Qualitative Phytochemical Screening, Fatty Acid Composition Analysis and Biological Studies of *Trianthema portulacastrum* L. Leaves

Vaskar Chowdhury, Nusrat Tabassum Shristy, Md. Hasanur Rahman and Tofail Ahmad Chowdhury

Department of Chemistry, Faculty of Science, University of Dhaka, Dhaka-1000, Bangladesh

(Received: April 20, 2022; Accepted: June 5, 2022; Published (Web): June 22, 2022)

ABSTRACT: *Trianthema portulacastrum* L. commonly known as 'horse purslane' and locally called 'shvetapunarnava' is an annual broadleaf plant of tropical areas throughout the world. In this study, determination of moisture and ash content of the plant leaves, as well as qualitative phytochemical test, cytotoxicity assay, antimicrobial activity, fatty acid composition, total phenolic content, total flavonoid content, total antioxidant capacity, and DPPH radical scavenging activity were carried out. Methanol extract of *T. portulacastrum* leaves was partitioned using Kupchan's method with different solvents of increasing polarity (*n*-hexane, dichloromethane, chloroform, ethyl acetate). The moisture and ash contents of the leaves were (81.74±0.49)% and (31.05±1.36)%, respectively. Initial phytochemical screening of *T. portulacastrum* showed the presence of alkaloids, tannins, flavonoids, and tri-terpenoids. In fatty acid composition analysis, both bound fatty acids and free fatty acids were isolated from n-hexane extract that showed the presence of the highest percentage of palmitoleic acid (67.095% and 48.843%, respectively) and stearic acid (12.14% and 20.437%, respectively). Dichloromethane and methanol extracts were found to be cytotoxic on HeLa cells in cytotoxicity assay against Vero, HeLa, and BHK-21 cell lines. The highest phenolic content was observed in ethyl acetate extract (68.55±0.038 mg/g) and the highest flavonoid content was found in chloroform extract (477.01±0.104 mg/g). The DCM fraction revealed the highest total antioxidant capacity (284.99±0.113 mg/g), and the IC_{50} value for ethyl acetate extract was the lowest (110.35±0.160 µg/ml) of all the extracts.

Key words: *Trianthema portulacastrum*, phytochemical, cytotoxicity, antimicrobial activity, antioxidant activity.

INTRODUCTION

In both traditional medicine and modern pharmaceutics, plants are a great source of natural treatments for maintaining human health. Traditional medicines are believed to be used by two-thirds of the world's population.[1] Medicinal plants naturally synthesize and accumulate natural products such as alkaloids, sterols, terpenes, flavonoids, saponins, glycosides, tannins, resins, lactones, quinines, volatile oils, and others.[2] There is now a consensus on the importance of medicinal plants and traditional health systems in addressing health-care issues, as well as the efficacy and safety of medicinal plants in treating a variety of ailments.[3]

Correspondence to: Md. Hasanur Rahman
E-mail: hasanur@du.ac.bd

Dhaka Univ. J. Pharm. Sci. **21**(1): 33-43, 2022 (June)
DOI: https://doi.org/10.3329/dujps.v21i1.60394

Trianthema is a genus of flowering plants belonging to the Aizoaceae family.[4] *T. portulacastrum* has a long history of usage against diseases and certain bioactive chemicals have been isolated from this plant.[5] It is a fast-growing, prostrate, abundantly branched, succulent annual broadleaf plant that grows in cultivated and wastelands generally during the rainy season.[6] The plant is commonly used as a potherb and also used for heart-related problems and anemia.[7] *T. portulacastrum* is an important source of medicinal natural products, particularly alkaloids and flavonoids.[8] The potential pharmacological properties of this plant have been reported to be substantial. *T. portulacastrum* has been found to have a number of biological properties, including antifungal and hypolipidemic properties.[9] The plant is also

hepatoprotective, nephroprotective, antinociceptive, anti-inflammatory, and antipyretic.[10] The herb is used to treat blood vessel diseases, bronchitis, inflammations, piles, and ascites. The plant has been used in traditional medicine for a variety of ailments such as liver obstruction, asthma, amenorrhea, dropsy, edema, and beriberi, to name a few.[11] The presence of various alkaloids and flavonoids may account for the bioactivity of the plant. According to a nutritional study, the plant *T. portulacastrum* is a good source of fiber, proteins, potassium, and iron, among other nutrients. The fact that it contains a wide range of nutrients makes it an excellent source of fodder for ruminants.[12] *T. portulacastrum* exhibits allelopathic activity against a variety of weeds and crops, including sorghum, pumpkin, eggplant, radish and wheat, by decreasing seed germination and seedling vigor. In terms of allelopathic potential, there is a positive correlation between *T. portulacastrum* and *Amaranthus viridis* when the density of *Amaranthus viridis* increases in the area infested by *T. portulacastrum.*[13] The preceding knowledge of the literature is addressed as a reference for crediting the appreciation of further research on the plant *T. portulacastrum*.

MATERIALS AND METHODS

Collection and processing of the plant materials. Plants of *T. portulacastrum* were collected from the local vegetable market of Chattogram, Bangladesh. The sample was authenticated by Dr. Mahbuba Sultana (Senior Scientific Officer) at Bangladesh National Herbarium, Dhaka. The leaves were separated from the collected plants and were washed thoroughly with water to remove mud and dust particles, first dried at room temperature for several days and then in the oven at 40ºC. The dried leaves were then ground to make mesh size by a grinder. The ground powder was stored for extraction in an airtight container for further studies. For moisture content determination, fresh leaves were used.

