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Evaluation of phytochemical constituents and antioxidant properties of *Trianthema portulacastrum* Linn

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Abstract
Trianthema portulacastrum Linn. (Biskhapra) is an annual herb belongs to the family Aizoacae, which is used to treat for many oxidative stresses related diseases. The current study was done to characterize the organoleptic and proximate characteristics and to estimate the phytochemical components and to investigate antioxidant activity of aqueous extracts of leaves of the plant. The results showed that total phenolic, flavonoids and tannin content are 112.96±0.93 mg Gallic Acid Equivalent/g Dry Weight, 164.84±0.91 mg Quercetin Equivalent/g Dry Weight and 11.79±0.37 mg Tannic Acid Equivalent/g Dry Weight, respectively. Total protein and amino acids contents are 193.56±2.68 mg BSA Equivalent/g Dry Weight and 128.79±3.19 mg Tyrosine Equivalent/g Dry Weight, respectively. Total polysaccharide, lipid and ascorbic acid content are 117.85±6.20 mg Dextrose Equivalent/g Dry Weight, 9.10% and 21.13 mg Ascorbic Acid Equivalent/g Fresh Weight, respectively. Inhibition percentage of antioxidants assays such as DPPH, ABTS and H_2O_2 are 80.68±0.83%, 77.27±0.93% and 72.83±0.86%, respectively. From the results may be concluded that the leaves of the plant possess biologically active secondary metabolites that can be highly applicable for the healing purposes of oxidative stress related diseases.

Keywords: *Trianthema portulacastrum*, organoleptic, proximate, nutritional, antioxidants

Introduction
From ancient time, traditional medicines are used by mankind and a huge number of traditional medicines are produced by the nutritive valuable plants in the rural areas. In case of human biological system plant based medicines has better adjustment. The demand for ethno medicinal treatment is increasing because it is cost effective as well as has minimal side effect. It is vastly important to search new sources of important phytochemical compounds of the natural drugs for therapeutic and industrial purposes [1, 2].
Trianthema portulacastrum Linn. (Biskhapra) an annual herbaceous plant belongs to the family Aizoacae. It is also known as Horse purslane in English. The plant is found in India, Baluchistan, Sri Lanka, and Africa, West Asia and Tropical America (tropical and subtropical countries) and endemic of South America. The plant is to 30-67 cm long, branched, prostate, diffused, and found on the ground areas such as wastelands, lawns, roadsides, gardens. The leaves of the plants are eaten as a vegetable and used as a traditional medicine. The plant is ascribed with analgesic, antipyretic, anti-inflammatory, and use as a wound-dressing because of the fleshy nature of leaves. *Trianthema portulacastrum* has a various number of essential phytochemical compounds because the plant is considered to be a traditional medicinal plant. Plant metabolites are naturally occurring substances that are present in plants and that have been reported to exhibit various biological activities. Therefore, it is very necessary to determine the amounts and estimate those bioactive compounds [3, 4].
The present course of study was designed to detect and estimate the organoleptic parameters, some proximate analysis, and to determine the phytochemical profiling and antioxidant activities of the plant aqueous extract which is very beneficial for medicinal system.

Materials and Methods
Chemicals and Reagents: In these experiments several chemicals and reagents were used which were analytical graded. From Merck Life Science Private Limited, Mumbai Folin-ciocalteu reagent, ascorbic acid and aluminum chloride were obtained.

From SD Fine-Chem Limited, Mumbai hydrogen peroxide and Gallic acid were purchased. From Tokyo Chemical Industry Co. Ltd., Japan. ABTS was obtained. DPPH, Quercetin, tannic acid was purchased from Sisco Research Laboratories Pvt. Ltd., Maharashtra.

Collection and Extraction

Leaves of the plant were used in all experiments. From university campus Salt Lake City, Kolkata, and West Bengal, India the fresh and green leaves were collected. At first with the distilled water the collected leaves were washed. Samples were dried for 25-30 days under shade at room temperature. Then the sample leaves crushed and get the powdered form of the leaves which was stored in airtight containers. By using mortar and pestle the aqueous leaf extract was extracted. Here one solvent was used and the calculated amount is 50 ml each for 1 g of powder. The sample solution of the extract was taken into the refrigerator at 4 °C and diluted the samples for further experiments. According to the needs for the specific phytochemical assay the diluted solution was used in further studies.

Organoleptic Characters

To observe the organoleptic characters of the leaf sample, standard methods were used [5, 6].

