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ORIGINAL ARTICLE

Estimation of Physicochemical Properties and Antioxidant Activity of Root Extract of *Physalis minima*

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ABSTRACT

Background: *Physalis minima* is used in a variety of ailments. Although, there have been a few studies on the leaves and fruits of the *Physalis minima*, there have been relatively few investigations on the roots.

Objectives: The objective of the present study is to investigate the physicochemical parameters, trace elements, FTIR, and antioxidant activity of *Physalis minima* root extracts.

Methodology: Standard procedures were used to conduct these studies on n-Hexane, chloroform, and methanolic extracts of roots. The antioxidant activity was performed by standard procedures as DPPH (2, 2-diphenyl-1-picrylhydrazyl), phosphomolybdenum method, FRAP (ferric reducing antioxidant power assay), and hydrogen peroxide scavenging activity.

Results: Phytochemical analysis showed moisture content as 4.93±0.98%, total ash 10.09±0.76%, sulfated ash 4.92±0.91%, acid insoluble ash 4.3±0.88%, water-soluble extractive value 4.60 ± 0.01%, and alcohol soluble extractive value 17.92 ± 0.01%. FTIR analysis showed the presence of alkyl, amino, and tertiary alcohol groups. Percentage scavenging activity was measured by DPPH method and it was found that methanol extract showed maximum 93.525±0.276% scavenging activity as compared to chloroform 60.248±0.847%, and n-hexane 50.0±0.547%. Phosphomolybdenum assay showed maximum potential 86.81± 0.521% in the methanolic extract, 60.07±0.645% in chloroform extract, and 49.33±0.841% in n-hexane extract. FRAP showed maximum reducing value for methanol extract 86.153±0.203%, in chloroform 47.180±0.352%, and n-hexane extract 30.26±0.703%. By hydrogen peroxide assay, methanol extract possesses a maximum percentage of inhibition 91.71±0.992%, chloroform extract 60.64±0.721%, and for n-hexane was 51.32±1.664%.

Conclusion: *Physalis minima* root extract can be utilized to treat a variety of ailments and malignancies.

Keywords: *Physalis minima*, root extract, anti-oxidant, physicochemical, secondary metabolites.

INTRODUCTION

The plant *Physalis minima,* commonly known as wild Cape gooseberry, native gooseberry, and pygmy groundcherry is one of the major genera of the well-known family Solanaceae with 80 to 100 species worldwide. It is an annual herb commonly found on the borders of waste and cultivated parts of the land. It is native to Baluchistan in Pakistan, India, Tropical Africa, Australia, Afghanistan, and Malaysia [1]. It is an annual upright herbaceous plant that usually grows with a size up to 15-45cm long, pubescent, erect, and a delicate herb with globular fruits enclosed in a bladder-like calyx. The fruiting and flowering season of this plant starts in April and continues up till the end of November [2].

Gooseberry is well known throughout the world due to its traditional use as abortifacient, diuretic, vermifuge, analgesic, purgative, analgesic, anthelmintic, and febrifuge. Steroidal lactones recognized from the plant have been observed to influence various pharmacological mechanisms like antipyretic, anti-malarial, lipase, cytotoxic hypoglycemic, alpha glycosidase inhibitors [3]. Other traditional uses of the *Physalis minima* are appetizing and growth enhancer, anti-gonorrhoeic, and as a curative agent for abdominal troubles and enlargement of spleen [3]. Syrup of the plant is used by the Malaysian public for anticancer management [4]. Further study suggests that the plant is reported to have cancer activity [5-8].

For the treatment of leprosy, *Physalis minima* are well known to the medical world. *Physalis minima* have many chemicals that can be used as antimycobacterial [9, 10], antileishmanial [11], antimicrobial [12] and anti-inflammatory activity [13]. Leaf and stem of *Physalis minima* are reported to contain flavonoid and phenolic compounds which imparts them high antioxidant properties [14]. Phenolic compounds are principally significant for nutritional applications [14]. To the best of our knowledge, the antioxidant activity of the root extract of *Physalis minima* has not been investigated properly. The aim of the present study is to evaluate the scavenging of radical capacity and other antioxidant properties of this plant. Other properties of plant-like determination of secondary metabolites, total ash contents have also been measured.