Chemicals and reagents. All solvents and analytical or laboratory-grade reagents used during the investigation were procured from E. Merck (Germany) and BDH (England).

Extraction. The powdered leaves (200.0 g) were extracted with methanol (MeOH) for 10 days at room temperature with occasional stirring and shaking in an airtight, clean flat-bottomed container. After that, the extract was filtered through Whatman No. 1 filter paper. The filtrate was collected in a glass container and evaporated using a rotary evaporator (Buchi Rotavapor R-3) at 40ºC into a sticky semi-solid extract. This crude extract was subjected to solvent-solvent partitioning by the modified Kupchan method.[14] The crude extract (5.89g) was triturated with 90% methanol. The prepared solution was then fractionated using solvents of increasing polarity, such as n-hexane (n-hex), dichloromethane (DCM), chloroform ($CHCl_3$), ethyl acetate (EA). All of the extracts were dried in rotary evaporators at 40°C before being stored in airtight containers for further analysis.

Moisture and ash contents. Fresh leaves of *T. portulacastrum* were taken in previously weighted porcelain crucibles and heated at 105ºC in a muffle furnace. This action was performed for three specimens. After heating, the residue was cooled to room temperature and weighted. The percentage of moisture was calculated from the mean value of the three specimens. After the moisture content measurement, these three specimens were then incinerated for 3 hours at 750ºC, cooled in a desiccator, and weighed. The ash content was calculated from the residue weight with respect to the weight of the sample.[15]

Phytochemical screening. The methanolic extract of *T. portulacastrum* was screened to detect the presence of alkaloids, phenols, tannins, saponins, flavonoids, anthraquinones, and tri-terpenoids based on the protocols available in the literature.[16]

Analysis of fatty acids. Fatty acid analysis was carried out for the n-hex extract of *T. portulacastrum*. The bound fatty acids (BFAs) and free fatty acids (FFAs) were both extracted from the plant leaves and converted into their corresponding methyl esters.[17] The prepared methyl esters of BFA and FFA were

analyzed by GLC (Shimadzu 9A, column-BP-50, Detector-FID, 170ºC-1min/ 4ºC - 270ºC - 30 min). The amount of BFAs and FFAs in the sample was measured and the relative percentages of each acid were calculated from the peak area of the gas chromatograms.

Cytotoxicity assay. Different extracts (MeOH, n-hex, DCM, EA, and AQ) of *T. portulacastrum* leaves were subjected to cytotoxicity assay against Vero (a kidney epithelial cell extracted from an African green monkey), HeLa (a human cervical carcinoma cell), and BHK-21 cell lines (a baby hamster kidney fibroblast cell) using Biological Bio Safety Cabinet (Model: NU-400E, Nuaire, USA), CO_2 Incubator (Nuaire, USA), Trinocular microscope with a camera (Optika, Italy) and Hemocytometer. The extracts were dissolved in 2.5% DMSO individually. The three cell lines were kept in DMEM (Dulbecco's Modified Eagles' medium) with 1% penicillin-streptomycin (1:1) and 0.2% gentamycin, as well as 10% fetal bovine serum (FBS). Vero cells (1.5×10^4/100 µl), HeLa Cells (2×10^4/100 µl), and BHK-21 cells (1.5×10^4/100 µl) were seeded onto a 96-well plate and incubated at 37 ºC + 5% CO_2. The next day, 25 µl of the sample (filtered) was added to each well. After 48 hours of incubation, cytotoxicity was examined under an inverted light microscope. For each sample, duplicate wells were used.[18]

Antimicrobial activity. Antimicrobial screening of the crude MeOH extract and different fractions (n-hex, DCM, EA, and AQ) was carried out against a gram-negative (*Escherichia coli*) and a gram-positive (*Staphylococcus aureus*) bacteria using DMSO as a control. The antimicrobial activities of different extracts were determined by their ability to inhibit the growth of microorganisms in discs that provide a zone of inhibition.[19]

Evaluation of antioxidant activity

Total phenolic content. The total phenolic content of various extracts of the sample (*T. portulacastrum* leaves) was determined using Folin-Ciocalteu's reagent with minor modifications to the method described by Singleton and Rossi.[20,21] 1mg/ml of each extract of the sample was taken in separated test tubes. Each test tube received 5ml Folin-Ciocalteu's reagent (1:10 v/v distilled water) and 4ml sodium bicarbonate solution (75 g/l). The solutions were then vortexed to ensure proper mixing and allowed to stand at 40°C for 30 min to develop color. The absorbance of each solution was determined using a double beam spectrophotometer (SHIMADZU UV-1800) in comparison to a blank, at a wavelength of 765 nm. Standard solutions of gallic acid of concentrations 6.25, 12.5, 25, 50, 100, 200, and 400µg/ml were prepared using gallic acid instead of extracts. The total phenolic content of each extract was expressed as mg GAE (gallic acid equivalent)/g of dry extract using a standard gallic acid calibration curve.

