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## **Phytochemical and in Vitro Biological Profiling of *Portulaca grandiflora* Whole Plant Extracts**

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**ABSTRACT:** *This study aimed to determine the phytochemicals and to examine the in vitro antibacterial, antifungal, anticancer and antioxidant potential of P. grandiflora. Crude methanolic, ethanolic and n-Hexane extracts of whole plant were used for this study. Antimicrobial properties were estimated through disk diffusion assay, antioxidant activity was determined by using 2,2-diphenyl-1-picryl-hydrazyl scavenging (DPPH), total antioxidant capability and reducing power assays. It was found that methanolic extract of P. grandiflora showed the highest zone of inhibition against Pseudomonas aeruginosa and Candida albicans i.e. 36 ± 1mm and 18 ± 1 mm respectively with MIC of 25 µg/ml. The highest amount of gallic acid equivalent phenolic and quercetin equivalent flavonoid content were found in methanolic extract of P. grandiflora i.e. 77 ± 0.68 µg GAE/mg and 63 ± 1.08 µg QE/mg extract respectively. The significant DPPH scavenging activity (P<0.05) was recorded for methanolic extract of P. grandiflora (87%). Considerably greater antioxidant capacity (10 ± 1.18 µg AAE/mg) and reducing power (4.06 ± 0.18 µg AAE/mg) was observed in methanolic extract. MTT cell viability assay methanolic extract of P. grandiflora showed the IC<sub>50</sub> of 42.70 µg/ml at 72h. It was concluded this plant extracts exhibit strong pharmacological properties.*

**Keyword:** Phenolic, Flavonoid, Antioxidant, DPPH, antimicrobial

## INTRODUCTION

Researchers acknowledge the role that traditional medical systems and herbal remedies play in mitigating the growing global health care crisis. History reveals that different species of plants have been utilized for health maintenance in different cultures as well as the derivation of most medicinal preparations (Gadir, 2012). In a tropical climate *Portulaca Grandiflora* is well growing plant. This herb mainly considered a problematic. The researchers encourage by this fact and others potencies that could be utilized from purslane herbs. To check the efficiencies on disease several studies have been conducted, and antibacterial activity is one of these efficacies. The antibacterial activity of purslane herbs has the potential to hasten the healing process and to be developed into topical medicines for the treatment of wound (Budiawan, 2023). Using DPPH and FRAP assays, the antioxidant effects of *Portulaca grandiflora* extracts were evaluated in order to pinpoint possible sources of compounds that might be helpful in thwarting the effects of free radicals.

Medicinal plants continue to be important in the fight against disease and are a viable source of medications,

particularly in underdeveloped nations (Mothana et al., 2019). Nearly all of the plant species in the world have outstanding antioxidant potential, and it is thought that two thirds of them have medicinal significance (Krishnaiah et al., 2011). There are about 3.4 billion herbal medicine users worldwide. The essential components of the antiquated conventional medical system were natural materials. The World Health Organization (WHO) defines a medicinal plant as any plant that has ingredients that are used for medical purposes (Asif et al., 2019; Shareef et al., 2016).

The free radical DPPH creates a violet solution in ethanol and is stable at room temperature. The foundation of the DPPH test is the stable DPPH free radical's capacity to interact with hydrogen donors. There is an intense UV-VIS absorption spectrum shown by the DPPH• radical. In this test, a radical solution is reduced with an antioxidant (AH) or a radical (R•) according to the following protocol, which results in decolorization of the solution:  $DPPH\cdot + AH \rightarrow DPPH\cdot -H + A\cdot$ ,  $DPPH\cdot + R\cdot \rightarrow DPPH\cdot - R$  (Bangbog, 2022). With a total phenolic content of 0.7346 mg GAE/g FW, the treatment using 100 kg/ha of NPK fertilizer had the greatest value. The extracts treated

with 100 kg/ha (FRAP, CUPRAC) and 200 kg/ha (DPPH, ABTS) with NPK fertilizer showed the largest increase in antioxidant activity. As a result, purslane's plant growth, total phenolic content, and antioxidant activity can all

be increased by using NPK fertilizer at recommended dosages. Based on the research, 100 kg/ha was indicated as the dose that produced the maximum antioxidant activity (FRAP, CUPRAC) and total phenolic (Putra, 2023).



**Fig. 1. A plant of *Portulaca grandiflora***

*P. grandiflora* is utilized for detoxification, as well as the treatment of skin rashes and sore throats. It is a purported immunostimulant that causes the immune system to become non-specifically activated, resulting in cytotoxic, antibacterial, and antitumor action. The effectiveness of *P. grandiflora* on a surface antigen of the hepatitis B virus has been documented. Furthermore, it has been observed that this plant has an antimutagenic impact on the mutations caused by cyclophosphamide and aflatoxin B1, two human carcinogens, in mice. It is important to note that *P. grandiflora* aqueous extracts are safe to use; studies

conducted on participants suggested a daily intake of 500 mg.

Various well-known medicines have been derived from different plants, such as morphine, aspirin, quinine, benzoin, vincristine, and vinblastine from *Papaver somniferum*, *Filipendula ulmavia*, *Cinchona pubescens*, *Slyrax tonkinensis*, and *Catharanthus roseus* (Michael et al., 1956) respectively.

