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# Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network



RESEARCH ARTICLE

BIOSCIENCE RESEARCH, 2020 17(3):2071-2079.

OPEN ACCESS

## Screening of antimicrobial, antioxidant activities and phytochemical analysis of *Myrtus communis* L plants used in Tehsil Utman Khel, District Bajaur Pakistan

Sher Hayat<sup>1</sup>, Imran Khan<sup>1</sup>, Amir Hasan Khan<sup>1\*</sup>, Ali Hazrat<sup>2</sup>, Ateeur Rahman<sup>1</sup>, Khaleeq Ahmad<sup>1</sup>, Ahmad Hassan Khan<sup>1</sup>, Qaisar ali<sup>1</sup>, Saira Naz<sup>1</sup>, Ashfaq Ahmad<sup>3</sup> and Zubir Shah<sup>1</sup>

<sup>1</sup> Department of Botany Shaheed Benazir Bhutto university, Sheringal, Dir upper, **Pakistan**

<sup>2</sup> Department of Botany, University of Malakand, KP. **Pakistan**

<sup>3</sup> Department of Forestry Shaheed Benazir Bhutto University, Sheringal, Dir upper, **Pakistan**

\*Correspondence: [amirhasankust@gmail.com](mailto:amirhasankust@gmail.com) Received 14-05-2020, Revised: 23-07-2020, Accepted: 30-08-2020 e-Published: 30-09-2020

The phytochemical and anti-microbial activity associated with leaves of *Myrtus communis* L were evaluated in this study. Phytochemical and antimicrobial screening analysis of *Myrtus communis* L showed the existence of Terpenoid, flavonoid, phenol, tannin, steroid and glycoside while saponins were absent and Terpenoid and steroid were contained in higher concentration. The zones of inhibition was ranged from 1mm to 7 against *Staphylococcus aureus* bacteria ethanol and n-hexane extracts was more efficient against *Staphylococcus aureus* ethanol 6.1 mm and n-hexane 7 mm and even though the smallest zone of inhibition was seen in methanol 1 mm the data furthermore indicate that methanol extract would be not a great efficient when evaluated with other extracts such as ethanol, n-hexane. The inhibition zones have been varied from 2.3mm to 3.4 against *Venturia inaequilis* fungi. Highest zone of inhibition percentage had been noticed for ethanol 3.4mm (50%) while the smallest zone of inhibition percentage was found in distal water 2.3 mm (34%) against *Venturia inaequilis* fungi. The antioxidant varied at various amount level at 60µg/ml (61.45±0.11) at 80µg/ml (65.23±0.10) as well as 100µg/ml (71.78±0.12) correspondingly. Total phenolic content of aqueous soluble fraction associated with the crude extract was (2.07%) of dehydrated extract. The outcome of this study stated the existence of antioxygenic substances in leaves, extracts exhibited potential as a natural antioxidant to restrict lipid peroxidation in foods.

**Keywords:** Medicinal plant, Antimicrobial activity, Phytochemical screening, Total phenol, antioxidant activity.

### INTRODUCTION

*Myrtus communis* L. belong to family (Myrtaceae) locally called it Mano. For thousands of years wild medicinal plants are in use in traditional medicine system, and a lot of medicines have been obtained. In developing countries, traditional use of plants as medicines

still play unique role in acquiring the basic health needs. In last few decades, natural medicinal use has been increased in developed countries (Verpoorte, 1998). Pakistan has a strong tradition of using herbal medicine and the rural populations still rely on a traditional medical system for health-related problems (Khattak et al., 1985). Pakistan