METHODOLOGY

Plant material

The plant was collected from Muridke and Nangre Bhatiyan (Village) and got authenticated by the Chairman Department of Botany Government College University, Professor Dr. Zaheer-ud-din Khan. A voucher specimen (G.C. Herb. Bot. 3387) was deposited in the herbarium of GCU. The plants were washed externally with tap water to remove mud and debris after which they were air-dried. The roots and stem were separated and shade - dried for almost 20 days. The dried plant roots were then homogenized to form a fine powder.

Chemicals and solvents

The analytical grade solvents and chemicals were used which include conc. H_2SO_4 (BDH, England), Conc. HNO_3 (BDH, England), sulphur powder, ferric chloride (E. Merck A.G Darmstadt, Germany), pyridine, sodium nitroprusside, folic & CiocalTeu's phenol reagent (Unichem Chemicals), dil. HCl, sodium hydroxide, butylated hydroxytoluene (Sigma life Sciences, Germany), quercetin (Sigma life Sciences, Germany), gallic acid (Sinochem, China), triton-X, potassium acetate extra pure (Merck, Germany), aluminium nitrate (Merck, Sigma Aldrich Germany), methanol (BDH, England) Riedel de Haen, chloroform (BDH, England & Merck, Germany), hexane (BDH, England) ammonium molybdate (Merck, Germany), ethyl acetate (BDH, England & Merck, Germany), ascorbic acid, DMSO, bovine serum, sodium phosphate (Mol wt. 137.99 g/mol), and 2,2-diphenyl-1-picrylhydrazyle (DPPH).

Physicochemical studies

A physicochemical examination of powdered plant material was carried out in accordance with USP criteria (2009). Moisture, total ash, acid insoluble ash, and sulfated ash were all measured. Different extractions were made in solvents including alcohol, water, and chloroform.

Estimation of primary metabolites in crude root powder

The powder material was analyzed to estimate primary metabolites content including total lipids [16], total proteins [17] and carbohydrates [18].

FTIR spectroscopy

The powder materials got from different batches of roots of the plants were analyzed using FTIR

spectrometer to estimate metabolic similarities or differences. For this purpose, *Physalis minima* root powder from different locations were subjected to FTIR analysis. The plant root powder was mixed with the 100mg potassium bromide and the mixture was ground to powder. The material was then taken to die and made a pellet (hydraulic press). IR range of FTIR spectra was set between 4000-400 cm^{-1}.

Trace elements analysis by atomic absorption spectroscopy

The 500 mg powdered sample was taken in a glass beaker with 8ml 65% (V/V) HNO_3 to digest the sample. For complete digestion, the blend was heated on a muffle furnace at 900 ^{0}C for 25 min. Then extract obtained was allowed to cool at room temperature. The final volume of 25 ml was achieved with the help of water. Digestion of reference and blank was followed by the same method. A stock solution of 1000 ppm was diluted to each element to 100 ppm to form standard solutions of Ca, K, Zn, Fe, Na, Mg, Mn, and Cu. According to the strength of the test sample, 100ppm solution was used for further dilutions of all trace elements to five different strengths.

For the analysis and detection/identification, atomic absorption spectroscopy was used. The wavelength for the heavy metal and trace element was adjusted according to the monograph and the lamp was lighted. The appropriate current slit width of adjustment. Gas flow was adjusted to proper conditions after a mixture of combustible gases was ignited. By nebulizing the blank solvent into flame, zero adjustments were done. After setting the wavelength and apparatus, the test solution of the sample which was formed after digestion was introduced into the flame, and the light absorption at the characteristics wavelength of the detecting element was measured. Precision and accuracy of the AASS were assured after the three results [19].

Organoleptic properties

The extracts were checked organoleptically throughout the research work to check their physical stability.

Qualitative analysis of extracts

1g of methanol, chloroform, and n-Hexane root extracts was dissolved in 100 ml of methanol, chloroform, and n-Hexane respectively for 24 h with occasional shaking. The contents were filtered and used for phytochemical analysis according to the specifications of USP 2009. The tests for alkaloids, saponins, tannins, glycosides, steroids, flavonoids, terpenoids, and reducing sugars were conducted.

