 H2O2 Scavenging Activity (%) of M. communis Leaves Extracts.

Extracts	Concentrations (µg/mL)				
	50	**100**	**150**	**200**	**250**
***n*-Hexane**	28±0.5	48±0.1	54±0.4	68±1.2	80±01
Methanol	30±0.3	52±0.5	62±0.3	70±1.3	84±01
Aqueous	28±0.2	48±0.2	62±0.2	65±0.2	78±02
Chloroform	18±0.1	32±0.3	45±0.2	52±1.1	62±01
Vitamin C	35±0.6	60±0.5	70±0.5	78±0.5	85±02

Data are average of three replicate ± SD.

Table 3 Percent inhibition rate of SOD-like activity of *M. communis* Leaves Extracts.

Extracts	Concentrations (µg/mL)				
	50	**100**	**150**	**200**	**250**
***n*-Hexane**	20±0.5	52±1	62±0.6	70±0.4	75±0.2
Methanol	22±1.4	62±1	70±1.5	78±0.1	85±0.5
Aqueous	15±0.8	40±2	58±0.3	68±0.6	74±0.4
Chloroform	12±0.1	25±1	34±0.5	52±0.2	60±00
Vitamin C	25±0.3	60±2	70±1.2	80±0.6	85±0.7

Data are average of three replicate ± SD.

Table 4 NO Scavenging Potential of *M. communis* Leaves Extracts.

Extracts	Concentrations (µg/mL)				
	50	**100**	**150**	**200**	**250**
***n*-Hexane**	20±0.2	40±0.5	50±1	70±0.1	72±0.9
Methanol	25±01	54±0.5	64±1	68±01	75±01
Aqueous	10±0.7	35±0.1	48±0.7	65±0.4	68±0.2
Chloroform	12±1.5	28±0.2	38±0.8	44±0.2	65±0.1
Vitamin C	25±0.5	48±0.9	65±0.1	70±0.5	75±0.8

Data are average of three replicate ± SD.