boasts an important biodiversity of different climate zones and various plants. There are four plant areas in Pakistan: Iran-Turanian (46%), China-Himalarians (10%), Saharo-Sindian (9.5%) and Indian elements (4.5%). There are about 6,000 wild plants in this country and about 400-600 species are considered medically important (Hamayun et al., 2003). *Myrtus communis* L leaves and fruits are indigenously utilized as antiseptic, hemostatic, stringent, laxative, blood purifier, hepato protective analgesic, anti-diarrheal, anti-hyperglycemic, anti-hemorrhagic and for vaginal lavage act. They are also used for treatment of peptic ulcers, headache, palpitation, bleeding, urethritis, leucorrhoea, respiratory diseases, anti-hemorrhagic, skin diseases and wound healing (Romani et al., 1999; Ali et al., 2017). Crude methanol extract from leaves, stem and berries possesses good antioxidant potential and higher flavonoids, phenolics and condensed tannins contents (Kanoun et al., 2014). Traditionally *Myrtus communis* L is used against cough, sinusitis and digestive diseases (Amenour et al., 2009). Typical myrtle is one of the Myrtaceae family members, such as approximately one hundred and forty five genera and above 5500 species (Snow et al., 2011). The flowering plant of genus *Myrtus* contains round about 16 species in regions of Asia (Twaij et al., 1998). *Myrtus communis* L. understood in reality myrtle is certainly highly significant aromatic and medicinal species of Myrtaceae family. It is a smaller tree, and about 1.8-2.5 m length, have a little bush and profoundly fissured copse (Mendes and Rodrigues, 2001). Important oils are fragrant and fickle substance reported just in 11% of the plant kingdom (Djilani and Dicko, 2012). Important oils and their elements might be hopeful biological agents, with their comparative protection, greater use through people and commercialization of potential useful utilization (Ormancey et al., 2001). The sum total important contents of plants is normally therefore minimal and hardly significantly more than 1% of biomass (Bowles, 2003). As an instance, the fundamental oil in flower, leaf, stalk of *Myrtus communis* L var. *Italica* were correspondingly 0.30%, 0.08% and 0.61%, (w/w) (Aidi et al., 2010). Important oils are complex mixtures including numerous different substances. Chemically these are generally produced by terpenes and their oxygenated ingredients (Prabuseenivasan et al., 2006).

## MATERIALS AND METHODS

### Plants collection and Extraction

Selected plants were shade dried and ground into a fine powder. From each plant 50g of powder were soaked in 200ml of different solvents (methanol, ethanol, distilled water and n-hexane) and powder of the selected plant was stored at room temperature for two weeks and was vigorously shaken each day. The impregnated plant material was first filtered through plain filter and whattmann filter paper # 41. By rotary evaporator under vacuum, solvents were completely vaporized to obtain the crude extracts. The filtrates acquired were kept in a refrigerator (Choudhary et al., 2011).

### Antibacterial activity

The antibacterial potential of *Myrtus communis* L plants was determined through a modified agar well diffusion assay reported by Khan et al., (2011).

### Requirements/ Materials

Plant samples, nutrient agar and broth, bacterial strain cultures, saline solution bacterial slants, sterile cork borer, disc (for diffusion), micropipette, Petri plates, organic solvent (DMSO), incubator, spirit lamp, Filter paper.

### Preparation of samples

Samples were prepared from three plants i.e, *Myrtus communis* L using methanol, ethanol, n-hexane and distilled water.

### Preparation of media for bacteria

1. Media was prepared through including 0.13g of nutrient broth to 10ml of dist. water, adjusting the pH to 7.0 & autoclaved.

2. Nutrient agar (NA) medium was prepared by dissolving 23gm/10ml nutrient agar in dist. water, adjusting the pH to 7.0 and autoclaved.

### Bacterial strains used

A gram positive strain called *Staphylococcus aureus* was used in the study. The bacteria were preserved at 4°C on nutrient agar medium.

### Antifungal assay

The antifungal activity of *Myrtus communis* L plant was determined through agar well diffusion assay reported by Khan et al., (2011) with some modifications.

**Microorganism used:**

Venturia inaequalis, ascomycetes fungi was used in the study

**Assay procedure**

Media was prepared by taking 6.5 g Sabouraud agar and dissolve in 100 ml of dist. water. The pH was adjusted to 5.6. Methanol is used as a negative control. 15 ml of each medium was poured into a P. dish (9cm), allowed to solidify. Subsequently, a 7-day-old well-tested fungal culture was obtained with a pre-sterilized cork drilling machine and placed upside down in the center of the plate. Petri dishes were place for 7 days in the dark at  $25 \pm 2$  ° C in incubator. Extension diameter (mm) was measured at 24-hour intervals for seven days. The assay was performed in triplicate.

