The Pharma Innovation Journal 2020; 9(7): 01-04

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2020; 9(7): 01-04

www.thepharmajournal.com
Received: 01-05-2020
Accepted: 03-06-2020

Sowparthani Kaliyaperumal
Department of Biotechnology, Valliammal College for Women, Chennai, Tamil Nadu, India

Radhika M
Department of Biotechnology, Valliammal College for Women, Chennai, Tamil Nadu, India

Corresponding Author:
Sowparthani Kaliyaperumal
Department of Biotechnology, Valliammal College for Women, Chennai, Tamil Nadu, India

Study of phytochemical analysis and antioxidant activity of *Spinach oleracea* L plant leaves

Sowparthani Kaliyaperumal and Radhika M

Abstract
Spinach is a leafy green flowering plant. The leaves are a common edible vegetable consumed by fresh leaves. The bioactive components present in the *Spinach oleracea* plant leaves are known to be responsible for its medicinal properties. The present study was undertaken to compare the effect of different extraction solvents to extract the active components like Carbohydrates, Tannin, Flavonoid, Phenol, Carbohydrate from the dried leaves of *Spinach oleracea* plant. The extraction solvents used were Ethyl acetate, Petroleum ether and Methanol. Phytochemical analysis, total flavnoid concentration, total phenol concentration and antioxidant activity have been identified to compare the efficiency of different extraction solvents. The results shows that using methanol as an extraction solvent works best for the extraction of various active phytochemicals, the total flavonoid and phenols concentration and the antioxidant activity showed the effective scavenging activity. This is the report that directly compares the three extraction solvents for the extraction of active components from the *Spinach oleracea* leaves and shows that method should be the solvent of choice.

Keywords: *Spinach oleracea*, methanol, ethyl acetae, petroleum ether, flavnoid

Introduction
Spinach is a green leaves vegetable and is grown in most parts of the world. Spinach leaves are best source of dietary magnesium. They help in energy metabolism, maintaining muscle, nerve function, maintaining heart rhythm, a healthy immune system and blood pressure [1, 2].
Several studies on the chemical composition of leafy vegetables have shown that, they contain enormous amount of micronutrients, several agronomic advantages and economic value. They also contain some chemical compounds that are having important medicinal uses, human well-being and healthy lifestyle. Some of the vegetables are also reported to cure more than one health problem. The medicinal values of vegetables and fruits are believed to be dictated by their phytochemical and other chemical constituents [3].
The nutritional value of Spinach indicates it to be a very nutrient dense food. It is low in calories yet very high in vitamins, minerals and other phytonutrients. Spinach is also packed with a number of antioxidants like polyphenols, flavonoids and carotenoids, which are shown to possess anti inflammatory effects, anti mutagenic potential, anti neoplastic effects as well as chemo preventive activities [4, 5].
Antioxidant compounds in food play an important role as a health protecting factor. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Some compounds such as gallates which have strong antioxidant activity, while others such as the monophenols are weak antioxidants. The main characterstic of an antioxidant is its ability to trap free radicals [6].

Materials and Methods
Collection of Plant sample
The *Spinach oleracea* leaves was collected from Tamil Nadu. The plant was identified and authenticated. Fresh plant material was washed with water, air dried and then blended fine powder (Fig 1and 2).

Fig 1: *Spinach oleracea*

Fig 2: *Spinach oleracea* (Leaves Powder)

Extraction Preparation

The leaves *Spinach oleracea* was dried in shade for 12days.The dried samples was grind into a coarse powder using a motor and pestle. The extraction with different solvents like Ethyl acetate, Petrolium ether and Methanol were done using Soxhlet apparatus. For every 100 ml for each solvent, 25 g of crushed leaves powder was used for soxhlet extraction. After extraction for 3 consecutive days the crude liquids were placed in water bath at 55 ºC for excess solvent evaporation

Phytochemical analysis

Phytochemical screening for major constituents was undertaken using standard qualitative methods. Screening test were performed for carbohydrates, tannins, saponins, flavonoid, alkaloids, quinines, glycosides, cardiac glycosides, terpenoids, phenols, coumarins, phlobatannins, anthraquinines, phytosteroids in the mentioned Spinach oleracea leaves extract by following procedure mentioned in this study as [7].

Test for carbohydrates

2ml of plant extract was added with 1ml of Molisch's reagent and few drops of concentrated sulphuric acid were added. Presence of purple or reddish colour indicates the presence of carbohydrates.

Test for tannins

1ml of plant extract, 2ml of 0.7M NaOH and few drops of Folin-Denis reagent was added into to the test tube. Formation of dark blue or greenish black indicates the presence of tannins.

Test for saponins

2ml of plant extract, 2ml of distilled water was added and shaken in a graduated cylinder for 15minutes lengthwise. Formation of 1cm layer of foam indicates the presence of saponins.

Test for flavonoids

2ml of plant extract, 1ml of 2N sodium hydroxide was added into to the test tube. Presence of yellow colour indicates the presence of flavonoids.