Quantitative analysis of extracts

The contents of total polyphenols were estimated according to a method of Singleion and Slinkard with some modifications [20]. Total flavonoids estimation was performed by a method of Chang *et al.* [21] with some modifications. The method of Heimer was used to determine the tannin contents. The method of Lowry *et al.* [22] was used to estimate protein contents in the sample. Total carbohydrates estimation in the sample were performed by a method of Hussain *et al.* [23]. The contents of total amides were estimated according to the method of Hussain *et al.* [24] with some modification. The method of Husain *et al*. 2008 was used to find out the total glycosaponins [23].

UV/Visible profiling of extracts

A stock solution of 1 mg/ml of extracts was prepared in methanol, chloroform, and n-Hexane. Then 100 µl of each of the stock solution was diluted with the respective solvent up to 1 ml. Finally, all the sample solutions were scanned using a UV visible spectrophotometer at 800-200 nm and results were analyzed to check the metabolomics.

Determination of antioxidant activity

Total antioxidant capacity assay by phosphomolybdenum

The assay was performed as described by the technique of Prieto *et al*. [25]. All readings were taken in triplicate and average values were used for calculations.

DPPH assay

DPPH (2, 2-diphenyl-1-picrylhydrazyl) is an organic compound made up of stable free radicals. Four milligrams of each extract in one milliliter of dimethyl sulfoxide (DMSO) were mixed to form a stock solution. 3.32 mg of solid DPPH was dissolved in 100 ml of methanol to form DPPH stock solution and 4 mg of ascorbic acid was dissolved in 1 ml of dimethyl sulfoxide (DMSO) to form a stock solution of ascorbic acid. The microplate was used, in which 20 µl of the different plant extracts were mixed in 180 µl of DPPH reagent, and volume was made up to 200 µl. Then the mixture was incubated at 37 ^{0}C for about 1 hour. Methanol was used as a negative control while ascorbic acid was used as a positive

control. After incubation of the sample, the reading was taken by using a microplate reader at 517 nm. The triplicate samples were used and the final scavenging percentages were calculated.

Ferric reducing assay

The method of Oyaizu [26] was adopted to calculate ferric reducing power activity of root extracts. Readings were taken in triplicate and average values were used for calculations.

Hydrogen peroxide scavenging activity

Method of [27] was used to find out the capacity of melatonin to scavenge H_2O_2. The percentage scavenging of H_2O_2 by melatonin and standard compounds was determined.

RESULTS

Physicochemical investigation of crude plant material

The results yielded are mentioned in Table **1** (Physicochemical properties of root powder of *Physalis minima*). The moisture content was found through the standard procedure and the result was observed to be 4.93%. The Total ash content was found through the standard procedure and the result was observed to be 10.9%. The Acid Insoluble ash contents were found through the standard procedure and the result was observed to be 4.3%. The sulfated ash contents were found through the standard procedure and the result was observed to be 4.92%.

Estimation of primary metabolites of crude root powder

The results of the primary metabolites found in the powdered form of the crude drug material of the plant *Physalis minima* are represented in Table **2** (Primary metabolites in *Physalis minima* powdered form).

FTIR Scanning

Numerous intensity bands of the strong, medium, and weak strength were observed on the IR spectrum obtained from the FTIR analysis. Possibly due to the presence of an alkyl group, a strong peak was demonstrated having a wavelength of 2928.82 cm^{-1}. At 1624.45 cm^{-1}, a medium peak was observed produced by the amino group. A second medium peak was observed at 1392.13 cm^{-1} which possibly indicated the tertiary alkyl group presence. A weak strength peak indicated the presence of a tertiary alcohol group and was observed 1120.26 cm^{-1} respectively. The mentioned peaks, however, were also indicative of the presence of both the groups of alcoholic as well as an amino group. Besides, the groups of methyl, alkyl, and alkyne were also present. The result of FTIR is shown in Figure **1** (FTIR analysis of root extract).

Trace elements in *Physalis minima* powdered material

Table **3** shows the mineral content found in the plant material of *Physalis minima*. The atomic absorption technique was used for the determination of mineral contents. The method of flame photometry was also used. The results are given in Table **3** (Mineral Contents of the *Physalis minima* root powder).

Percentage yield in different solvents

The percentage yield of different solvents is given in Table **4** (Percentage yield of various extracts of *Physalis minima*).

