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QUANTITATIVE ESTIMATION OF TOTAL PHENOLIC CONTENT OF TWO SPECIES OF PORTULACA OBTAINED BY USING MICROWAVE ASSISTED EXTRACTION AND ITS VALIDATION

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ABSTRACT: *Portulaca oleracea* and *Portulaca quadrifida* have been used traditionally have been used for their pharmacological properties and consumed as a part of the diet. Anti-oxidative property of phytochemicals might play a role in their observed benefits, and phenolic compounds are known to be the major contributors towards the anti-oxidant property of phytochemicals. The objective of this study was to quantify the total phenolic content of extracts obtained by microwave extraction of these plant species using a validated technique. The plant species were collected and authenticated. Microwave-assisted extraction of separate plant parts using different solvents was carried out. The total phenolic content was measured using a modified Folin-Ciocalteau method, and the method was validated for linearity, range, limit of detection, limit of quantification, recovery, and precision. Amongst the tested extracts, the ethanolic extract of *P. oleraceae* seed contained the highest amount of total phenolic compounds. The stated method for quantifying total phenolic compounds was found to be precise and reliable, and the further study of the anti-oxidant property of these two plant species need to be conducted.

INTRODUCTION: *Portulaca oleraceae* L. (Family: Portulacaceae), commonly known as purslane, might have originated in Asia and spread to other parts of the world including Africa and the Mediterranean region. The plant belongs found to grow like a weed, turf- grass, or field- crop [1-4]. It is primarily consumed as a part of a salad, soup, or pickles [4]. The plant comprises of carbohydrates, protein, fats, minerals, and vitamins.

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Phytochemical analysis of the plant revealed the presence of tannins, saponins, oxalate, urea, alkaloids, sitosterols, mono, di and triterpenes, phenolic compounds and omega-3 fatty acids. Reported pharmacological activities of the plant include anticancer, antidiabetic, hypocholesteremic, neuroprotective, hepatoprotective, nephroprotective, anti-inflammatory, antiulcer, antimicrobial, and anti-oxidant activity. Traditionally, it has been used for wound healing, uterine bleeding control and wormicidal and insecticidal activities [5].

Portulaca quadrifida Chepahije also belongs to the family Portulacaceae and is commonly called as chickweed. It is found to grow in tropical parts of India as a small diffused, succulent, annual herb. It is used as a vegetable for preparation of salad or

soup and is used in traditional systems of medicine for treatment of asthma, cough, urinary discharges, inflammations and ulcers, hemorrhoids. Phytochemical constituents of the plant include alkaloids, saponins, flavonoids, triterpenoids/ steroids, tannins and glycosides [6].

Free radicals are generated in the human body as a result of various biochemical reactions [7]. However, the body's defense mechanism of enzymes such as catalases, superoxide dismutases, peroxidases, and glutathione cycling mechanism ensure that the free radicals are scavenged before they can cause any deleterious effects to the biomolecules. An unbalanced production of free radicals, however, is observed in many disorders such as atherosclerosis, arthritis, Alzheimer disease, cancer, *etc*. Reactive oxygen species (ROS) and nitrogen (RNS) species are the most frequent free radicals generated either by normal metabolism or are induced by external factors. These free radicals react with biomolecules leading to lipid peroxidation, complex formation with polypeptides and nucleic acids, *etc*. [7-9] Such ill effects of free radicals can be prevented by intake of antioxidant substances. These antioxidant substances have been reported in plant materials and artificially synthesized supplements. Since these plant materials containing antioxidants can be consumed as part of our diet, they offer greater benefit as compared to synthetic ones [9-15]. As can be seen from the above-mentioned phytoconstituents found in *P. oleraceae* and *P. quadrifida*, they contain a variety of phenolic compounds. As such, their chemical composition needs to be investigated as a potential source of anti-oxidant compounds [16].

To investigate the phytochemical phenolic compounds, different extraction techniques are employed. One of these techniques is microwave assisted extraction which interestingly offers advantages over Soxhlet extraction and maceration. The most noticeable one being volumetric heating of sample which leads to a reduction in time and solvent consumed [17].