Plants naturally yield a vast range of chemical constituents that have a strong medicinal value and therefore are used in pharmaceutical industries. The existence of such bioactive metabolites is also responsible for several other properties to plant particular order due

to the terpenoids; flower color is because of the presence of betalains, quinones, and tannins, and chilies have a flavor due to the presence of terpenoid capsaicin (Gülcin, 2012). Latterly the hunt for effective antibacterial representatives has been moved to herbs. The traditional evaluation recommends that 10% of all the flowering plants have been in use for at least one time by the native population but only 1% were noticed by world scientists for medicinal purposes (Kunin, 1993). More than one hundred human diseases including arthritis, CNS injury, AIDS, cancer, atherosclerosis, ischemia, and gastritis are the consequence presence of free radicals in the human body (Cook and Samman, 1996; Kumpulainen and Salonen, 1999). Numerous factors are responsible for the production of free radicals including pollution, chemicals, radiation, toxins, spicy food, and also physical stress which then weakened the immune system of the body, mutation in genes that changes their expression, and also produces unusual proteins. Reduction in the natural antioxidant in the immune system consumption of antioxidants and free radicals might be important (Halliwell, 1994; Kuhnau, 1976; Kumpulainen and Salonen, 1999; Younes and Siegers, 1981).

*P. grandiflora* is a small herbaceous annual herb from Portulacaceous family (Fig. 1). Sun plant, Rose Moss and Moss Rose are the common names of this plant (Brickell, 2003). A few years ago, the aqueous extract of *P. grandiflora* was used to examine its toxic effects on anti-adenoviruses activities, in vitro anti-herpes simplex viruses (Chiang et al., 2003), and Wistar rats (Chavalittumrong et al., 2004). Additionally, the water formulation of *P. grandiflora* enhances the proliferation of lymphocytes in vitro, suggesting a part in the modulation of the immune system (Sriwanthana et al., 2007). Compared to its close relative *P. oleracea* (Dkhil et al., 2011; Lim and Quah, 2007; Sanja et al., 2009; Uddin et al., 2012), comprehensive studies on the characterization, as well as health benefits of *P. grandiflora* limited. Perhaps, the anticancer activity of *P. grandiflorais* still not reported.

The use of natural antioxidants is gaining importance due to unhealthy and negative impacts of artificial antioxidants like butylated hydroxy anisole (BHA), gallic acid esters, etc., (Barlow, 1990; Branen, 1975). The presence of natural antioxidants in the diet decreases the threat of heart disorders, cancer, as well as aging diseases (Kritchevsky, 1999; Lauro and

Francis, 2000; Pszczola, 1998; Rao and Agarwal, 1999). Numerous human disorders including cancer have been treated by natural medicinal products e.g., etoposide, Taxol, paclitaxel, vincristine, and irinotecan are the drugs obtained for plants (Da Rocha et al., 2001). The study aimed to determine the phytochemicals and to examine the *in vitro* antifungal, antibacterial, antioxidant and anticancer potential of *Portulaca grandiflora*.

## MATERIAL AND METHODS

### Plant Collection

*P. grandiflora* was collected in the summer season from the home garden. Identification of the plant was done by Prof Dr. Mir Ajab Khan, Department of Plant Sciences, Quaid-i-Azam University Islamabad, Pakistan.

### Reagents and Chemicals

Analytical grade solvents have been used for the present study, Ethanol, Methanol, and *n*-hexane (Sigma USA). Merck provided all the reagents used for the study including gallic acid,

quercetin, sodium carbonate, DPPH, aluminum chloride, potassium ferricyanide, ferric cyanide, Folin-Ciocaltau (F-C) reagent, ammonium molybdate, sodium phosphate, trichloroacetic acid, sulfuric acid, ascorbic acid and potassium acetate (Merck KGaA, Germany).

### Extract Formation

*P. grandiflora* was rinsed scrupulously with simple tap water then air dried and crushed to ultra-fine particles. Process of maceration was used to make extracts using 3 solvents *n*-hexane (NH) Ethanol (EL), and methanol (ML) with different polarity levels from non-polar to polar solvents respectively. 50grams of powdered material was soaked in 500ml of extraction solvents. All solvent extraction was performed thrice. Subsequently, filter paper (Whatman filter paper No.1) was used to filter all the mixtures. The rotatory evaporator was used at decreased pressure and 45°C to vaporize the mixtures for thickening (Buchi, Switzerland).

**Table 1: *P. grandiflora* extraction in different organic nonpolar to polar solvents with percent yield**

Serial No.	Sample code	Solvent	% Yield (g)
1	NH	<i>n</i> -hexane	1
2	EL	Ethanol	3
3	ML	Methanol	3.5

## **Phytochemical screening**

### **Determination of total phenolic contents (TPC)**

The determination of total phenolic content in samples was done by the previously defined procedure (Clarke et al., 2013; Qader et al., 2011; Zhang et al., 2010). Immediately the stock solutions (4 mg/ml) of test samples were concocted in DMSO. Each extract (20 $\mu$ l) was moved to all wells of 96 well plate. 90  $\mu$ L of F-C reagent diluted with double distilled water was added to each well for 5 minutes. A 7.5 % of 90  $\mu$ l of Na<sub>2</sub>CO<sub>3</sub> solution was also added to the reaction mixture. After five minutes, the whole plate was incubated for 60 minutes and the microplate reader (Bioteck) was used to measure the absorbance at 650nm. The gallic acid in DMSO as well as blank DMSO as standard were run concurrently. The resulting TPC was calculated as  $\mu$ g GAE/mg extract.