Qualitative analysis of extracts

The phytochemical qualitative analysis was carried out for methanol, chloroform, and n-Hexane extracts of *Physalis minima*. The results mentioned in Table **5** (Qualitative assessment of phytochemical constituents of *Physalis minima* roots extracts) showed the presence or absence of various phytochemical constituents in all extracts.

Quantitative analysis of extracts

The results are indicating that there is a high percentage of glycosaponins in the methanolic extract which is 28% as compare to chloroform 23% and n-Hexane 17%. Similarly, in all cases, percentages of secondary metabolites are higher in methanolic extract than chloroform and n-hexane as shown in Table **6** (Quantitative assessment of phytochemicals constituents of *Physalis minima* root extracts).

UV-Visible metabolomics comparison

Extracts were scanned at 800-200nm for comparison of UV visible profile. The overlay spectra are shown in the Figure **2** (UV visible spectra of *Physalis minima* extracts). Among the extracts of methanol, chloroform, and n-hexane, Methanol extract showed a slightly different profile as compared to chloroform and n-hexane. Chloroform and n-hexane showed a similar profile indicating the pharmacological activities of these extracts may be similar.

Antioxidant activity

Determination of total antioxidant capacity by phosphomolybdenum

The antioxidant activity of *Physalis minima* was checked by phosphomolybdenum method and results are shown in Table **7** (Evaluation of Antioxidant activity of *Physalis minima* extracts and standard using different assays). The Methanol extract showed maximum antioxidant capacity that is (86.81±0.521%) followed by chloroform extract (60.07±0.645%), and n-hexane extract (49.33±0.841).

Determination of antioxidant activity by DPPH assay

The methanol extract showed maximum percentage inhibition of (93.525±0.276) followed by chloroform extract (60.248±0.847), n-hexane extract (50.0±0.547). Table **7** shows results of DPPH assay.

Investigation of antioxidant activity by ferric reducing power assay

We also reported the antioxidant activity of these fractions by using the ferric reducing power. The total antioxidant activities of these are shown in Table **7**. The methanol extract showed maximum percentage inhibition of (86.153±0.203) followed by chloroform extract (47.180±0.352), and n-hexane extract (30.203±0.703).

Investigation of antioxidant activity by hydrogen peroxide scavenging activity

The methanol extract showed maximum percentage inhibition of (91.71±0.992) followed by chloroform extract (60.64±0.721), n-hexane extract (51.32±1.664) as shown in Table **7**.