Total flavonoid content. The extracts' total flavonoid content was determined using the aluminum chloride colorimetric method.[22] Quercetin was used to make the standard calibration curve for determining total flavonoid content. 5 ml of each extract solutions (1 mg/ml) were mixed with 2.5 ml $AlCl_3$ reagent (2% $AlCl_3$ and 1 mol/l NaOAc). The mixtures were vortexed and allowed to settle at room temperature for 30 min. The absorbance of the reaction mixtures was determined using a UV-Vis spectrophotometer against a blank at 430 nm. The total flavonoid content of each extract was determined and expressed as mg QE (quercetin equivalent)/g of dry extract using the standard quercetin calibration curve.

Total antioxidant capacity. The total antioxidant capacity of the sample *T. portulacastrum* extracts was evaluated by the phosphomolybdenum assay method.[23,24] The reagent solution (3 ml) was allowed to mix with 0.3 mL of each extract (0.6 mol/l H_2SO_4, 28 mmol/l Na_3PO_4, 4 mmol/l ammonium molybdate). The reaction mixture was incubated at 95°C for 90 minutes. After cooling the solution to room temperature, the absorbance of each solution was measured at 695 nm using a UV-Vis spectrophotometer in comparison to a blank solution containing the same amount of solvent (ascorbic acid) but without the presence of extract. Using a standard ascorbic acid calibration curve, the total

antioxidant capacity was determined and expressed as mg ascorbic acid equivalents per gram of dry extract.

DPPH free radical scavenging activity. The antioxidant activity of plant extracts is measured using DPPH (1, 1-diphenyl-2-picrylhydrazyl), a stable organic free radical with a deep violet color.[25] The free radical scavenging activities of the sample extracts on the DPPH radical were estimated by the method introduced by Brand-Williams.[26] 2.0 ml of the methanol solution of each extract were mixed with 2.0 ml of the DPPH methanol solution. The mixtures were kept at dark for 30 min and the absorbance of each solution was measured using a double beam UV-Vis spectrophotometer against a blank at 517 nm. The antioxidant potential was determined by the bleaching of the purple-colored solution of DPPH radical by the sample extracts as compared to that of the standard butylated hydroxytoluene (BHT). The % inhibition of the sample was calculated using the following equation:

$$\% \text{ Inhibition} = (1 - A_{sample}/A_{control}) \times 100$$

Where, $A_{control}$ and A_{sample} indicate the absorbance of the DPPH methanol solution and the reaction mixture respectively. A plot comparing percent inhibition to extract concentration yielded the effective dose required to neutralize 50% of the DPPH radical solution (IC_{50}).

RESULTS AND DISCUSSION

Extractive value. Extractive values give an idea about the chemical constituents of crude drugs and also help in estimating definite constituents soluble in a particular solvent. The extractive values are shown in Figure 1.

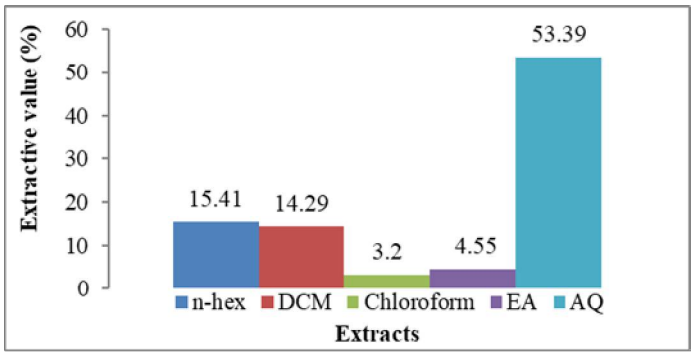

Figure 1. Extractive values of the sample in different solvents.

The results indicate that the sample contains most of the extractable compounds in polar aqueous fractions. The highest proportion of aqueous fraction (53.39%) indicates that the sample contains a higher number of polar elements, such as phenolics and flavonoids, which are responsible for antimicrobial, antioxidant, and other biological activities. DCM and n-hexane fractions have moderate extractive values which imply the presence of non-polar secondary metabolites also.

Moisture and ash contents. The shelf life of any material is determined by the moisture content. The results showed that the research plant possesses high moisture content (81.74±0.49) %. In another investigation, the moisture content of this plant was estimated to be 90%.[27] The ash content value of the leaves is (31.05±1.36) % which refers that it contains inorganic material in high proportion. The presence of inorganic minerals is important because the amount of minerals determines physiochemical qualities, and these minerals can be employed to treat a variety of ailments.[28]

Phytochemical screening. The results of phytochemical screening of the methanolic leaves extract showed the presence of various secondary metabolites such as alkaloids, tannins, flavonoids, and tri-terpenoids which are shown in Table 1.

These phytochemicals have a wide range of pharmacological and biochemical effects. Alkaloids

are plant-derived chemicals that act as a repellent to predators and parasites. Plants that contain alkaloids are also effective pain relievers. Tannins, which are astringent in nature, are used to treat digestive illnesses such as diarrhea and dysentery.[29] Flavonoids are powerful anticancer agents that scavenge free radicals and protect cells from oxidative damage.[30] Plant terpenoids are also useful for their aromatic qualities and play a role in traditional herbal medicines.[31] This study shows that *T. portulacastrum* leaves are rich in phytochemicals and their use should be strongly recommended for health purposes.

Fatty acid analysis. The fatty acid composition analysis of *T. portulacastrum* leaves is a very new work with respect to the literature. Fatty acids are important components having biological and functional roles.[32] The n-hexane extract was used for the analysis of fatty acid composition and the amount of BFA and FFA obtained are shown in Figure 2.