The rate of fungal inhibition was determined by the following formula:

$$\% \text{ inhibition of fungi growth} = \frac{\text{Linear growth in test sample}}{\text{Linear growth in control}} \times 100$$

**Phytochemical assay**

Phytochemical process is carried out after plant extract preparation by using the detailed protocol is as follows:

**Test forsaponins**

A total of 3ml from extract was added to 5ml of dist. water in a test tube and shaken vigorously thus will result in a stable foam, a +v signs for saponins.

**Test for steroid**

To 5 ml of extract, 2 ml of chloroform and conc. H<sub>2</sub>SO<sub>4</sub> was added. Red color production in chloroform lower layer indicates steroids presence.

**Test for Glycosides**

3ml of extract was mixed with 2ml of chloroform, then 2ml of conc. H<sub>2</sub>SO<sub>4</sub> was added carefully and gently shaken. A reddish brown color was obtained which shows the presence of steroidal ring.

**Test forTerpenoids**

A total of 5 ml extract was mixed in 2ml of chloroform and dissolve till dryness. Then 2ml of conc. H<sub>2</sub>SO<sub>4</sub> was included and heat up for approximately two minutes. A grayish colour appeared which indicate the existence of terpenoids.

**Test for Flavonoids**

5 ml of extract had been included to 2ml of 2% NaOH solution which resulted in yellow colour. Then a small number of drops of dilute acid were put into make the solution colourless which is a positive notice for the existence of flavonoids.

**Test for Phenols and Tannins**

From extract a total of 0.5 ml was combined 2ml of water and 2 to 3 drops of FeCl<sub>3</sub>were added. The appearance of blackish color indicates the existence of tannins and phenols.

**Total phenolic assay**

The magnitude of total phenol was identified through the use of Folin-Ciocalteu assay reported by (Oliveira *et al.*, 2008). Plant sample extract 0.1g were added with 60 to 80ml of distilled water. Now it is heated and boiled for 30 minutes in a sand bath and filtered. Final volume was up to 100ml with distilled water. From the above extract 0.1ml added to test tube (screw cap) plus 3.9ml dist water is added and agitated. Add 1ml of FolinCiocalteu reagent and then added 5ml of 20% of sodium carbonate and shake vigorously. Keep it at room temperature for 20 minutes. After that O.D of sample will be observed at 720nm wave length. Experiments were performed in triplicate.

**Antioxidant assay**

Antioxidant assay was performed by scavenging effect of 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Oliveira *et al.*, 2008). A total 0.25 ml DPPH 0.8 mm in MeOH was included with test tubes to accurately weigh the weighted amounts of the extracts break up in 3.75 ml MeOH, accordingly to an extraction concentration ranging from 0.01 and 0.2 mg / ml. ml. Subsequently preparing, the samples were kept at room temperature for half an hour in the dark. Absorbance at 517 nm was calculated utilizing a UV / Vis V-530 spectrophotometer and examined having a non-extracted control. A blank for every sample was ready utilizing methanol rather than of the DPPH solution. Ascorbic acid had been utilized as the reference compound. Antioxidant activity was shown as a percentage of DPPH radical inhibition and was measured by comparison:

$$\% \text{ scavenging DPPH free radical} = 100 \times \frac{(1-AS)}{AD}$$

These values are defined as the inhibitory concentration of the extract required to reduce the first DPPH radical concentration by 50% and shown in mg/ml.