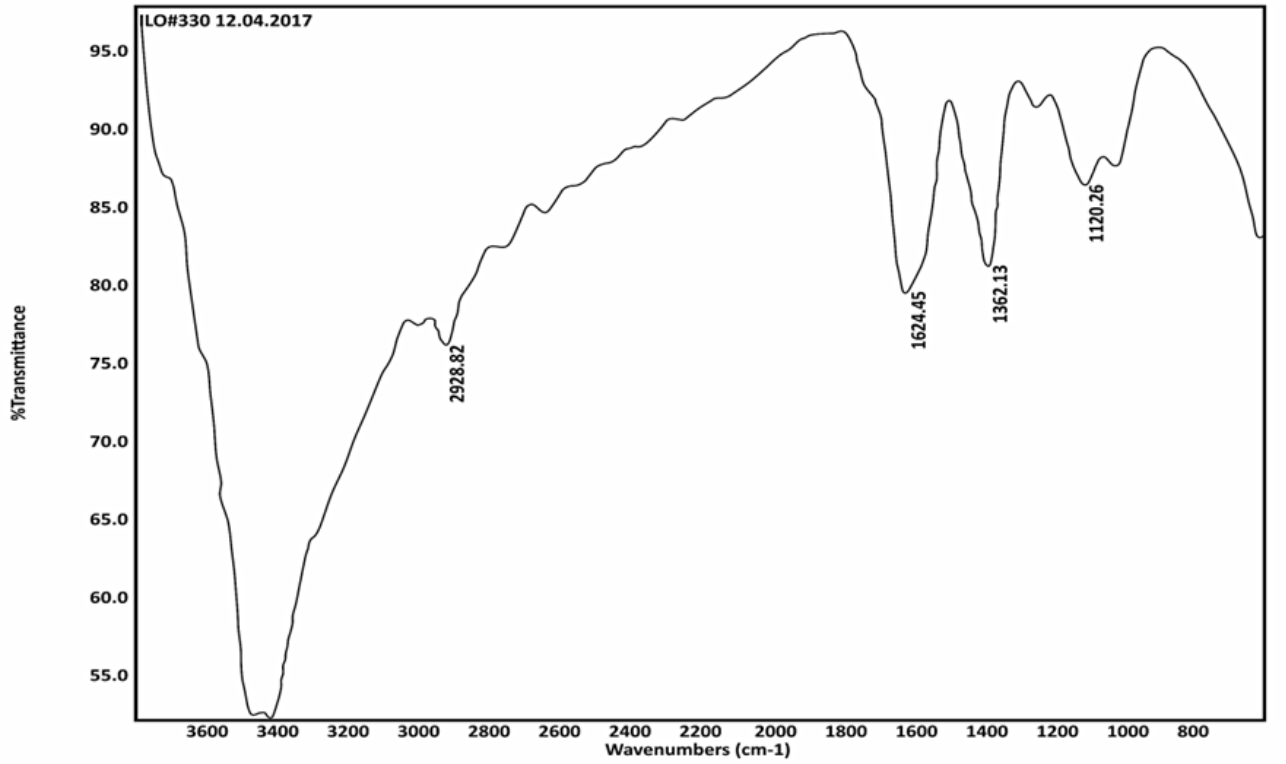

Figure 1. FTIR analysis of root extract.

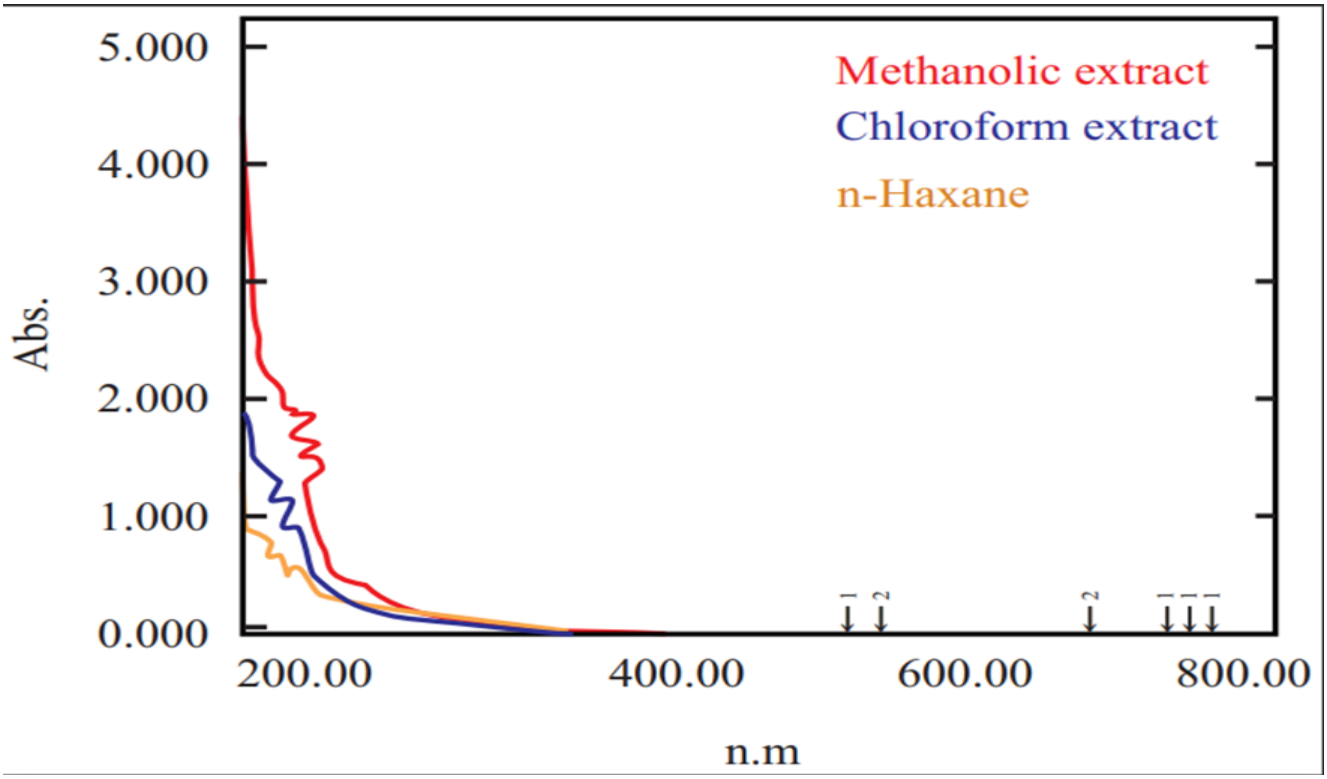

Figure 2. UV visible spectra of *Physalis minima* extracts.