Proximate Analysis

Determination of Extractive Value

To determine Extractive standard protocol was used. The Extractive value was calculated in percentage by using this formula [7]:

Extractive Value (%) = (Weight of dried extract / Weight of plant material) × 100

Determination of Total Moisture Content

To determine the Total moisture content standard method was used. It is calculated by the following formula: [8, 9]

$MC_{WB} = (W_i - W_f) / W_i \times 100$

Where, MC = Moisture Content, W_i = Initial Weight of Sample, W_f= Final Weight of Sample

Determination of Relative Water Content

To determine relative water content standard method was used. It is calculated by the help of the following formula [10]:

RW_{WB} = (FW-DW) / (TW-DW) × 100

Where, FW = Fresh weight, TW = Turgid weight, DW = Dry weight

Determination of pH

To observe the pH value in the sample, in 10 ml deionized water 5 gm of fresh leaves was homogenized. This solution was filtered and determine the pH after calibrating pH meter with buffer solution of pH of 4and 9 [11].

Determination of Conductivity

To observe the conductivity value in the sample, in 10 ml deionized water 5 g of fresh leaves was homogenized. This solution was filtered and determines the conductivity after calibrating conductivity meter with buffer solution of pH of 7 and double distilled water.

Determination of Water Solubility and Insolubility

100 mg plant sample was crushed with 10 ml of double distilled water and filtered by using Whatman no.1 filter paper. Before using the filter paper the weight of the filter paper was taken and after using it, again weight was taken. From there two weights were obtained filtrated and infiltrated. To determine the soluble and insoluble part of the leaf in water, the following formula was used:

Insoluble part (W_i) =W_2 - W_1,

Soluble part (W_s) = 100 – W_2

Where, weight of filter paper =W_1, weight of filter paper with infiltrated part =W_2

Phytochemical Screening

Qualitative Analysis

To detect flavonoids [12], alkaloids [13, 14], reducing sugars [13, 15], protein [16], saponin [17], tannins [15], phenolic compounds [18], carbohydrates [19], Volatile oil [18, 20], Cardiac glycosidase [21], anthocyanin and Betacyanin [22] and terpenoids [19] standard methods were used.

Quantitative Analysis

Quantification of Total Phenolic Compounds

To determine the total polyphenolic content, the Folin-Ciocalteu method was used. The concentration of total polyphenolic compounds of the samples was expressed as mg Gallic Acid Equivalent/g dry weight [23].

Quantification of Total Flavonoids Content

The determination of total flavonoids quantity was done by the help of aluminium chloride colorimetric method with slight modification. Total flavonoids quantity was measured in mg Quercetin Equivalent/g dry weight of the sample [24].

Quantification of Total Tannins Content

Measurement of total tannin was investigated by using standardized assay. The amount of tannins was expressed in mg Tannic Acid Equivalent/g dry weight [25].

Nutritional Analysis

Determination of Total Polysaccharides Content

To estimate the carbohydrate content of the extract standard protocol was used. The total carbohydrate content was expressed as mg dextrose Equivalent/g dry weight of the sample [26].

Determination of Lipid Content

To determine the total lipid content Bligh and Dyer Method, 1959 was used. To determine the total lipid content in the extract, the following formula was used [27].

Total lipid content = (Weight of the lipid in aliquot × Volume of chloroform layer) / Volume of aliquot %

Determination of Total Protein Content

To estimate the total amount of protein content in the plant extract Lowry's protein estimation method was used. Total protein amount was measured in mg BSA Equivalent/g dry weight of the sample [28].

Determination of Amino Acid Content

The total free amino acid of the extract was determined

according to the protocol of Sircel J *et al*. The total amino acid content was expressed as mg tyrosine Equivalent/g dry weight of the sample [29].

Determination of Ascorbic Acid Content

To determine ascorbic acid content iodine titration method was used with proper adjustments. The total ascorbic acid content was expressed by ascorbic acid Equivalent/g fresh weight of the sample. To calculate the amount of ascorbic acid $V_1S_1 = V_2S_2$ formula was used [30, 31].

Determination of Antioxidant Activity

DPPH Radical Scavenging Test

The free radical regulating effect was carried out by standard protocol, using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) with slight modifications. DPPH radical scavenging capacity was measured in terms of Ascorbic Acid Equivalent, as percentage of inhibition expressed by the below mentioned formula: [32]

% Inhibition of DPPH= OD of control – OD of sample/ OD of control*100

ABTS Radical Scavenging Assay

ABTS radical cation decolorization assay was used with slight modifications for free radical inhibiting ability of extract by applying standard protocol. ABTS scavenging capacity was measured in terms Ascorbic Acid Equivalent, as percentage inhibition calculated by the formula: [33]

% Inhibition of ABTS= OD of control – OD of sample/ OD of control*100

H_2O_2 Radical Scavenging Assay

Hydrogen peroxide scavenging ability was determined by using a standard method with slight modification. H_2O_2 radical scavenging effect was measured in terms Gallic Acid Equivalent and as percentage inhibition expressed by the below mentioned formula: [34]

% Inhibition of H_2O_2= OD of control – OD of sample/ OD of control*100

Statistical Analysis

All the experimental measurements were executed in triplicate manner and showed as the average ± standard deviations. The means, standard curve and standard deviations were analyzed and calculated by using MS Excel Software.