The percentage of FFA in the extract is moderately high which indicates that the sample if converted to oil form may be degraded easily in presence of lipolytic enzymes. The compositions of different fatty acids were determined by GC analysis of FFA and BFA. The results are shown in Figure 3 and Figure 4.

Table 1. List of phytochemicals present in *T. portulacastrum*.

Phytochemicals	Alkaloids	Tannins	Flavonoids	Triterpenoids
Inference	+	+	+	+

Where the (+) sign indicates the presence of phytochemicals.

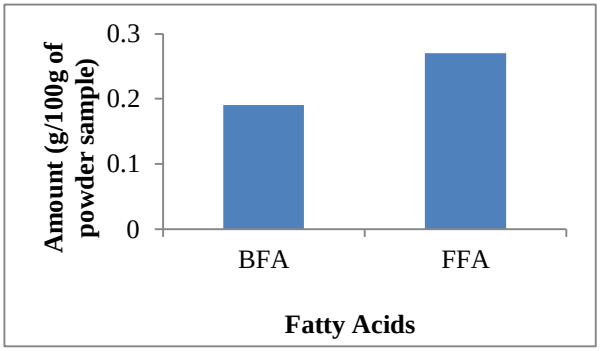

Figure 2. Amount of BFA and FFA in the sample.

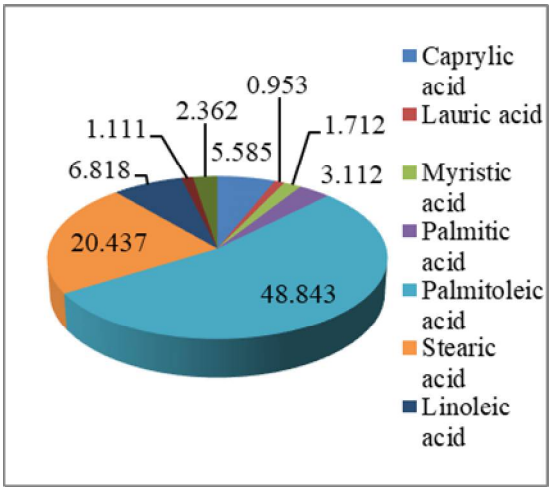

Figure 3. Relative percentages of FFA.

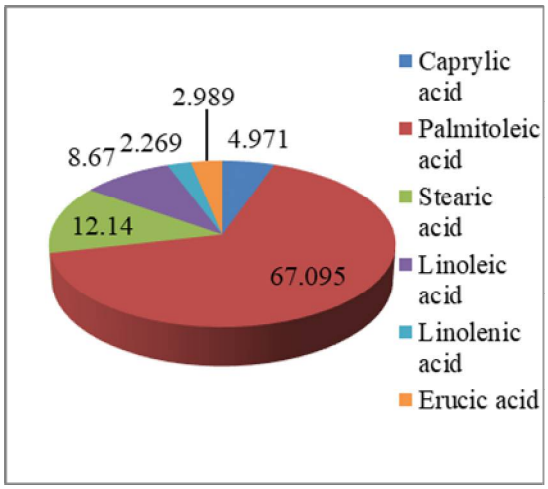

Figure 4. Relative percentages of BFA.

From the fatty acid composition analysis, it was found that the plant contains a high amount of palmitoleic acid (48.843% and 67.095%) which is an important monounsaturated fatty acid for pharmaceutical applications. It was also reported to have anti-thrombotic properties, which could aid in stroke prevention.[33] The plant also contains a moderate proportion of stearic acid (20.437% and 12.14%) which have commercial benefits as it is used to manufacture products like emulsifiers, lubricants, ointments, lotions, emollients, detergents, and so on. Linoleic acid present in the plant is helpful to reduce LDL cholesterol.[34] Linolenic acid is an essential fatty acid that belongs to the omega-3 fatty acids group.[35] Therefore, it can be proposed that the plant *T. portulacastrum* can be a major source of obtaining useful fatty acids and commercial production of fatty acids.

It was also found that both the BFA and FFA contain a high proportion of unsaturated fatty acids and a relatively low percentage of saturated acids, which indicates that consumption of this plant will be advantageous for the human body. Various studies have shown that eating food, rich in unsaturated fats instead of saturated fats lower blood cholesterol levels, which can help to reduce the risk of heart diseases and type 2 diabetes.[36]

Cytotoxicity. The analysis was carried out against three cell lines (Vero, HeLa, and BHK-21) and the results are shown in Table 2.

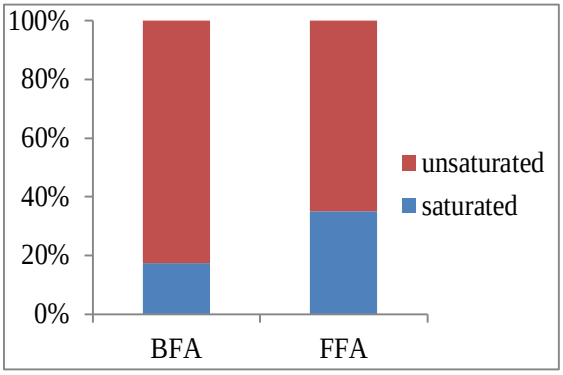

Figure 5. Percentage of saturated and unsaturated acids present in FFA and BFA.

Table 2. Cytotoxicity analysis for different extracts of *T. portulacastrum* dissolved in DMSO (2.5%).