## STATISTICAL ANALYSIS

Standard deviation was applied on the data and recorded in mean values then converted into percent. The Formula of calculation in percent is as follows:

$$\text{Percent inhibition} = \frac{\text{Zone of inhibition of extract (mm)}}{\text{Zone of inhibition of standard (mm)}} \times 100$$

## RESULTS AND DISCUSSION

### Antibacterial activity of *Myrtus communis* L plants extracts

The outcomes obtained within the current study find out that the observed *Myrtus communis* L indispensable oil contain possibilities antibacterial properties and antifungi activity against certain particular bacterial strains showed on (Tables 1). Analysis regarding the data shown that methanol, n-hexane and Distilled Water extracted samples had a efficient inhibitory impact against *Staphylococcus aureus* in comparison to the ethanol extracted samples. Methanol and n-hexane extracts were more efficient against *Staphylococcus aureus* when compared to the n-hexane extracts, 7 mm (50% Z.I) and by distal water 6.3 mm (45% Z.I) and 6.1mm Ethanol (44% ZI) and 1 mm (7% Z.I) by Methanol in the amount level of just one and 2mg disc-1 (Table 1, 2) respectively. The antimicrobial activity of *Myrtus communis* L is impacted mainly through specific types of bacterial strain and extraction solvent. Amongst the entire of the extracts n-hexane and distal water extracts reflect maximal antibacterial activity against *Staphylococcus aureus* at mean of zone of prohibition. In graphical representation the highest zone of inhibition was found at n-hexane extract while the lowest zone of inhibition observed in Methanol extract (Fig 1) This might be because of the capability of n-hexane and methanol to extract certain partially polar dissolve factor of plants which have active characteristics. in the past, (Majhenic *et al.*, 2007) stated methanol and ethanol contain highly antimicrobial properties of medicinal plants. thus, the outcome specify in the current study is sustained through earlier scientific reports (Andrews, 2006). The greatest antibacterial properties of solvent such as methanol between polar solvent and n-hexane as of non-polar solvent extract against bacterial strain might because of the capability associated with the correlation with solvent to extract many partial polar and non-polar solvable part of plants which contain active attributes. Consequently, *Myrtus communis* L essential oil extract has

antibacterial activity (Ya *et al.*, 1998).

### Anti-fungal activity

In the current study, the inhibitory effectuation of various extract (distilled water, methanol, ethanol and n-hexane) of *Myrtus communis* L. were analyzed against fungal strains. The antifungal activity was find out utilizing agar well diffusion method. The activity had been quantitatively approximated according to the inhibition zone and their activity index was additionally determined using minimum inhibitory concentration. Methanol with no extract was utilized as negative control. Different zone of inhibition were observed against *Venturia inaequilis* fungi . Zones of inhibition were ranged from 2.3mm to 3.4mm in *Myrtus communis* L plant extracts .(Tabl 3). Maximal zone was noticed with Ethanol 3.4mm (50%) and n-hexane 3.3mm (49%), methanol 3.1mm (46%) and distal water 2.3mm (34%). but minimum zone of inhibition had been observed at distilled water 2.3mm against *Venturia inaequilis* fungi (Table 3, 4). In graphical representation the maximum zone of inhibition was found at ethanol extract while the lowest zone of inhibition was found in distal water extract (Fig 2). *Myrtus communis* L showed maximum antifungal activity for ethanol plant extract. Medium antifungal activity was seen in methanol extract and a small amount of inhibition in distilled water extract was seen against the fungal strains. The current research is within decide with (Cannas *et al.*, 2013). That stated that myrtle extracts exhibited antifungal activity against different *Candida* species.

### Phytochemical study of *Myrtus communis* L.

*Myrtus communis* L. phytochemical screening revealed the existence of terpenoid, flavonoid, phenol, tannin, steroid and glycoside but saponins were found absent (Table 5). Terpenoid and steroid were current in higher concentration. The same scientific studies were carried out by many scientist who revealed their results. *Myrtus communis* L leaves were seen which contain small concentration of phenolic acids, flavonoid (Al-Abache *et al* 2012). One other study confirmed the existence of tannins, polyphenolic and glycosides within the leaves of *Myrtus communis* L (Yoshimura *et al.* 2008). Major Terpenoid and its particular derivatives were present in leaves of *Myrtus communis* L (Khani and Basa, 2012).