MATERIAL AND METHODS:

Material: Gallic acid, Folin Ciocalteu reagent, and Sodium bicarbonate were purchased from Sisco Research Laboratories. Ethanol was purchased from SD Fine Chem Limited. The microwave system manufactured by Catalyst and spectro-photomter manufactured by Shimadzu was used.

METHODS:

Collection of Plant: The plants *Portulaca oleraceae* L. and *Portulaca quadrifida* Chepahije were collected from a local farm of Walva taluka, District Sangli, State Maharashtra where they were growing like a weed. The plant specimens were authenticated by Dr. Dhanaji S. Pawar, Associate Professor, Department of Botany, M. H. Shinde Mahavidyalaya, Tisangi, State Maharashtra with voucher number V01 for *P. oleraceae* and voucher number V04 for *P. quadrifida*. Some of the *P. oleraceae* plants were dried in the shade, and their seeds were separated and stored for further use.

Extraction: Microwave-assisted extraction was carried out in a controlled Catalyst microwave system having maximum power output 800 Watt, 50 gram (g) sample and 120 milliliters (ml) solvent for 20 min [17]. The extracts obtained were as follows-

- ✓ Aqueous extract, ethanolic extract, methanolic extract and butanolic extract of *Portulaca oleraceae* whole plant.
- ✓ Ethanolic *Portulaca oleraceae* dry whole plant.
- ✓ Ethanolic *Portulaca quadrifida* fresh whole plant.
- ✓ Ethanolic *Portulaca oleraceae* seed.

The extracts were evaporated to dryness and stored at minus 20 degree Celsius (°C) deep freezer until required.

Estimation of Total Phenolic Content:

Preparation of Standard Solution: Gallic acid was used as the standard which represents the phenolic compound in the plant specimen. 10 milligram (mg) of gallic acid monohydrate was dissolved in 100 ml of methanol to give a concentration of 100 microgram/ml (µg/ml).

Preparation of Calibration Curve: Aliquots of 0.1, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, 0.50. ml from the above stock solution were taken in six different 10 ml volumetric flasks. To each flask 2.5 ml of 1 normal (N) Folin- Ciocalteu reagent and 2 ml of 20 percent (%, weight/volume) sodium carbonate were added.

The mixture was allowed to stand for 15 min and the volume was made up to mark with water to get a concentration ranging from 1-5 µg/ml. The absorbance of the resulting solutions was measured at 765 nanometers (nm) against reagent blank. A standard calibration curve of Gallic acid was prepared by plotting absorbance against concentration.

Preparation of Sample Solution: 10 mg of extract was dissolved in 10 ml of methanol to get 1 mg/ml solution. 0.30 ml of this stock solution was added to a 10 ml volumetric flask, and color development was carried out the same as that for the standard. The absorbance of the test solution was measured at 765 nm against blank. The concentration of total phenol in the test sample was determined by extrapolation from the calibration graph.

Validation of Developed Method:

Linearity and Range: The standard stock solution containing 100 µg/ml each of gallic acid was further diluted to get linearity concentration of 1-5 µg/ml for gallic acid. Each concentration was analyzed in triplicates. A calibration curve was plotted by taking concentration on x-axis and absorbance on the y-axis. The relation between drug and its absorbance is expressed by equation y = mx+b, where m=slop, and b= intercept.

Limit of Detection (L.O.D.) and Limit of Quantification (L.O.Q.): LOD and LOQ of the drug were derived by calculating the signal-to-noise ratio (S/N, 3.3 for LOD and 10 for LOQ) using the following equation as per ICH guidelines. The residual standard deviation of regression line or standard deviation of Y-intercept of regression lines was used to calculate LOD and LOQ.

$$LOD = 3.3 \times D/S$$

$$LOQ = 10 \times D/S$$

Where, D = Standard deviation of blank (n = 8), S = Slope of calibration curve.

Recovery Studies: It was carried out by standard addition method at three different levels. A known amount of drug was added to the pre-analyzed sample, and percentage recoveries were calculated.

Precision: The intra-day precision was determined by estimating the corresponding response 3 times on the same day for gallic acid, whereas the inter-day precision was determined by estimating the corresponding response on 3 different days throughout 1 week. The results are reported in terms of relative standard deviation (R.S.D.).