Results and Discussions

Organoleptic Characters

By using standard method, in case of this current study different Organoleptic Characters like Color, Size (Length& Width), Shape, Odor and texture of the leaves of the medicinal plants has been determined and represented in the table 1.

Table 1: Organoleptic characteristics of leaf samples

Plant Name	Shape	Size		Odor	Color	Texture
		Length (cm)	Width (cm)			
Trianthema portulacastrum	Elliptical	3.53	2.52	Vegetative	Green	Leathery

Proximate Analysis

In the present course of study proximate parameters extractive value, total moisture content, relative water content, pH, conductivity, water solubility and insolubility were determined by using standard methods. The results are represented in table 2.

Table 2: Proximate parameters are shown in table

Extractive Value (mg)	Total Moisture Content (%)	Relative Water Content (%)	pH	Conductivity (mS/cm)	Water Soluble & Insoluble (%)	
					Soluble	Insoluble
10	82	78.45	6.69	1.34	89	11

Qualitative Assay

For qualitative screening the leaves is extracted in the aqueous solution and obtained the results are presented in Table 3. To detect different phytochemicals total 12 qualitative tests were done. All the tests were done in aqueous medium. Out of 12 tests 9 compounds were present and these are flavonoids, phenols, proteins, reducing sugar, saponin, terpenoids, tannin, alkaloids and carbohydrates. Three (3) compounds were absent such as cardiac glycosides, anthocyanin and volatile oil. Therefore, the result shows that the experimental plant carries a lot of significant possibility for pharmaceutically important secondary metabolites.

Table 3: Results of qualitative assays

Parameters	Results
Flavonoids	+
Alkaloids	+
Reducing sugar	+
Protein	+
Saponin	+
Tannin	+
Polyphenols	+
Carbohydrates	+
Terpenoid	+
Volatile oil	-
Cardiac glycosidase	-
Anthocyanin	-

Where, '+' Present, '-' Absent

Quantitative Assay

In the present course of study for quantitative analysis, the leaves of the plant is extracted in the aqueous solution and concentration of flavonoids, polyphenols and tannin were determined based on the standard methods. Standard curve for total polyphenol content, total flavonoid content and total tannin content were prepared taking Gallic acid, Quercetin and Tannic acid as standard reagent, respectively (Table 4).

Table 4: Standard curve equation and the total phytochemical contents

Phytochemical Assays	Standard Curve Equation	R^2 Value	Phytochemical Contents
Polyphenols	Y=0.658x+0.107	0.999	112.96±0.93
Flavonoids	Y=0.087x+0.041	0.994	164.84±0.91
Tannin	Y=5.338x+0.130	0.996	11.79±0.37

The total polyphenolic content was quantified to be 112.96±0.93 mg GAE/g dry weight for aqueous extract. Polyphenols are reactive species towards oxidation and lead the physiological activity. The oxidation process and free radicals generation proceeds for microbial infections, cancer, diabetes and other diseases. The action of polyphenols against those oxidative stress-related causing processes can have helpful therapeutic application in the pharmaceutical industry. Plants having more phenolic content showed significant antioxidant properties [23, 32, 35].

Total flavonoids quantity was measured as 164.84±0.91 mg QE/g dry weight for aqueous extracts. The antioxidant activities of flavonoids are because of different mechanisms such as scavenging of free radicals, chelating of metal ions and for free radical generation, the inhibitions of enzymes are responsible. Flavonoids can inhibit the reactive oxygen species as its structure supports this [24, 32, 35].

Total tannin quantity was showed that 11.79±0.37 mg TAE/g dry weights for aqueous extracts. Tannins are observed in the stem of plants instead of leaves. High amount of tannin tannin presence shows the antioxidant properties. The tannin-protein complex shows the persistent antioxidant property [25, 32, 35].

Nutritional Analysis

For nutritional analysis, the leaves of the plant are extracted in the aqueous solution. Standard curve for total polysaccharide content, total protein content and total amino acid content were prepared by taking dextrose, BSA and tyrosine as standard reagent, respectively (Table 5). Some of important nutritional analysis like lipid was calculated in percentage and ascorbic acid were determined by using titration method which is presented in table 6.