### Total Phenol Contents of *Myrtus communis* L.

The total phenolic amount associated with

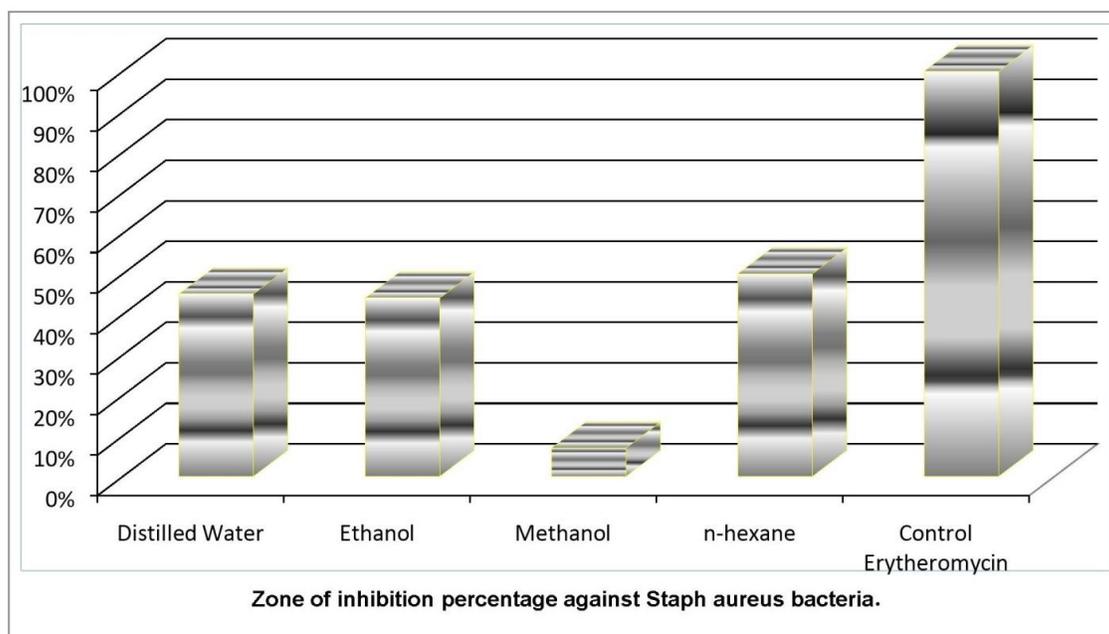
aqueous extracts was found through using Folin-Ciocalteu reagent.

**Table 1: Zone of inhibition of *Myrtus communis* L plants extracts against *Staphylococcus aureus* bacteria.**

Name of the Organism	Plants extract	Zone of Inhibition in (mm)	Mean± Standard Deviation	Chi –Square
<b>Staphylococcus aureus</b>	Distilled Water	6.3	6.3±0.21	0.5
	Ethanol	6.1	6.1±0.18	
	Methanol	1	1.0±0.11	
	n-hexane	7	7.0±0.15	
	Control Erytheromycin	14	14±0.17	

**Table 2: Percentage inhibition of *Myrtus communis* L plants extracts against *Staphylococcus aureus* bacteria.**

Name of the Organism	Plants Extract	Percentage (%)
<b>Staphylococcus aureus</b>	Distilled Water	45%
	Ethanol	44%
	Methanol	7%
	n-hexane	50%
	Control Erytheromycin	100%



**Fig 1. Zone of inhibition percentage against *Staphylococcus aureus* bacteria.**

Table3: Zone of inhibition in (mm) against fungal strain *Venturia inaequilis*.

Name of the Organism	Plant Extract	Zone of Inhibition in (mm)	Mean± Standard Deviation	Chi -Square
<i>Venturia inaequilis</i>	Distilled Water	2.3	2.3±0.152	0.5
	Ethanol	3.4	3.4±0.115	
	Methanol	3.1	3.1±0.208	
	n-hexane	3.3	3.3±0.115	
	Control (methanol)	6.8	6.8±0.152	

Table4: Percentage inhibition of *Myrtus communis* L plants extracts against fungal strain *Venturia inaequilis*.