### **Determination of total flavonoid content (TFC)**

Because medicinal plants can scavenge free radicals, there has been a favorable correlation found between their antioxidant activity and overall phenolic contents (Geethalakshmi et al., 2013).

20  $\mu$ l (4 mg/mL DMSO) of test samples and 10  $\mu$ l of 10 % AlCl<sub>3</sub> as well as 10  $\mu$ l of 1M CH<sub>3</sub>CO<sub>2</sub>K were mixed to determine the total flavonoid content of different extracts. Distilled water was mixed with the different extracts to attain a total volume of 200  $\mu$ l. The obtained solution was incubated for 30 minutes (Incubator IC83 Yomato, Japan). Absorbance was calculated at 415nm at 37°C by microplate reader. Quercetin was used as the standard and results were articulated as  $\mu$ g QE/mg extract (Clarke et al., 2013).

## **Biological assessment**

### **DPPH free radical scavenging assay**

DPPH reagent was used to evaluate the free radical scavenging property of the collected samples. Concisely, standard solutions of 4 mg/mL of experimental samples were set ready in DMSO (Clarke et al., 2013; Qader et al., 2011; Zhang et al., 2010). A 10  $\mu$ l of each sample was added in 190  $\mu$ L of 0.004% DPPH in ML and incubated for 1 hr. The absorbance was taken at 515 nm with the help of a microplate reader. Ascorbic acid as well as DMSO were used as control. Each sample was calculated at final concentration of 200  $\mu$ g/ml. A sample that shows strong quenching activity ( $\geq$  50%) was again

used with a lower concentration to calculate IC<sub>50</sub>. By following the formula percent inhibition was measured:

Percent inhibition of the test sample = % scavenging activity =  $(1 - Ab_s / Ab_c) * 100$

Where

Ab<sub>s</sub>= Absorbance of DPPH solution with the sample,

Ab<sub>c</sub>= Absorbance of negative control (containing the reagent except for the sample).

Version 4 of Table Curve software was used to evaluate the IC<sub>50</sub>.

### **Determination of total antioxidant capacity (TAC)**

The TAC of test samples was calculated by mixing 900 µl of a reagent comprising 0.6 M H<sub>2</sub>SO<sub>4</sub>, 4 mM (NH<sub>4</sub>)<sub>2</sub>MoO<sub>4</sub>, and 28 mM NaPO<sub>4</sub> with 100 µl of stock solution (4mg/ml in DMSO) and incubated for one and half hour at 95°C. Solution Mixtures were kept to cool at 37°C and with the help of a microplate reader, the OD was calculated at 695 nm. DMSO (100 µl) was used as a blank. Different concentrations of ascorbic acid were used along with DMSO for a standard curve. The attained TAC was measured as µg AAE/mg extract (Aliyu et al., 2009).

### **Reducing power assay**

The determination of reduction potential was done by the already defined method (Aliyu et al., 2009). 100 µl of all test samples prepared as 4 mg/ml extract in DMSO were added with 250 µl of 1% C<sub>6</sub>N<sub>6</sub>FeK<sub>3</sub> as well as 200 µl of 0.2 M phosphate buffer (pH 6.6). The incubation of the resulting solution was done at 50°C for 20 minutes. After the incubation period, 200 µl of TCA (10%) was used to acidify the reaction mixture and centrifuged for ten min at 3000 rpm. 150 µl of supernatant was collected and added to 50 µl of 0.1% FeCl<sub>3</sub>. Afterward, 200 µl of reaction mixtures were moved to the respective wells of the 96-well plate. The absorbance was evaluated at 630 nm with the help of a microplate reader. As ascorbic acid was a positive control, the results were calculated as µg AAE/mg extract.

### **Antibacterial Activity**

Susceptibility of all samples was verified against 6 multidrug-resistant bacterial strains i.e., 2-gram+ve (Methicillin-resistant *Staphylococcus aureus* and *Enterococcus. spp*) and 4-gram-ve (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Serratia marcescens*). The bacterial strains were cultured in N-broth and after culturing the strains were swabbed

onto the nutrient agar plates. Filter paper discs were soaked in five microliters (20mg/mL DMSO) of samples were put down on nutrient agar plates and incubated for 1 day. After incubation, a clear area of the zone of inhibition appeared around the discs which were measured in millimetre sand noted (Haq et al., 2012). MIC of samples showing an inhibition zone of  $\geq 10.0$  mm was further verified at 100, 75, 50, and 25  $\mu\text{g/ml}$  by Microtiter Plate Broth Dilution Method.

### **Antifungal activity**

The antifungal property of all samples under investigation was tested against the given fungal cultures (*Aspergillus niger* FCBP# 0198, *Mucor* specie FCBP# 0300, *Fusarium solani* FCBP# 0291, *Aspergillus flavus* FCBP# 0064 and *Candida albicans* FCBP-478) grown on SDA medium (Sabouraud Dextrose Agar; Merck Germany). Before the determination of sensitivity, fungal spores were cultured in 0.02% Tween 20 solution.