RESULTS:

Estimation of Total Phenolic Content: The calibration curve of gallic acid as standard over the concentration range of 1 µg/ml to 5 µg/ml was plotted using concentration against absorbance measured at 765 nm as follows **Fig. 1**:

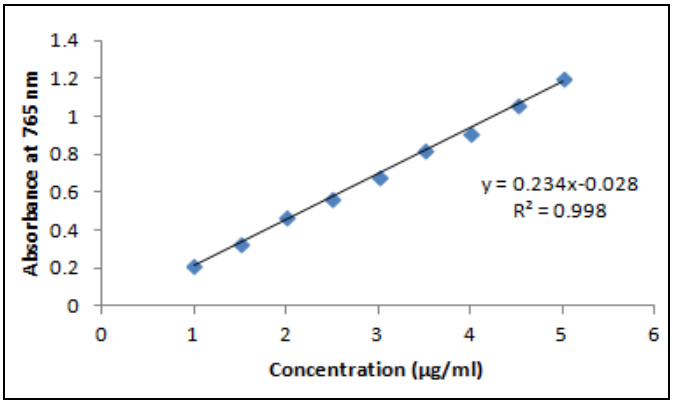

FIG. 1: CALIBRATION CURVE OF STANDARD GALLIC ACID AGAINST ABSORBANCE MEASURED AT 765 nm

Using the equation of the standard curve y = 0.234x-0.028, the total phenolic content of all the extracts was calculated as follows in **Table 1**.

TABLE 1: CALCULATED TOTAL PHENOLIC CONTENT OF DIFFERENT EXTRACTS EXPRESSED AS GALLIC ACID EQUIVALENT (GAE) PER mg OF EXTRACT

S. no.	Sample	Total phenolic content (µg GAE/mg of extract)
1	Aqueous *P. oleraceae* fresh whole plant	6.19 ± 0.21
2	Ethanolic *P. oleraceae* fresh whole plant	15.67 ± 0.82
3	Methanolic *P. oleraceae* fresh whole plant	11.92 ± 0.26
4	Butanolic *P. oleraceae* fresh whole plant	10.64 ± 0.34
5	Ethanolic dry *P.oleraceae*	10.45 ± 0.09
6	Ethanolic fresh *P. quadrifida*	12.92 ± 0.09
7	Ethanolic *P. oleraceae* seed	16 ± 0.05

Expressed as Mean ± standard error of the mean (S.E.M.), n = 3.

Linearity and Range: The linearity of the response for the gallic acid standard was

determined over the range of 1 µg/ml through 5 µg/ml **Fig. 1** and relative standard deviation for replicates was calculated and was found to be below 10% **Table 2**. Hence, the current method was found to be linear over this concentration range of 1 µg/ml through 5µg/ml.

TABLE 2: RELATIVE STANDARD DEVIATION OF ABSORBANCE OF THE REPLICATES OF THE STANDARD OVER THE CONCENTRATION RANGE WITH LINEAR RESPONSE

Conc. (µg/ml)	Absorbance 1	Absorbance 2	Absorbance 3	Mean Absorbance	Standard Deviation (S.D.)	Relative Standard Deviation (R.S.D.)	Mean R.S.D.
1.000	0.201	0.213	0.240	0.218	0.020	9.163	6.556
1.500	0.308	0.380	0.320	0.336	0.039	11.481	
2.000	0.500	0.420	0.500	0.473	0.046	9.758	
2.500	0.550	0.620	0.540	0.570	0.044	7.647	
3.000	0.650	0.750	0.660	0.687	0.055	8.021	
3.500	0.800	0.850	0.830	0.827	0.025	3.044	
4.000	0.880	0.950	0.930	0.920	0.036	3.919	
2.000	1.090	1.110	1.000	1.067	0.059	5.493	
5.000	1.210	1.200	1.200	1.203	0.006	0.480	

Limit of Detection and Limit of Quantification: The L.O.D. and L.O.Q. Values for the current method were calculated as 0.072 µg/ml and 0.218 µg/ml, respectively.

Recovery Studies: The recovery studies were carried by spiking the extracts with the gallic acid standard at three different concentration levels (50%, 100%, 150%). The results are as follows in **Table 3**.