Table 1. Physicochemical properties of root powder of *Physalis minima*.

Physicochemical Properties	Percentage Content ± S.D
Moisture Contents	4.93 ±0.98%
Total Ash Contents	10.9±0.76%
Acid Insoluble Ash	4.3±0.88%
Sulphated Ash	4.92±0.91%
Alcohol extractive value	17.92 ± 0.01%
Water extractive value	4.60 ± 0.01%

Table 2. Primary metabolites in *Physalis minima* powdered form.

Primary Metabolites	Percentage Yield (% w/w) +SD
Total Proteins	8.0 ± 0.3
Total Carbohydrates	72.14 ± 2.08
Total Lipids	3.93 ± 0.2

Table 3. Mineral Contents of the *Physalis minima* root powder.

Elements	mg/L
Magnesium	111
Iron	298.25
Sodium	1800
Zinc	57.5
Manganese	77.0
Copper	15.0
Potassium	23.2
Calcium	80.95

Table 4. Percentage yield of various extracts of *Physalis minima*.

S No	Extract Name	Percentage Yield (% w/w)
1.	Chloroform	2. 89
2.	Methanol	82.8
3.	n-Hexane	5.88

Table 5. Qualitative assessment of phytochemical constituents of *Physalis minima* roots extracts.

Phytochemicals	Extracts		
	Methanol	Chloroform	n-Hexane
Alkaloid	+	+	+
Saponins	+	+	+
Tannins	+	+	+
Glycosides	+	+	+
Steroids	+	+	_
Flavonoids	+	+	+
Terpenoids	+	+	+
Reducing Sugars	_	_	_

Table 6. Quantitative assessment of phytochemicals constituents of *Physalis minima* root extracts.

S No.	Parameters	Methanol extract (% age)	Chloroform extract (% age)	n-Hexane extract (% age)
1	Total glycosaponins	28	23	17
2	Total proteins	51.3	20.4	17.6
3	Total polysaccharides	2.6	1.7	1.4
4	Total amides	29.4	19.3	12.2
5	Total tannins	44.5	26.8	20.8
6	Total flavonoids	1.8	1.5	1.4
7	Total polyphenols	0.7	0.5	0.4

Table 7. Evaluation of Antioxidant activity of *Physalis minima* extracts and standard using different assays.

Extracts	Antioxidant Activity			
	Phosphomolybdenum	DPPH assay	Ferric reducing capacity	Hydrogen peroxide scavenging activity
Methanol extract	86.81±0.521	93.525±0.276	86.153±0.203	91.71±0.992
Chloroform extract	60.07±0.645	60.248±0.847	47.180±0.352	60.64±0.721
n-hexane extract	49.33±0.841	50.0±0.547	30.203±0.703	51.32±1.664

DISCUSSION

The moisture content of the crude plant material showed a value of 4.93%. The total ash content was found to have a value of approximately 10.9%. The literature states that total ash contents provide a good insight into the properties of the drug material. The ash value is a strong representative of salts of organic nature that are naturally found in the crude sample of gooseberry. The ash value may also be due to the occurrence of adulterants. The possible adulterants in the crude sample might be silica, oxalate, and powder of chalk [28]. The acid-insoluble contents of the plant material indicate the non-physiological content of the total ash contents and are an indication of particles having an insoluble nature such as herbal-mineral material in sand, soil, and silica. The acid-insoluble content value of gooseberry crude extract was found out to be having a value of 4.30%. The value for the

sulfated ash of crude drug sample of gooseberry was found out to be 4.92%.