Name of the Organism	PLANT EXTRACTS	Percentage (%)
<i>Venturia inaequilis</i>	Distilled Water	34%
	Ethanol	50%
	Methanol	46%
	n-hexane	49%
	Methanol (-ve contr)	100%

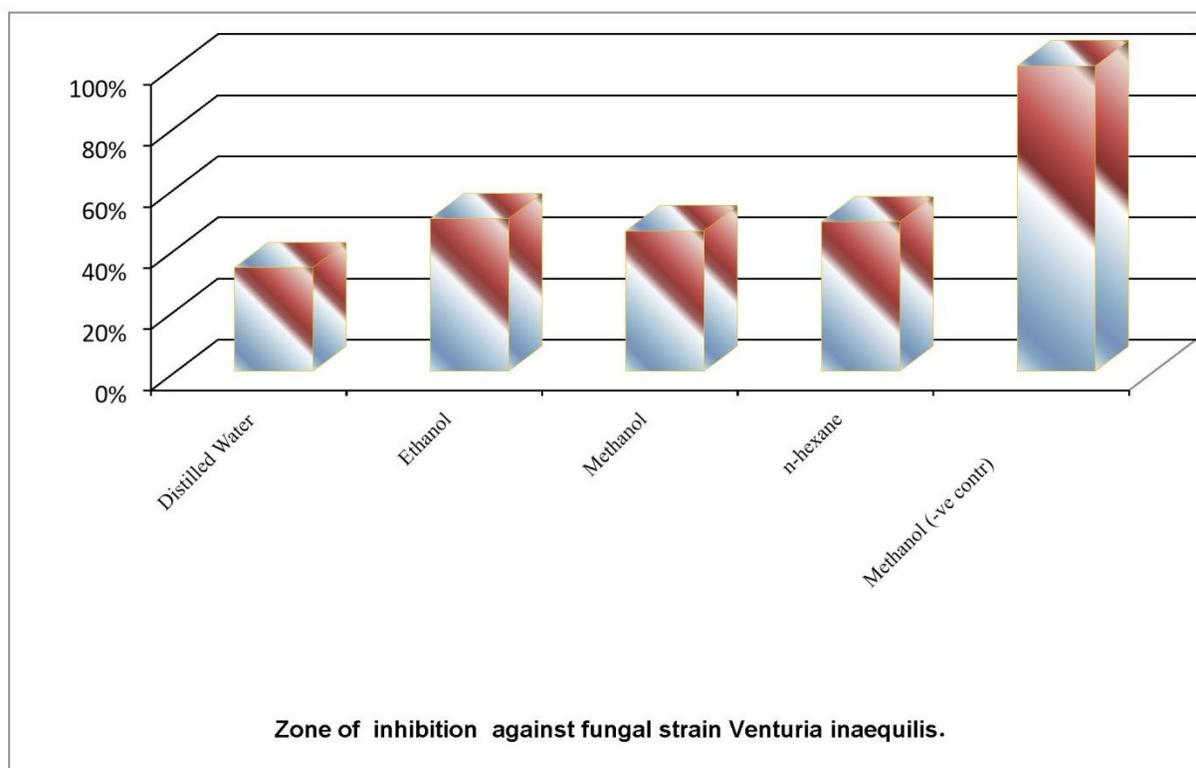
Fig 2. Zone of inhibition Percentage of against fungal strain *Venturia inaequilis*.

Table 5: Phytochemical screening of *Myrtus communis* L.

Sr. No	PHYTOCHEMICAL COMPOUND	Methanol extract
1	Saponin	—
2	Steroid	++
3	Glycoside	+
4	Terpenoid	++
5	Flavonoid	+
6	Phenol and Tannin	+

Table 6: Total phenolic analysis of *Myrtus communis* L plants extracts by Folin-Ciocalteu assay

Treatment	Sample weight (g)		O.D	Blank	O.D Blank	X= y + 0.0018 / 0.0012	Total phenol (ppm)	%age
<i>Myrtus communis</i> L. 1	0.11		0.145	0.113	0.032	28.16	14806.15	1.47±0.99
<i>Myrtus communis</i> L. 2	0.11	0.153	0.113	0.04		34.83	17950	1.80±1.21

Table 7: Antioxidant potential of aqueous plant extracts of *Myrtus communis* L plant.