Sample	Survival of cells			Observation
	Vero	HeLa	BHK-21	Cell cytotoxicity was observed for sample DCM and MeOH on the HeLa cell line.
Solvent (-)	100%	100%	100%	
DMSO (solvent) (+)	>95%	>95%	>95%	
n-hex	>95%	>95%	>95%	
DCM	>95%	<5%	>95%	
MeOH	>95%	10%-20%	>95%	
AQ	>95%	>95%	>95%	
EA	>95%	>95%	>95%	

Cytotoxicity assay showed that the fractions are not toxic for Vero and BHK-21 cell lines which indicate that the research plant can be used for the dietary purpose. DCM and MeOH fractions showed

cytotoxicity on the HeLa cell line which is a human cervical carcinoma cell line. These results imply that the use of *T. portulacastrum* as an anti-cancer drug might appear to be a great convenience.

Antimicrobial activity. The crude methanol extracts and different fractions i.e., n-hex, DCM, EA, and AQ were subjected to antimicrobial screening with a concentration of 10 mg/mL/disc in every case. No samples showed a zone of inhibition against *E. coli* and *S. aureus* in this concentration according to the disc diffusion method. A better result may be obtained by varying the concentration of the fractions.

Total phenolic content. Phenolic antioxidants are highly significant plant constituents as they act as free radical scavengers. Hydroxyl groups are responsible for their ability to scavenge free radicals.[37] Total phenolic contents of different extracts are represented in Table 3.

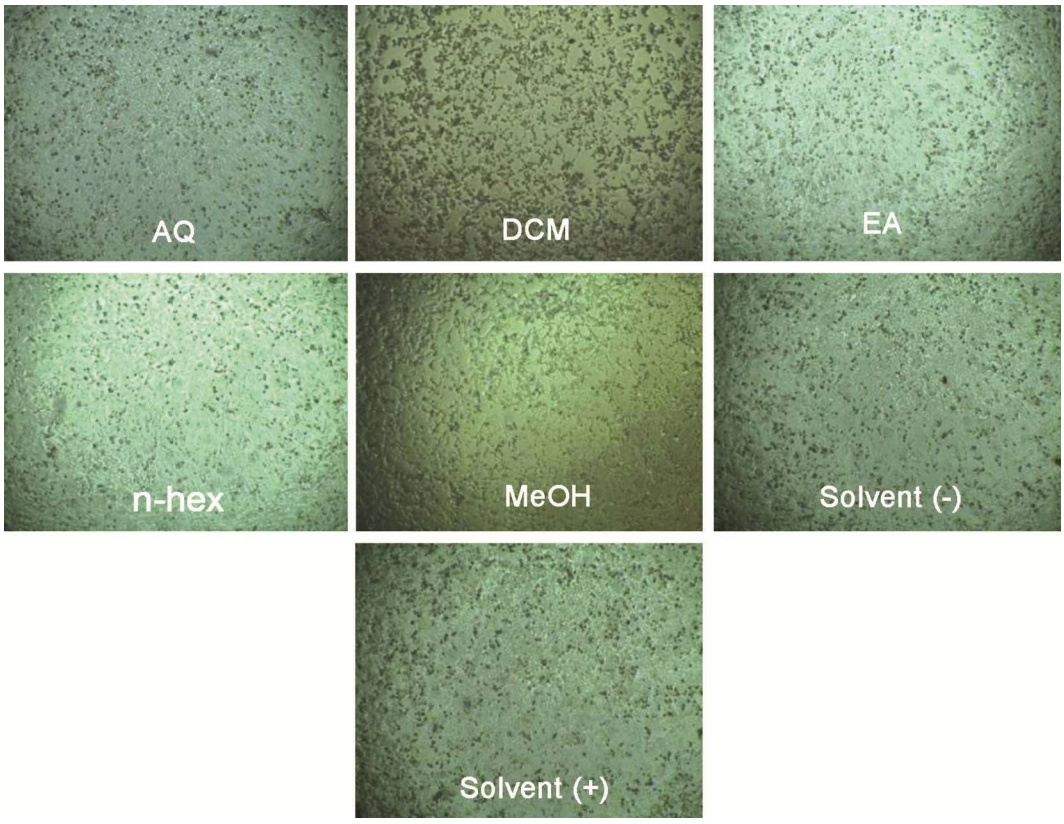

Figure 6. Cytotoxic assay of different extracts of *T. portulacastrum* against HeLa cell line.

Table 3. Total phenolic content (mean ± SD, n=3) of different extracts of *T. portulacastrum*.

Extracts of *T. portulacastrum*	MeOH	n-hex	DCM	$CHCl_3$	EA	AQ
TPC (mg/g)	10.71 ± 0.095	7.73 ± 0.065	35.06 ± 0.075	18.19 ± 0.061	68.55 ± 0.038	10.58 ± 0.061

Among the five fractions, the EA extract of *T. portulacastrum* was found to contain the maximum amount of total phenolics (68.55 ± 0.038mg GAE/g of dry extract). The n-hex extract was found to contain the lowest amount of phenolic content (7.73 ± 0.065mg GAE/g of dry extract accordingly). The DCM and $CHCl_3$ extracts were found to contain moderate amounts of phenolic content. As the EA

extract revealed the presence of the highest amount of phenolic content, it may be assumed that this extract has promising antioxidant activity.[38] It might be assumed that the presence of phenolics is responsible for the claimed therapeutic applications of *T. portulacastrum.*

Total flavonoid content. Flavonoids are polyphenols that have strong antioxidant qualities due to their reducing ability when it comes to neutralizing free radicals, scavenging radicals.[39] Flavonoids are also been proven to provide antidiabetic, anticancer, anti-inflammatory, cardioprotective, anti-aging, and other health benefits.[40] The total flavonoid contents of the different extracts are represented in Table 4.