The turbidity of fungal spores was modified based on McFarland 0.5 turbidity standard. 100  $\mu\text{l}$  inoculum was used to dab the plates having SDA medium. 5 $\mu\text{l}$  from each sample was used to infuse the filter-paper discs and 2.5 microliters (4mg/ml) of standard antifungal terbinafine was employed as

standard discs, and incubated for 1 day at 28°C. By using Vernier caliper clear area as the zone of inhibition was measured and noted after a given time (Ul-Haq et al., 2012).

### **Anticancer /Cytotoxic Activity Assay**

#### **MTT Assay**

The antiproliferative effects of polar and non-polar extracts of *P. grandiflora* were evaluated through an MTT cell viability assay as described previously (Batool et al., 2017).

The % cell viability was calculated through the following formula:

$$\% \text{ Cell viability} = (\text{OD of treated cell} / \text{OD of control}) \times 100$$

Where OD = Optical Density

#### **Statistical analysis**

All the trials were performed in triplicate. The data were expressed as mean  $\pm$  standard deviation (SD). Graphpad prism version 6.01 was used to test IC<sub>50</sub>. The graphical presentation was done by Origin 8.5 software.

## **RESULTS**

A total of 3 extracts of *P. grandiflora* whole plant were prepared in three different solvents. Table 1 described the sample code as well as the % yield. The highest extraction production was gained with the methanol (ML) while n-



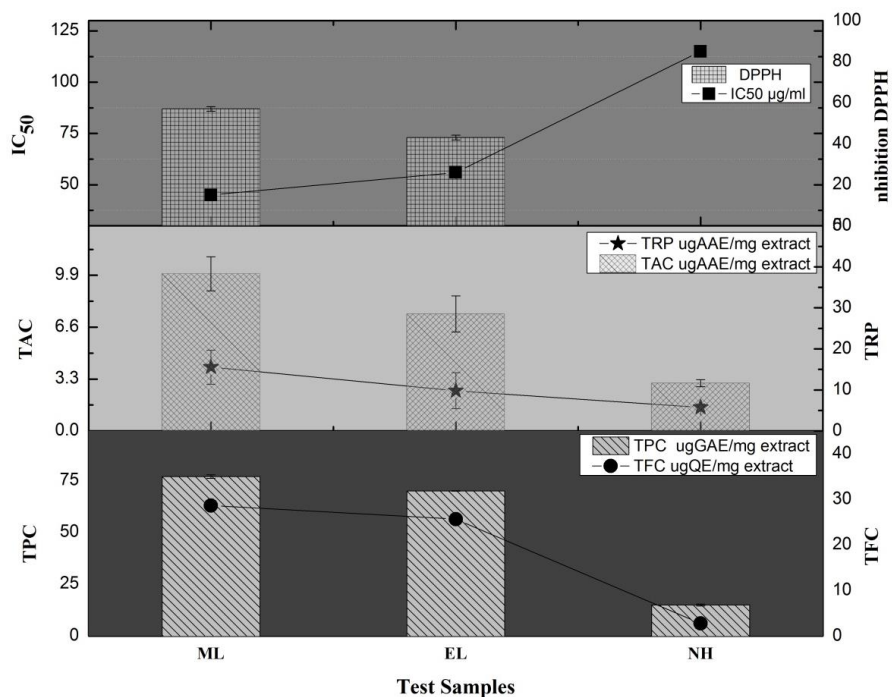
hexane (NH) extract was obtained in the least quantity among others.

## 1. Phytochemical evaluation

### Total phenolic and flavonoid content (TPC: TFC)

Among all phytochemically analyzed solvent extracts, the maximum quantity of gallic acid corresponding TPC was examined in ML ( $77 \pm 0.68 \mu\text{g}/\text{mg}$ ) and

EL extracts ( $70 \pm 0.58 \mu\text{g}/\text{mg}$ ) while the minimum amount of TPC was examined in n-hexane NH ( $15 \pm 0.23 \mu\text{g GAE}/\text{mg}$ ) (Fig. 2). The quercetin equivalent (QE) total flavonoid content was found to be varied greatly from 6.08-63  $\mu\text{g}/\text{mg}$ . The maximum amount was found in ML ( $63 \pm 1.08 \mu\text{g}/\text{mg}$ ), EL ( $56.45 \pm 2.15 \mu\text{g}/\text{mg}$ ), and a lower value was shown by NH ( $6.08 \pm 3.08$ ) (Fig. 2).



**Fig. 2. Total flavonoids and phenolic content and antioxidant property of various non-polar as well as polar solvent extracts of *P. grandiflora***

## 2. Biological evaluation

### DPPH radical scavenging activity

The % DPPH scavenging property of all samples lie between 24 to 87% with highly significant percentage activity ( $p < 0.05$ ) being examined for ML (87%:

IC<sub>50</sub>= 45 $\mu\text{g}/\text{ml}$ ), EL extracts (73%: IC<sub>50</sub>= 56  $\mu\text{g}/\text{ml}$ ). The lowest scavenging activity of 24% scavenging potential was found in the NH extract (Fig. 2).

### Phosphomolybdenum based total antioxidant capacity (TAC)

The maximum antioxidant capacity, when measured in comparison with ascorbic acid, was found for ML ( $10 \pm 1.18 \mu\text{g AAE/mg}$ ). However, EL showed ( $7.44 \pm 1.54 \mu\text{g AAE/mg}$ ) while NH extract ( $3.04 \pm 1.69 \mu\text{g AAE/mg}$ ) showed the least TAC.