TABLE 3: RECOVERY STUDIES CARRIED OUT BY SPIKING EACH EXTRACT WITH THREE DIFFERENT CONCENTRATIONS OR LEVELS OF GALLIC ACID STANDARD EXPRESSED AS MEAN ± S.E.M. (n = 3)

Extract used	Standard	Amount of standard taken (µg/ml)	Amount of drug added (%)	% Recovery
Aqueous microwave *Portulaca oleraceae* fresh whole plant	Gallic acid	2	50	97.07 ± 0.4
			100	98.12 ± 0.40
			150	98.51 ± 0.51
Ethanolic Microwave *Portulaca oleraceae* fresh whole plant	Gallic acid	2	50	98.27 ± 0.60
			100	97.26 ± 0.85
			150	98.23 ± 0.94
Methanolic Microwave *Portulaca quadrifida* fresh whole plant	Gallic acid	2	50	97.24 ± 0.31
			100	98.41 ± 0.75
			150	98.25 ± 0.81
Microwave butanolic *Portulaca oleraceae* fresh whole plant	Gallic acid	2	50	98.06 ± 0.44
			100	98.40 ± 0.62
			150	98.50 ± 0.50
Ethanolic Microwave *Portulaca oleraceae* dry whole plant	Gallic acid	2	50	98.21 ± 0.30
			100	98.40 ± 0.70
			150	97.31 ± 0.80
Ethanol Microwave *Portulaca quadrifida* fresh whole plant	Gallic acid	2	50	98.31 ± 0.66
			100	97.29 ± 0.89
			150	98.31 ± 0.95
Ethanolic Microwave *Portulaca oleraceae* seed	Gallic acid	2	50	98.23 ± 0.34
			100	97.45 ± 0.70
			150	98.31 ± 0.82

Precision: Precision studies were carried out in two stages of intraday and interday analysis. The relative standard deviation values were calculated as follows in **Table 4**.

TABLE 4: INTER- DAY AND INTRA- DAY PRECISION EXPRESSED AS RELATIVE STANDARD DEVIATION (n = 6)

Sample	Inter- day (% RSD)	Intra- day (% RSD)
Aqueous *P. oleraceae* fresh whole plant	2.14	2.18
Ethanolic *P. oleraceae* fresh whole plant	3.27	3.25
Methanolic *P. oleraceae* fresh whole plant	3.07	3.09
Butanolic *P. oleraceae* fresh whole plant	7.53	7.53
Ethanolic dry *P. oleraceae*	1.97	1.98
Ethanolic fresh *P. quadrifida*	1.59	1.54
Ethanolic *P. oleraceae* seed	0.96	0.95

DISCUSSION: A new simple, rapid, sensitive, precise and economic spectrophotometric method in visible region has been developed for the determination total phenolic content using microwave extraction using aqueous, ethanol, methanol, and butanol as a solvent of

- ✓ *Portulaca oleraceae* fresh whole plant.
- ✓ *Portulaca oleraceae* dry whole plant.
- ✓ *Portulaca quadrifida* fresh whole plant.
- ✓ *Portulaca oleraceae* seed.

These methods obey Beers- Lambert's law in concentration ranges (1-5 µg/mL) employed for evaluation. The result of analysis has been validated statistically, and the Limit of detection and Limit of quantification was within the limit as per International Conference on Harmonization guidelines [18]. Percentage recoveries recovery studies confirmed the accuracy of the proposed method. The total phenolic content of *Portulaca oleraceae* was earlier reported to range from 127 ± 13 to 478 ± 45 mg GAE/100 g fresh weight of plant [19]. Another independent group of researchers reported the total phenolic content to range from 174.5 ± 8.5 to 348.5 ± 7.9 mg GAE/100 g [20]. As regards to *Portulaca quadrifida*, reported preliminary phytochemical investigations to show the presence of phenolic compounds such as alkaloids, tannins, triterpenoids in aqueous as well as ethanolic extracts [21]. The phenolic content of *P. oleraceae* contributes to its nutritive value and as such may vary with growth conditions of the plant [22].

CONCLUSION: The present study reports a validated quantification of total phenolic content of different extracts of *Portulaca oleraceae* and *Portulaca quadrifida* harvested from the Western part of India.