Among the extracts, the $CHCl_3$ extract of *T. portulacastrum* was found to contain the maximum amount of total flavonoid (477.01 ± 0.104mg QE/g of dry extract). The AQ extract was found to contain the lowest amount of flavonoid content (66.38 ± 0.266mg QE/g of dry extract accordingly). The other extracts contain a moderate amount of flavonoid content. As the total flavonoid content of $CHCl_3$ extract is high, it can be estimated that the extract contains flavonoids which are polar compounds with antioxidant activity.

Total antioxidant capacity. Antioxidants' primary purpose is to protect other molecules from being oxidized by free radicals by inhibiting the initiation or propagation of oxidizing chain reactions, and they may help to decrease oxidative damage to the human body.[41] The total antioxidant capacities of the different extracts are represented in Table 5.

Table 4. Total flavonoid content (mean ± SD, n=3) of different extracts of *T. portulacastrum*.

Extracts of *T. portulacastrum*	MeOH	n-hex	DCM	$CHCl_3$	EA	AQ
TFC (mg/g)	170.56 ± 0.099	234.96 ± 0.176	103.4 ± 0.198	477.01 ± 0.104	143.33 ± 0.202	66.38 ± 0.266

Table 5. Total antioxidant capacity (mean ± SD, n=3) of different extracts of *T. portulacastrum*.

Extracts of *T. portulacastrum*	MeOH	n-hex	DCM	$CHCl_3$	EA	AQ
TAC (mg/g)	164.42 ± 0.118	259.31 ± 0.304	284.99 ± 0.113	241.75 ± 0.316	172.32 ± 0.104	28.32 ± 0.188

Table 6. IC_{50} values of standard butylated hydroxytoluene (BHT) and fractions of the leaves of *T. portulacastrum*.

Fractions	BHT	MeOH	n-hex	DCM	$CHCl_3$	EA	AQ
IC_{50}	280.59±0.362	455.15±1.885	427.64±0.746	480.41±1.414	317.01±2.219	110.35±0.160	374.47±1.525

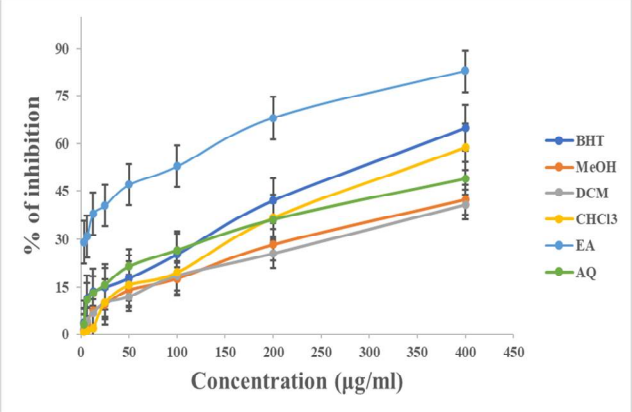

Figure 7. DPPH free radical scavenging activity of different fractions of *T. portulacastrum* leaves.

The DCM fraction of *T. portulacastrum* leaves was found to have the maximum amount of total antioxidant capacity (284.99± 0.113mg ascorbic acid equivalent per gram of dry extract). On the contrary, the AQ extract was found to contain the lowest amount of total antioxidant capacity (28.32 ± 0.188mg ascorbic acid equivalent per gram of dry extract accordingly). MeOH, n-hex, $CHCl_3$ and EA fractions showed 164.42, 259.31, 241.75, 172.32 mg/g antioxidant capacity respectively. All of the fractions were found to possess a significant amount of total antioxidant capacity.

DPPH free radical scavenging activity. The DPPH antioxidant assay is based on the scavenging of DPPH radicals.[42] The DPPH free radical-scavenging by antioxidants is due to their hydrogen-donating ability.[43] The IC_{50} of the standard and different extracts are represented in Table 6.

In the DPPH method, the IC_{50} values differ in different fractions are ranging from (110.35±0.160 µg/ml) to (480.41±1.414 µg/ml). Among the fractions, EA showed the highest free radical scavenging activity with an IC_{50} value of 110.35±0.160 µg/ml as compared to the IC_{50} value of standard BHT (280.56±0.362 µg/ml). $CHCl_3$ fraction also showed higher activity with an IC_{50} value of 317.01±2.219 µg/ml compared to the BHT.

CONCLUSION

Phytochemical screening of *T. potulacastrum* has shown the presence of various important secondary metabolites. Exploration of the individual phytochemicals found in *T. portulacastrum* extracts could lead to more effective and commercially available therapies for the prevention of a variety of diseases. The presence of palmitoleic acid and other fatty acids was found in the fatty acid analysis. This study is expected to provide useful information for ongoing research into this intriguing species and its potential as a source of beneficial fatty acids. The cytotoxicity assay has shown the presence of cytotoxicity in the extracts. Evaluation of the cytotoxic activity of the plant on other carcinoma cells and determination of its effective and lethal doses could pave the way to the initiation of a natural cure for life-threatening cancers. Total flavonoids and total phenolics were found to be highest in EA and $CHCl_3$ extract respectively. The DCM fraction of *T. portulacastrum* leaves showed the maximum amount of total antioxidant capacity and the EA extract showed the highest free radical scavenging activity. Due to its vast availability in all seasons, harsh environments, and ease of collecting at low cost**,** the plant could serve as a 'lead' for the development of novel drugs with good efficacy in many illnesses in the future.