Concentration	Anti-oxidant activity (%)	
	Mean	Mean± Standard Deviation
60 µg	38	38.17±0.05
80 µg	43	43.32±0.07
100 µg	50	49.67±0.41

Within the assay, gallic acid was applied like being a standard substance and absolute quantity of phenolic contents were indicated like mg/g gallic acid equivalent utilizing the standard curve equation:  $y = 0.0012x - 0.0018$ , where y is absorbance at 720 nm and x is total phenolic content in *Myrtus communis* L extracts shown in mg/gm. The outcomes indicated that *Myrtus communis* L contain (1.80%) phenolic contents (Table 6). *Myrtus communis* L may be viewed due to the fact the majority providing plant species for natural herbal sources of antioxidants a high potent importance for the making of medicines. The same scientific studies were performed on *Myrtus communis* L by Mahato et al., (2018); Akin, et al., (2013); Hagos et al., (2017) who reported that these plants are a possible source to obtain phenolic compounds and therefore justifying our finding.

#### Antioxidant activity

The antioxidant potential of *Myrtus communis* L was identified utilizing aqueous extracts of the plant by scavenging effect assay. Various concentration (60, 80 and 100 µg/ml) of the extracts were utilized within the assay as exhibited in table 7. At 60µg/ml the aqueous

fraction of *Myrtus communis* L indicated the greatest significant scavenging activity (41.23±0.08). At 80 and 100µg/ml, a maximum antioxidant activity (65.23±0.10 & 71.78±0.12) for the aqueous extracts of *Myrtus communis* L with moderated antioxidant possibilities (48.21±0.15 & 52.48±0.21) was documented correspondingly (Table 7). All around a concentration reliant activity was reported for every extract. The efficient antioxidant feature of plant extracts is because of the characteristics of having hydroxyl groups existing into the phenolic substances (Cai et al 2004). Various reports that antioxidant activity 26 of phenolic compounds contained in plants have redox characteristics that allow it to act as reducing agents and hydrogen donators (Mustafa et al., 2010). The finding have been in accordance towards the results published by Hagos et al., (2017) where a concentration dependant antioxidant activity were recorded for *Myrtus communis* L being the absolute most effective one. The outcome obtained into the assay revealed that these plants are particularly significant from medicinal view-point, and it also requires more phytochemical qualitative screening to isolate the anti-oxidant compounds. (Mustafa et al. 2010).

## CONCLUSION

Current study have confirmed the use of *Myrtus communis* L plant in the indigenously therapeutic systems regarding cure of various contagion. The results of this study revealed that the crude extracts of the *Myrtus communis* L plant might have very important antimicrobial activity, particularly against fungal strains utilized into the study. The plant may contain potent antimicrobial substance, excellent for the cure of different fungal and bacterial infections. But, more assessment is needed towards the natural ingredient isolation, toxic effect and healthcare provider to be able to use the magnificent compound(s) that efficient antimicrobial agents.

## CONFLICT OF INTEREST

The authors declared that current study was carry out in the absence of all conflict of interest.

## Funding sources.

This research study was sponsored through Prime minister Reimbursement scheme, Pakistan.

## ACKNOWLEDGEMENT

This research work was sponsored by Prime minister Reimbursement scheme, Pakistan which is highly acknowledged. The authors extend their high appreciation and acknowledgment to the NIFA staff by giving moral support to the authors.

## AUTHOR CONTRIBUTIONS

All author contributed in the paper here. Sher Hayat designed and carry out the experiments AHK wrote the manuscript. Ahmad Hasan, Saira Naz, Qaisar ali, Ateeq ur rahman, Zubir shah performed Data analysis, Ashfaq Ahmad and Khaleeq Ahmad helped in plant collection identification dehydration and powder preparation. Imran Khan and Ali Hazrat provide technical suggestions on the draft and identified the language and grammatical mistakes. IK supervised all the stages. All authors read and approved the final version.

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