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CONFLICT OF INTEREST: None

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REFERENCES:

1. Masoodi MH, Ahmad B, Mir SR, Zargar BA and Tabasum N: *Portulaca oleracea* L. a review. Journal of Pharmacy Research 2011; 4: 3044-3048.
2. Kamal-Uddin MD, Juraimi AS, Begum Mahfuza, Ismail MR, Rahim AA and Radziah O: Floristic composition of weed community in turfgrass area of West Peninsular Malaysia. International Journal of Agriculture and Biology 2009; 11(1): 13-20.
3. Uddin MK, Juraimi AS, Ismail MR and Brosnan JT: Characterizing weed populations in different turfgrass sites throughout the Klang Valley of Western Peninsular Malaysia. Weed Technology 2010; 24(2): 173-181.
4. Uddin M, Juraimi AS, Hossain MS, Un A, Ali M and Rahman MM: Purslane weed (*Portulaca oleracea*): a prospective plant source of nutrition, omega-3 fatty acid, and antioxidant attributes. The Scientific World Journal of 2014.
5. Syed S, Fatima N and Kabeer G: *Portulaca oleracea* L.: a mini review on phytochemistry and pharmacology. International Journal of Biology and Biotechnology 2016; 13(4): 637-641.
6. Syed KM, Liyakha TA and Swamy P: Neuro-pharmacological effects of ethanolic extract of *Portulaca quadrifida* Linn. in mice. International Journal of PharmTech Research 2010; 2(2): 1386-1390.
7. Weidinger A and Andrey V K: Biological activities of reactive oxygen and nitrogen species: oxidative stress versus signal transduction. Biomolecules 2015; 5(2): 472-484.
8. Nimse SB and Pal D: Free radicals, natural antioxidants, and their reaction mechanisms. Rsc Advances 2015; 5(35): 27986-28006.
9. Zhang H and Rong T: Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects. Current Opinion in Food Science 2016; 8: 33-42.
10. Shahidi F and Priyatharini A: Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects-A review. Journal of functional foods 2015; 18: 820-897.
11. Croft KD: Dietary polyphenols: antioxidants or not? Archives of biochemistry and biophysics 2016; 595: 120-124.
12. Amiot MJ, Riva C and Vinet A: Effects of dietary polyphenols on metabolic syndrome features in humans: a systematic review. Obesity Reviews 2016; 17(7): 573-586.
13. Valdés L: The relationship between phenolic compounds from diet and microbiota: impact on human health. Food & function 2015; 6(8): 2424-2439.
14. Singh RK: Influence of diet on the gut microbiome and implications for human health. Journal of translational medicine 2017; 15(1): 73.

15. Prior RL: Oxygen radical absorbance capacity (ORAC): New horizons in relating dietary antioxidants/bioactive and health benefits. Journal of Functional Foods 2015; 18: 797-810.
16. Pisoschi AM and Aneta P: The role of antioxidants in the chemistry of oxidative stress: a review. European journal of medicinal chemistry 2015; 97: 55-74.
17. T Michel: Microwave-Assisted Extraction in RSC Green Chemistry. University of Nice Sophia Antipolis 2013; 113-156. DOI: 10.1039/9781849737579-00113
18. Validation of analytical procedures: Text and Methodology Q2 (R1).International Conference on Harmonization Tripartite Guideline. Version Step 4.
19. Lim YY and Quah EPL: Antioxidant properties of different cultivars of *Portulaca oleracea*, Food Chemistry 2007; 103(3): 734-740.
20. Uddin M K, Juraimi AS, Ali ME and Ismail MR: Evaluation of antioxidant properties and mineral composition of purslane (*Portulaca oleracea* L.) at different growth stages. International journal of molecular sciences 2012; 13(8): 10257-10267.
21. Mulla SK and Swamy P: Preliminary pharmacognostic and phytochemical evaluation of *Portulaca quadrifida* Linn. International Journal of Pharmtech Research 2010; 2(3): 1699-1702.
22. Simopoulos AP, Norman HA, Gillaspy JE and Duke JA: Common purslane: a source of omega-3 fatty acids and antioxidants. Journal of the American College of Nutrition 1992; 11(4): 374-382.

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