ACKNOWLEDGEMENT

The first author wishes to express his sincere gratitude to the Ministry of Science and Technology, Government of the People's Republic of Bangladesh for supporting this research through the NST fellowship program.

REFERENCES

1. Alves, R. and Rosa, I.M. 2007. Biodiversity, traditional medicine and public health: where do they meet? *J. Ethnobiol. Ethnomed.* **3**, 1-9.
2. Denthamil Selvan, P., Vellavan, D.S. and Sakunthala, P. 2015. Analysis of phytochemical component and nutrients component in ethanol extracted *Oldenlandia corymbosa*. *World J. Pharm. Res*. **4**, 1960-1967.
3. Abe, R., and Ohtani, K. 2013. An ethnobotanical study of medicinal plants and traditional therapies on Batan Island, the Philippines. *J. Ethnopharmacol*. **145**, 554-565.
4. Shivhare, M.K., Singour, P.K., Chaurasiya, P.K. and Pawar, R.S. 2012. *Trianthema portulacastrum* Linn. (bishkhapra). *Pharmacogn. Rev*. **6**, 132.
5. Balyan, R.S. and Bhan, V.M. 1986. Emergence, growth, and reproduction of horse purslane (*Trianthema portulacastrum*) as influenced by environmental conditions. *Weed Sci.* **34**, 516-519.
6. Johnson, D.H., Jordan, D.L., Johnson, W.G., Talbert, R.E. and Frans, R.E. 1993. Nicosulfuron, primisulfuron, imazethapyr, and DPX-PE350 injury to succeeding crops. *Weed Technol*. **7**, 641-644.
7. Chowdhury, S.S., Tareq, A.M., Tareq, S.M., Farhad, S. and Sayeed, M.A. 2021. Screening of antidiabetic and antioxidant potential along with phytochemicals of *Annona* genus: a review. *Future J. Pharm. Sci.* **7**, 1-10.