### Reduction potential

Reducing the power of test samples depend upon the color of extracts that deviates to either blue or green. The higher the absorbance greater will be reducing power. Among all analyzed samples, Methanol (ML) and ethanol (EL) soluble extracts were shown to exhibit higher reduction potential when expressed as equivalent of ascorbic i.e.,  $4.06 \pm 0.18$  and  $2.56 \pm 0.29 \mu\text{g AAE/mg}$  respectively. NH extract ( $1.5 \pm 0.10 \mu\text{g AAE/mg}$ ) showed the lowest reducing power.

### Antimicrobial activity

The sensitivity of samples against different bacterial as well as fungal strains was evaluated by the well diffusion method. According to our findings, ML showed the highest activity against *MRSA* and *Pseudomonas aeruginosa* with  $29 \pm 1.6$  (MIC:  $25 \mu\text{g/ml}$ ) and  $36 \pm 1$  (MIC:  $25 \mu\text{g/ml}$ ) of the zone of inhibition followed by EL and NH shown in Table 1, minimum inhibition. Against different fungal strains ML extract was observed to exhibit the highest sensitivity against *Candida albicans* with  $18 \pm 1$  mm growth inhibition zone with MIC  $25 \mu\text{g/ml}$ . While the lowest activity was shown by NH against *Fusarium Solani* and *Mucor* specie (Table 2). The absence of a clear zone confirmed that DMSO did not showed any toxic effect.

Table 1: Antibacterial activity of *Portulaca grandiflora* extracts against MDR bacterial strains (Data Values are presented as mean +SD (n=3))

PG	S. aureus	MIC $\mu\text{g/ml}$	S. marcescens	MIC $\mu\text{g/ml}$	K. pneumonia	MIC $\mu\text{g/ml}$	E. coli	MIC $\mu\text{g/ml}$	P. aeruginosa	MIC $\mu\text{g/ml}$	E. aerogenes	MIC $\mu\text{g/ml}$
MtOH	$29 \pm 1.6$	25	$14 \pm 1$	75	$14.5 \pm 0.71$	75	$18 \pm 1$	50	$36 \pm 1$	25	$32 \pm 2$	25
EtOH	$28.7 \pm 1.5$	25.0	0	0	0	0	$16 \pm 1.7$	50	$28.7 \pm 1.5$	25	0	....
N-Hexane	$29 \pm 1$	25.0	$14.7 \pm 2.5$	75	$13.5 \pm 3.5$	75	$18 \pm 1$	50	$35 \pm 1.7$	25	0	....

Table 2: Antifungal activity of *Portulaca grandiflora* extracts against 5 fungal strains (values are expressed as Negative control = DMSO (no zone of inhibition), Positive control = Terbinafine, mean  $\pm$ SD (n=3))

PG	<i>F. solani</i>	MIC ug/ml	<i>A. flavus</i>	MIC ug/ml	<i>A. niger</i>	MIC ug/ml	<i>M. specie</i>	MIC ug/ml	<i>C. albicans</i>	MIC ug/ml
MtOH	8.3 $\pm$ 0.19	0	11.67 $\pm$ 0.19	75	13.00 $\pm$ 1	50	11 $\pm$ 0.58	75	18 $\pm$ 1	25
EtOH	10 $\pm$ 0.58	75	9.33 $\pm$ 0.51	0	9.67 $\pm$ 0.51	75	12 $\pm$ 0.58	75	11.67 $\pm$ 0.51	75
N-Hexane	9 $\pm$ 0.65	0	10.7 $\pm$ 1.5	75	11.5 $\pm$ 1.7	75	9 $\pm$ 0.71	0	13 $\pm$ 0.95	75
Terbinafine	22 $\pm$ 0.806		12 $\pm$ 0.455		11 $\pm$ 0.56		15 $\pm$ 0.77		10 $\pm$ 0.706	

**MTT/Cytotoxicity assay**

It was analyzed the effects of ML, EL, and NH extracts of *Portulaca grandiflora* on the viability of Hep2 cells and also on normal human corneal epithelial cell line (HCEC) through MTT assay. The cell line was treated to 0, 50, 100, 200, and 400  $\mu$ g/ml of ML, EL, and NH extracts of *Portulaca grandiflora*, for 24, 48, and 72 h

proceeded by calculation of cellular viability by the colorimetric-based MTT assay. The data shown in Fig. 3A to 3C indicated that ML extract has better cytotoxic activity against Hep 2 cell lines, with the lower IC<sub>50</sub> (inhibitory concentration at which cell growth is 50% reduced) of 42.70  $\mu$ g/ml as compared to the other extracts, after 72 h.