Table 5: Standard curve equation and total nutritional contents

Nutritional Assays	Standard Curve Equation	R^2 Value	Nutritional Contents
Polysaccharides	Y=1.456x+0.083	0.997	117.85±6.20
Protein	Y=0.569x+0.109	0.990	193.56±2.68
Amino acid	Y=0.055x+0.048	0.996	128.79±3.19

The total polysaccharide content was quantified to be 117.85±6.20 mg DE/g of dry weight for aqueous extracts by using regression equation obtained from the calibration curve. Polysaccharide is the principle and essential source of energy. Due to polysaccharides content the plant sample can be used as a good food source [26].

Protein is always considering as the major ingredients of human diet and it gives high nutrition. Plant protein supplements are widely used in current period. Proteins are not only supports growth but also repairs and maintain the body tissue. The total protein content was quantified to be 193.56±2.68 mg BSAE/g of dry weight for aqueous extracts [28].

Amino acids are building blocks of proteins. These are plays many critical roles in human body. Amino acids are needed for vital processes like the building of proteins, neurotransmitters and synthesis of hormones. The total amino acid content was quantified to be 128.79±3.19 Tyr Equivalent mg/g of dry weight for aqueous extracts [29].

Table 6: Total amount of Lipid and Ascorbic acid

Nutritional Assays	Nutritional Contents
Lipid	9.10%
Ascorbic acid	21.13 mg AA/g of FW

To determine the total lipid content cold extraction of the sample was used and chemicals Chloroform, methanol, water was used in 2:2:1 ratio. The total lipid content was quantified to be 9.10%. Lipids play diverse and important role in nutrition and health and it provides large amount of energy. Lipids in food also act as carrier of fat-soluble vitamins [27].

The total ascorbic acid content in plant sample was done by titration method. The total ascorbic acid content was quantified to be 21.13 g AA/g of FW. Development and growth of the body cells is always dependent on the ascorbic acid. Plants having more ascorbic acid content show significant antioxidant and healing properties [31].

Antioxidant Activity

Phytomolecules can act as antioxidants by scavenging the free radicals. DPPH is a free radical scavenging assays which is widely used to investigate the free radical scavenging capacity of natural antioxidants. DPPH antioxidant assay is depending on the reduction of free radicals in methanol due to the production of the non-radical shape of stable free radical in the presence of hydrogen-donating antioxidants [24, 32]. The inhibitory percentage of DPPH radical scavenging capacity was showed to be 80.68±0.83% for aqueous extract as compared to inhibition percentage for standard reagent.

In case of scavenging capacity of the ABTS cation radical has been compared with ascorbic acid. For a stable form of ABTS radical cation in the assay potassium persulfate was used [33]. Inhibition percentage of ABTS assay was resulted to be 77.27±0.93% for aqueous extract.

In case of H_2O_2 assay, the process is based on the reduction of stable free radicals in phosphate buffer solution (pH 6.8) due to the formation of the non-radical form of standard stable free radical in the presence of hydrogen-donating antioxidants [34]. Inhibition percentage of H_2O_2 assay was showed to be 72.83±0.86% for aqueous. These *in vitro* antioxidants research study findings support the previous investigations as well which concluded that the antioxidant properties are mainly dependent on several bioactive compounds such as polyphenols, flavonoids and tannins [12, 15, 18].

Table 7: Standard curve equation and inhibition percentages of antioxidant assays

Antioxidant Assays	Standard Curve Equation	R^2 Value	Inhibition Percentages (%)
DPPH	Y=183.8x+3.256	0.996	80.68±0.83
ABTS	Y=188.5x+5.653	0.994	77.27±0.93
H_2O_2	Y=175.2x+6.914	0.994	72.83±0.86

Conclusions

In the current research study, the leave extracts of *Trianthema portulacastrum* showed the high secondary metabolites presence. It possesses a countable quantity of phytochemical compounds presence as well as it showed higher range of antioxidant activity. The results demonstrated the higher content of phytochemical constituents like polyphenols, flavonoids, tannins, saponins, proteins, carbohydrates and higher antioxidant property for stable free radical inhibiting assays. The antioxidant ability of the decoctions may be ascribed to their free radical scavenging ability.

The antioxidant properties of the extracts depended on the polyphenolic substances and other phytochemicals. Mainly the higher amount of flavonoids and polyphenols content is responsible for significant antioxidant activities, which was known in previous research investigations also. From the present research investigation and according to the results obtained, it can be concluded that leaves of the plant has vast source of natural phytochemicals, nutraceuticals and natural antioxidants. Also the plant could be used to develop pharmaceutical products against various oxidative stress-related diseases.

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Conflict of interest

The author of the present research article declares no conflict of interest.

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