The extractive values of the sample are a measure of the presence of chemical constituents. The values of extraction for the sample of gooseberry was found out to be 17.92 ± 0.01% in ethanol, whereas, the extractive values observed in chloroform water were found out to be 4.60 ± 0.01%. The highest extractive value was found to be observed in ethanol. A superior yield is given by extractive values and the reasons for this is the fact that ethanol has a higher evaporation probability than either chloroform water, and a majority of the constituents found in the extract of gooseberry are soluble in organic solvents and therefore yield better results with the performance of extractive values. Total protein has a value of 8.0 ± 0.3%. The powder material has a total carbohydrate content of 72.14 ± 2.08 % and lastly the material is composed of the lipid content of 3.93 ± 0.2%. The estimation of protein, carbohydrates, and lipids provides nutritional as well as commercial importance of *Physalis minima.* Carbohydrates contents were more than lipids and protein contents so it's the energy-rich source.

Physalis minima were analyzed by atomic absorption spectroscopy and flame photometry for the detection of metals and trace elements and minerals. Atomic Absorption Spectrophotometer was used for Mn, Fe, Cu, Mg, Zn and flame photometer for Na, K, Ca. Trace elements are the elements that occur only in small amounts within a given matrix, whereby the question of what constitutes a trace amount is a matter of convention and usage. However, in biological systems, there is a deeper meaning to the term since trace elements within living bodies are often not coincidental contaminants but fulfill primary functions. As the plant was collected from only the selected place that is Muridke, maybe if the plant is collected from other places composition may vary because soil properties, climatological conditions, environmental conditions, and water properties affect the overall trace elements concentration in the plant. In our experiment Manganese content was 77 mg/L. Manganese is similar to Mg in its biochemical function and is involved in activating enzyme-catalyzed reactions including reductions, phosphorylations, hydrolysis, and decarboxylations reactions and consequently, affects processes such as amino acid synthesis, respiration, lignin biosynthesis, and the intensity of hormones in plants [29].

The plant material has an iron content of 298.25 mg/L of the plant material. The iron metal is necessary for the synthesis of red blood cells. It is also an important component of enzymes like cytochromes found in cells. *Physalis minima* contains very limited trace elements except for Na is 1800 mg/L. So *Physalis minima* can be used as a tonic in case of Na and potassium deficiency. The overall quantity of Ca (80.95%) observed in the sample was high.

The pharmacological behavior of that plant is only due to the existence of saponins, steroids, terpenoids, and phytochemical ingredients. Saponins present in the plan talso have uses like as emulsifying agent and expectorant [30]. Alternatively, their soap-like characteristics make them valuable surfactants [31]. Steroids and terpenoids have shown analgesic properties [32].

The linear regression equation was used to determine the total protein contents, polysaccharides, total amide contents, total tannins, total flavonoid, total polyphenol contents, and phenolic compounds linear curves were obtained from the standard curve of bovine serum albumin. The methanolic extract was having maximum content as compare to chloroform and n-hexane. These results were similar to the study conducted by [33]. The presence of phytochemicals is used for a variety of diseases which is present in many plants. Plants use phytochemicals formation for defense in and repairing in their natural ecosystem. The anti-diarrheal activity has been reported in phenols, tannins, and flavonoids [34] and minimized the chances of disorders related to oxidative stress [35]. Antiseptic behavior is found in tannins and can speed up the healing of wounds. It can also use against parasites as body resistance [36]. Flavonoids are strong water-soluble anti-oxidants and free radical scavengers which avoid oxidative cell damage and have strong anticancer activity.

The human population is exposed to H_2O_2 employing the environment, nearly about 0.28 mg/kg/day with intake frequently from leaf crops. Hydrogen peroxide may enter into the human body through the breathing of vapor or mist and the skin or eye contact. H_2O_2 is quickly decayed into oxygen and water, this may construct hydroxyl radicals (-OH) that can start lipid peroxidation and cause DNA damage in the body. But this plant showed a significant presence of antioxidant properties as observed from all four methods of antioxidant measurement. The overall results reflected that the plant has significant antioxidant

potential and in future can be used for different diseases like cancer.

CONCLUSION

Qualitative and quantitative analysis of root extracts revealed that significant secondary metabolites are present in significant amount in the plant. Moreover, the concentrations of flavonoids are directly proportional to the phenolic compounds in the plant and the anti-oxidant activity is attributed to the phenolic compounds. Further studies are required to ascertain the concentration of secondary metabolites in various species of the genus and to compare their antioxidant potential with synthetic products available in the market.

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