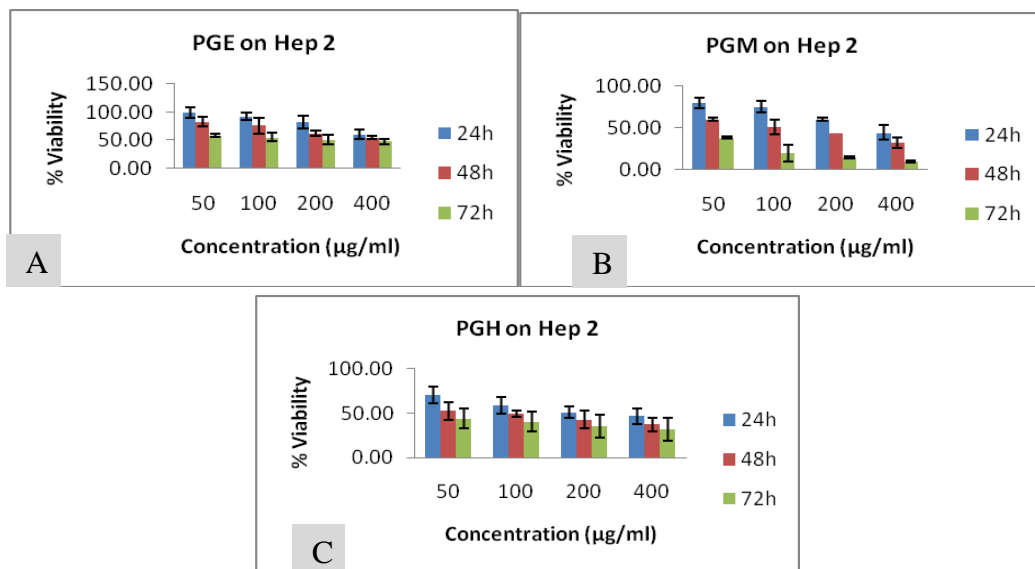


Fig. 3. Anti-proliferative effect of three different extracts of *P. grandiflora* in Hep 2 cells as shown in graph A, B, and C

## DISCUSSION

In the trial of Metabolite Profiling Analysis and the Correlation with Biological Activity of Betalain-Rich *P. grandiflora*, Sporna Kucab et al found antioxidant properties against gram negative bacteria of compounds extracted from *P. grandiflora*. In the present study, *P. grandiflora* showed high zone of inhibition that showed its effectiveness against *Pseudomonas aeruginosa* and *Candida albicans* also showed high MIC and results are in agreement with Spórna-Kucab et al. (2022).

This result showed that this plant can be used in pharmaceuticals industries with some others solvents. Secondary metabolites or phytochemicals of plants are contributing actively to the treatment of several diseases, nowadays they are considered the major part of both modern as well as traditional systems of medicine. In the present study, polarity-based extract efficacy disparity has been estimated which indicates the effect of the nature of the solvent on the phytochemicals extracted, possibly due to the occurrence of delocalized electrons that make the majority of the plant constituents extremely polarisable (Lim and Quah, 2007). A battery of assays to determine the antioxidant activity was employed in this study

because a single test cannot completely assess the rationality behind the activity. In this study, a number of antioxidant assays like total reducing power as well as DPPH free radical scavenging assay were used to evaluate the antioxidant property of various extracts. A useful association between the TFC, TPC, and DPPH hunting potential of ethanolic as well as methanolic extracts of *P. grandiflora* has been established which is consistent with the already reported positive correlation between TFC, TPC, as well as quenching potential. The outcomes of this study firmly correlate with the literature where maximum antioxidant activities have been observed in polar extracts such as ethanol as well as methanol. Therefore, it can be recommended that the compounds of this plant have antioxidant activity and are quite polar. These results also revealed that *P. grandiflora* extracts had comparable free radical scavenging activity in *P. oleracea* [IC<sub>50</sub> = 0.89 ± 0.07 mg/ml] as reported by Lim and Quah (Lim and Quah, 2007).

Phenolic compounds have several biological implications such as antioxidant activities, antibacterial, antitumor, and antimutagenic, and they have a hydroxyl group present on their aromatic hydrocarbon ring (Kolář et al.,

2002; Shui and Leong, 2002). Flavonoids and phenolics have been described have being related to antioxidant activity in natural systems. High concentration of phenolics as well as flavonoids contents in methanolic as well as ethanolic extracts of *Portulaca grandiflora* respectively also described by the previous study of *P. grandiflora* (Lim et al., 2014), and their connection with antioxidant capability show our interest in determining the antioxidant potential of the given test samples. The overall antioxidant property of the extracts can be credited to the total flavonoids as well as phenolic contents (Cai et al., 2004; Hendra et al., 2011).

In the given study numbers of bacterial and fungal strains were used to check the antimicrobial potential of *P. grandiflora*. Results were comparable with the earlier research where the highest zone of inhibition was shown against *Pseudomonas aeruginosa* and *Candida albicans* similar to our results (Shinde et al., 2014). Reactive oxygen species (ROS) are generated by other metabolic reactions as well as the electron transport chain of mitochondria. These free radicals also affect the deoxyribosyl backbone of DNA along nucleic acid bases causing genotoxicity and ultimately mutations. Mutations are the major contributor to

carcinogenesis and tumor formation (Valko et al., 2004). Polysaccharides scavenge the buildup of free radicals as well as restrain immunity functions also (YouGuo et al., 2009), Polysaccharides from *Portulaca oleracea* a very close relative of *P. grandiflora* exhibit many biological properties, like anti inflammation, antioxidant, anticancer, and immunity-enhancing assets (Chen et al., 2010; Liu et al., 2009; Yang et al., 2008; Zhu and Wu, 2009). As there is no such information reported previously about the anticancer activity of *P. grandiflora*, based on studies regarding its counterpart *Portulaca oleracea* it is assumed that *P. grandiflora* also has anticancer activity due to the presence of polysaccharides.