8. Kirtikar, K.R. and Basu, B.D. 2003. In: *Indian medicinal plants with illustration.* International Book Distributors, Dehradun, 2nd edition, Volume 11, pp. 3747-3749.
9. Abd El-Gawad, A.M., El Gendy, A.G., Elshamy, A.I. and Omer, E.A. 2016. Chemical composition of the essential oil of *Trianthema portulacastrum* L. Aerial parts and potential antimicrobial and phytotoxic activities of its extract. *J. Essent. Oil-Bear. Plants.* **19**, 1684-1692.
10. Mandal, A. and Bishayee, A. 2015. *Trianthema portulacastrum* Linn. displays anti-inflammatory responses during chemically induced rat mammary tumorigenesis through simultaneous and differential regulation of NF-κB and Nrf2 signaling pathways. *Int. J. Mol. Sci.* **16**, 2426-2445.
11. Vohora, S.B., Shah, S.A., Naqvi, S.A.H., Ahmad, S. and Khan, M.S.Y. 1983. Studies on *Trianthema portulacastrum*. *Planta Med*. **47**, 106-108.
12. Ara, A., Akram, A., Ajmal, M., Akhund, S. and Nayyar, B. G. 2021. Pharmacological, nutritional and allelopathic attributes of noxious weed, *Trianthema portulacastrum* L. (Horse purslane). *Pure Appl. Biol*. **4**, 340-352.
13. Putnam, A.R. and Weston, L.A. 1986. Adverse impacts of allelopathy in agricultural systems. *The Science of Allelopathy*, pp. 43-56.
14. Ramjan, A., Hossain, M., Runa, J.F., Md, H. and Mahmodul, I. 2014. Evaluation of the thrombolytic potential of three medicinal plants available in Bangladesh, as a potent sources of thrombolytic compounds. *Avicenna J. Phytomed.* **4**, 430.
15. Hassan, L.G. and Umar, K.J. 2006. Nutritional value of Balsam Apple (*Momordica balsamina* L.) leaves. *Pak. J. Nutr*. **5**, 522-529.
16. Pande, J., Kanakiya, A., Padalia, H. and Chandra, S. 2018. Physicochemical, phytochemical and pharmacognostic evaluation of a halophytic plant, *Trianthema portulacastrum* L. *Int. J. Curr. Microbiol. App. Sci*. **7**, 1486-502.
17. Al Mamun, M.R., Ahmed, T., Reza, M.S.A. and Rahman, M. H. 2021. Phytochemical investigation, fatty acid analysis and *in vitro* membrane stabilizing activity of the roots of *Amaranthus spinosus* L. *Dhaka Univ. J. Sci.* **69,** 59-62.
18. Reza, M.S.A., Rahman, M.H. and Chowdhury, T.A. 2020. Isolation of secondary metabolites from *Leucas aspera* and investigation of biological activity. *Dhaka Univ. J. Sci.* **68**, 101-104.
19. Shandhi, S.P., Roy, A.C., Rahman, H. and Chowdhury, T.A. 2020. Isolation of alkaloids chabamide I, piperine and chingchengenamide A: from *Piper chaba* H. leaves and their *in vitro* antimicrobial activities. *J. Pharmacogn. Phytochem.* **9**, 2811-2814.
20. Singleton V.L. and Rossi J.A. 1965. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *Am. J. Enol. Vitic*. **16**, 144–158.
21. Rahman, M.H., Rashid, M.A. and Chowdhury, T.A. 2019. Studies of biological activities of the roots of *Bombax ceiba* L. *Bangladesh Pharm. J.* **22**, 219-223.
22. Chang, C.C., Yang, M.H., Wen, H.M. and Chern, J C. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Anal.* **10**, 3.
23. Zou, Y.P., Y.H. Lu and D.Z. Wei 2004. Antioxidant activity of a flavonoid-rich extract of *Hypericum perforatum* L. *in vitro*. *J. Sci. Food Agric*. **52**, 5032–5039.
24. Rahman, M., Rahman, M.H. and Chowdhury, T.A. 2020. Phytochemical and biological activity studies of *Tinospora crispa* stem. *Dhaka Univ. J. Sci.* **68**, 167-170.
25. Ilahi, Samar S, Khan I and Ahmad I. 2013. *In vitro* antioxidant activities of four medicinal plants on the basis of DPPH free radical scavenging. *Pak. J. Pharm. Sci*. **26**, 949-952.
26. Molyneux, P. 2004. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin. J. Sci. Technol*. **26**, 211-219.
27. Gupta, S., Lakshmi, A.J., Manjunath, M.N. and Prakash, J. 2005. Analysis of nutrient and antinutrient content of underutilized green leafy vegetables. *Food Sci. Technol.* **38**, 339-345.
28. Soetan, K.O., Olaiya, C.O. and Oyewole, O.E. 2010. The importance of mineral elements for humans, domestic animals and plants-a review. *Afr. J. Food Sci.* **4**, 200-222.
29. Eyong, E.U., Agiang, M.A., Atangwho, I.J., Iwara, I.A., Odey, M.O., and Ebong, P. E. 2011. Phytochemicals and micronutrients composition of root and stem bark extracts of *Vernonia amygdalina* Del. *J. Med. Sci.* **2**, 900-903.
30. Benavente-Garcia, O. and Castillo, J. 2008. Update on uses and properties of citrus flavonoids: new findings in anticancer, cardiovascular, and anti-inflammatory activity. *J. Agric. Food Chem.* **56**, 6185-6205.
31. Cox-Georgian, D., Ramadoss, N., Dona, C. and Basu, C. 2019. Therapeutic and medicinal uses of terpenes. In: *Medicinal Plants.* Springer, Cham, pp. 333-359.
32. Nagy, K. and Tiuca, I.D. 2017. Importance of fatty acids in physiopathology of human body. In: *Fatty acids*. Intech. pp. 3-22.
33. Hu, W., Fitzgerald, M., Topp, B. Alam, M., and O'Hare, T. J. 2019. A review of biological functions, health benefits, and possible de novo biosynthetic pathway of palmitoleic acid in macadamia nuts. *J. Funct. Foods*. ***62***, 103520.
34. Weststrate, J.A. and Meijer, G.W. 1998. Plant sterol-enriched margarines and reduction of plasma total-and LDL-cholesterol concentrations in normocholesterolaemic and mildly hypercholesterolaemic subjects. *Eur. J. Clin. Nutr*. **52**, 334-343.
35. Uddin, M., Juraimi, A.S., Hossain, M.S., Un, A., Ali, M. and Rahman, M.M. 2014. Purslane weed (*Portulaca oleracea*): a prospective plant source of nutrition, omega-3 fatty acid, and antioxidant attributes. *Sci. World J.* **2014**, 6.
36. Willett, W.C. 2012. Dietary fats and coronary heart disease. *J. Intern. Med.* **272**, 13-24.

37. Ghafar, F., Nazrin, T.T. N.N., Salleh, M.M.R., Hadi, N.N., Ahmad, N., Hamzah, A. A. and Azman, I.N. 2017. Total phenolic content and total flavonoid content in *Moringa oleifera* seed. *Sci. Herit. J.* **1**, 23-25.

38. Sarikurkcu, C., Uren, M.C., Tepe, B., Cengiz, M., and Kocak, M.S. 2014. Phenolic content, enzyme inhibitory and antioxidative activity potentials of *Phlomis nissolii* and *P. pungens* var. *pungens*. *Ind. Crops Prod*. **62**, 333-340.

39. Ha, P.T.T., Tran, N.T.B., Tram, N.T.N. and Kha, V.H. 2020. Total phenolic, total flavonoid contents and antioxidant potential of common bean (*Phaseolus vulgaris* L.) in Vietnam. *Agric. Food*. **5**, 635-648.

40. Ganesan, K. and Xu, B. 2017. Polyphenol-rich dry common beans (*Phaseolus vulgaris* L.) and their health benefits. *Int. J. Mol. Sci.* **18**, 2331.

41. Ismail, A., Marjan, Z.M. and Foong, C.W. 2004. Total antioxidant activity and phenolic content in selected vegetables. *Food Chem.* **87**, 581-586.

42. Sharma, O.P. and Bhat, T.K. 2009. DPPH antioxidant assay revisited. *Food Chem.* **113**, 1202-1205.

43. Singh, N. and Rajini, P.S. 2004. Free radical scavenging activity of an aqueous extract of potato peel. *Food Chem.* **85**, 611-616.