## CONCLUSION

It was concluded that *Portulaca grandiflora* is of great importance as it possesses several pharmacological properties by displaying strong antimicrobial, antioxidant, and anticancer potential associated with its diverse chemical constituents.

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## CONFLICT OF INTEREST

Authors declare there is no conflict of interest.

## REFERENCES

1. Aliyu A, Ibrahim M, Musa A, Ibrahim H, Abdulkadir I, Oyewale A (2009). Evaluation of antioxidant activity of leave extract of *Bauhinia rufescens* Lam. (Caesalpiniaceae). *J. Medi. Plants Res.* 3(8): 563-567.
2. Asif K, Rahman M, Annanya K (2019). Extraction and evaluation of phytochemicals from banana peels (*Musa sapientum*) and banana plants (*Musa paradisiaca*). *Malaysi. J. Hal. Res. J.* 2.
3. Barlow SM (1990). Toxicological aspects of antioxidants used as food additives. *Food Antioxid.* 253-307.
4. Batool R, Salahuddin H, Mahmood T, Ismail M (2017). Study of anticancer and antibacterial activities of *Foeniculum vulgare*, *Justicia adhatoda* and *Urtica dioica* as natural curatives. *Cellul. Mol. Biol.* 63(9): 109-114.
5. Branen A (1975). Toxicology and biochemistry of butylated hydroxyanisole and butylated hydroxytoluene. *J. Amer. Oil Chem. Soc.* 52(2): 59-63.
6. Brickell C (2003). *Royal Horticultural Society AZ encyclopedia of garden plants*: Dorling Kindersley.
7. Cai Y, Luo Q, Sun M, Corke H (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sci.* 74(17): 2157-2184.
8. Chavalittumrong P, Chivapat S, Attawish A, Bansiddhi J, Phadungpat S, Chaorai B, Butraporn R (2004). Chronic toxicity study of *Portulaca grandiflora* Hook. *J. Ethnopharmacol.* 90(2-3): 375-380.
9. Chen T, Wang J, Li Y, Shen J, Zhao T, Zhang H (2010). Sulfated modification and cytotoxicity of *Portulaca oleracea* L. polysaccharides. *Glycoconjugate J.* 27(1): 635-642.
10. Chiang LC, Cheng HY, Liu MC, Chiang W, Lin CC (2003). In vitro anti-herpes simplex viruses and anti-adenoviruses activity of twelve traditionally used medicinal plants in Taiwan. *Biol. Pharmaceu. Bull.* 26(11): 1600-1604.
11. Clarke G, Ting KN, Wiart C, Fry J (2013). High correlation of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric reducing activity potential and total phenolics content indicates redundancy in use of all three assays to screen for antioxidant activity of extracts of plants from the Malaysian rainforest. *Antioxid.* 2(1): 1-10.
12. Cook NC, Samman S (1996). Flavonoids—chemistry, metabolism, cardioprotective effects, and dietary sources. *J. Nut. Biochem.* 7(2): 66-76.

13. Da Rocha AB, Lopes RM, Schwartsmann G (2001). Natural products in anticancer therapy. *Curr. Opin. Pharmacol.* 1(4): 364-369.
14. Dkhil MA, Moniem AA, Al-Quraishy S, Saleh RA (2011). Antioxidant effect of purslane (*Portulaca oleracea*) and its mechanism of action. *J. Med. Plants Res.* 5(9): 1589-1563.
15. Gadir SA (2012). Assessment of bioactivity of some Sudanese medicinal plants using brine shrimp (*Artemia salina*) lethality assay. *J. Chem. Pharm. Res.* 4(12): 5145-5148.
16. Geethalakshmi R, Sakravarthi C, Kritika T, Arul Kirubakaran M, Sarada D (2013). Evaluation of antioxidant and wound healing potentials of *Sphaeranthus amaranthoides* *Burm. f.* *BioMed. Res. Int.*
17. Gülcin I (2012). Antioxidant activity of food constituents: an overview. *Arch. Toxicol.* 86(1): 345-391.
18. Halliwell B (1994). Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? *Lanc.* 344(8924): 721-724.
19. Hendra R, Ahmad S, Oskoueian E, Sukari A, Shukor MY (2011). Antioxidant, anti-inflammatory and cytotoxicity of *Phaleria macrocarpa* (Boerl.) Scheff fruit. *BMC Complem. Altern. Medi.* 11(1): 1-10.
20. Kolář P, Shen JW, Tsuboi A, Ishikawa T (2002). Solvent selection for pharmaceuticals. *Fluid Phase Equil.* 194(1): 771-782.
21. Krishnaiah D, Sarbatly R, Nithyanandam R (2011). A review of the antioxidant potential of medicinal plant species. *Food Bioprod. Proces.* 89(3): 217-233.
22. Kritchevsky SB (1999).  $\beta$ -Carotene, carotenoids and the prevention of coronary heart disease. *J. Nut.* 129(1): 5-8.
23. Kuhnau J (1976). Flavonoids. A class of semi-essential food components: Their role in human nutrition. *World Rev. Nut. Dibet.*
24. Kumpulainen JT, Salonen JT (1999). Natural antioxidants and anticarcinogens in nutrition, health and disease: Elsevier.
25. Kunin CM (1993). Resistance to antimicrobial drugs—a worldwide calamity. *Ann. Int. Med.* 118(7): 557-561.
26. Lauro GJ, Francis J (2000). Natural food colorants: Sci. and Technol. CRC press.
27. Lim CK, Tiong WN, Loo JL (2014). Antioxidant activity and total phenolic content of different varieties of *Portulaca grandiflora*. *Int. J. Phytopharm.* 4(1): 1-05.
28. Lim YY, Quah EP (2007). Antioxidant properties of different cultivars of *Portulaca oleracea*. *Food Chem.* 103(3): 734-740.

29. Liu Y, Liu C, Tan H, Zhao T, Cao J, Wang F (2009). Sulfation of a polysaccharide obtained from *Phellinus ribis* and potential biological activities of the sulfated derivatives. *Carbohydr. Polym.* 77(2): 370-375.
30. Michael A, Thompson C, Abramovitz M (1956). *Artemia salina* as a test organism for bioassay. *Sci.* 123(3194): 464-464.
31. Mothana RA, Khaled JM, El-Gamal AA, Noman OM, Kumar A, Alajmi MF, Al-Rehaily AJ, Al-Said MS (2019). Comparative evaluation of cytotoxic, antimicrobial and antioxidant activities of the crude extracts of three *Plectranthus* species grown in Saudi Arabia. *Saud. Pharmaceut. J.* 27(2): 162-170.
32. Pszczola D (1998). Natural colors: pigments of imagination. *Food Technol. (USA)*.
33. Qader SW, Abdulla MA, Chua LS, Najim N, Zain MM, Hamdan S (2011). Antioxidant, total phenolic content and cytotoxicity evaluation of selected Malaysian plants. *Molec.* 16(4): 3433-3443.
34. Rao A, Agarwal S (1999). Role of lycopene as antioxidant carotenoid in the prevention of chronic diseases: a review. *Nut. Res.* 19(2): 305-323.
35. Sanja S, Sheth N, Patel N, Patel D, Patel B (2009). Characterization and evaluation of antioxidant activity of *Portulaca oleracea*. *Int. J. Pharm. Pharm. Sci.* 1(1): 74-84.
36. Shareef M, Ashraf MA, Sarfraz M (2016). Natural cures for breast cancer treatment. 24: pp. 233-240): Elsevier.
37. Shinde P, Wagh K, Patil P, Bairagi V (2014). Pharmacognostic standardization and antibacterial potential of aerial herbs of *Portulaca grandiflora* Hooker (Portulacaceae). *World J. Pharmaceut. Sci.* 2(12): 1871-1885.
38. Shui G, Leong LP (2002). Separation and determination of organic acids and phenolic compounds in fruit juices and drinks by high-performance liquid chromatography. *J. Chromatogr. A.* 977(1): 89-96.
39. Spórna-Kucab A, Tekieli A, Grzegorzczuk A, Świątek Ł, Rajtar B, Skalicka-Woźniak K, Starzak K, Nemzer B, Pietrzkowski Z, Wybraniec S (2022). Metabolite profiling analysis and the correlation with biological activity of betalain-rich *Portulaca grandiflora* Hook. extracts. *Antioxid.* 11(9): 1654.
40. Sriwanthana B, Treesangsri W, Boriboontrakul B, Niumsukul S, Chavalittumrong P (2007). In vitro effects of Thai medicinal plants on human lymphocyte activity. *In vitro*, 29: 1.
41. Uddin MK, Juraimi AS, Ali ME, Ismail MR (2012). Evaluation of



- antioxidant properties and mineral composition of purslane (*Portulaca oleracea* L.) at different growth stages. *Int. J. Mol. Sci.* 13(8): 10257-10267.
42. Ul-Haq I, Ullah N, Bibi G, Kanwal S, Ahmad MS, Mirza B (2012). Antioxidant and cytotoxic activities and phytochemical analysis of *Euphorbia wallichii* root extract and its fractions. *Iranian J. Pharmaceut. Res.* 11(1): 241.
43. Valko M, Izakovic M, Mazur M, Rhodes CJ, Telser J (2004). Role of oxygen radicals in DNA damage and cancer incidence. *Mol. Cellul. Biochem.* 266: 37-56.
44. Yang X, Zhao Y, Yang Y, Ruan Y (2008). Isolation and characterization of immunostimulatory polysaccharide from an herb tea, *Gynostemma pentaphyllum* Makino. *J. Agric. Food Chem.* 56(16): 6905-6909.
45. YouGuo C, ZongJi S, XiaoPing C (2009). Evaluation of free radicals scavenging and immunity-modulatory activities of Purslane polysaccharides. *Int. J. Biol. Macromol.* 45(5): 448-452.
46. Younes M, Siegers CP (1981). Inhibitory action of some flavonoids on enhanced spontaneous lipid peroxidation following glutathione depletion. *Planta Med.* 43(11): 240-244.
47. Zhang H, Jiang L, Ye S, Ye Y, Ren F (2010). Systematic evaluation of antioxidant capacities of the ethanolic extract of different tissues of jujube (*Ziziphus jujuba* Mill.) from China. *Food Chem. Toxicol.* 48(6): 1461-1465.
48. Zhu J, Wu M (2009). Characterization and free radical scavenging activity of rapeseed meal polysaccharides WPS-1 and APS-2. *J. Agric. Food Chem.* 57(3): 812-819.