Test for alkaloids

2ml of plant extract, 2ml of concentrated hydrochloric acid was added into to the test tube. Then few drops of Mayer's reagent were added. Presence of green colour or white precipitate indicates the presence of alkaloids.

Test for quinines

1ml of extract and 1ml of concentrated sulphuric acid was added. Formation of red colour indicates presence of Quinines.

Test for glycosides

2ml of plant extract, 3ml of choloroform and 10% ammonia solution was added. Formation of pink colour indicates presence of glycosides.

Test for cardiac glycosides

0.5ml of extract, 2ml of glacial acetic acid and few drops of 5% ferric chloride were added. This was under layered with 1 ml of concentrated sulphuric acid. Formation of brown ring at the interface indicates presence of cardiac glycosides.

Test for terpenoids

0.5ml of extract, 2ml of chloroform was added and concentrated sulphuric acid was added carefully. Formation of red brown colour at the interface indicates presence of terpenoids.

Test for phenols

1ml of the extract, a few drops of Phenol-Denis reagent was added followed by 2ml of 15%Sodium carbonate solution. Formation of blue or green color indicates presence of phenols.

Test for coumarins

1 ml of extract, 1ml of 10% NaOH was added. Formation of yellow colour indicates presence of coumarins.

Test for steroids and phytosteroids

1ml of plant extract equal volume of chloroform is added and subjected with fewdrops of concentrated sulphuric acid appearance of brown ring indicates the presence of steroidsandappearance of bluish brown ring indicates the presence of phytosteroids.

Test for phlobatannins

1ml of plant extract few drops of 2% HCL was added appearance of red colour precipitate indicates the presence of phlobatannins.

Test for anthraquinones

1ml of plant extract and few drops of 10% ammonia solution was added, appearance pink colour precipitate indicates the presence of anthraquinones.

Quantification of total flavnoids content

Total Flavanoid content in the extracts was determined using the method described by [8]. The flavonoid content was determined by aluminium chloride method using Quercetin as

standard. Extracts and Quercetin were prepared in (10 mg/ mL). 0.1 mL of extract was mixed with 0.9 mL of distilled water in test tubes, followed by addition of 75 µL of 5% sodium nitrite solution. After 6 minutes, 150 µL of 10%aluminium chloride solution was added and the mixture was allowed to stand for further 5minutes after which 0.5 mL of 1M Sodium hydroxide was added to the reaction mixture. Then add 2.5 ml of distilled water and mixed well. The absorbance was measured immediately at 510 nm using a spectrophotometer. A calibration curve was generate during various concentrations of Quercetin (20-100µg). Blank consist of all the reagents, exceptfor the extract or Quercitin is substituted with 0.1ml of Results were expressed as the Quercetin equivalence (QE) of the sample was expressed in mg/g of the extract.

Total phenolic content

The amount of phenolic compounds in the extracts was determined by the Folin Cio calteu colorimetric method and calculated from a calibration curve obtained with gallic acid as standard (1mg/1ml) [9]. From the standard solution 20 to 100 µl was taken and added to different test tubes. Extract was added in a separate test tube at a concentration of 10 mg/ml. To 0.1ml of each extract, 5ml of folins – ciocalteu (1:10 dilution) was added and the contents were mixed thoroughly and incubated in dark for three minutes. 5ml of sodium carbonate (11.25/150ml) was added and the mixture was incubated in dark for 60 minutes. The absorbance was measured at 760nm in a UV-Visible Spectrophotometer The results were expressed in Gallic acid equivalence of the samples (GE) µg/mg of the extract.

Anti oxidant activity

Free radical scavenging activity was determined by DPPH radical scavenging assay as described by [10]. 4mg/100ml of DPPH in methanol was prepared and 1.5 ml of this solution was mixed with 1.5 ml of extractives in methanol at different concentrations (20-100). The reaction mixture was vortexed thoroughly and left in the dark for 20 minutes. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid was used as reference standard. Percentage DPPH radical scavenging activity was calculated by the following equation

$$[(A_{control} - A_{extract}) / (A_{control})] \times 100$$

Results and Discussion

Phytochemical analysis

Qualitative analysis performed with *Spinach oleracea* leaves in three solvents namely Petroleum ether, Ethyl acetate, and Methanol based on the polarity showed positive results for Carbohydrates, Tannins, Saponins, Flavonoids, Alkaloids, Glycosides, Cardiac glycosides, Terpenoids and Phenols.

The present study was conducted with an objective to identify the best extraction solvent, which can be used to extract the maximum amount of the phytochemicals from the dried *Spinach oleraceae* plant leaves. The three extracts of *Spinach olreaceae* leaves commonly showed the presence of phytochemical such as Carbohydrate, Tannin, Flavonoid Terpenoid and Phenol(Table 1). *Spinach oleraceae* is a well known medicinal plant known to have health benefits against many diseases.. Qualitative biochemical estimation were conducted to detect the presence of different phytochemicals in the dried *Spinach oleraceae* plant leaves extract obtained by using different solvents Petroleum ether, ethyl acetate and methanol. Our results highlights that all the extracts formed by using different solvents from *Spinach oleraceae* plant leaves contain phytochemicals like carbohydrate, tannin, flavonoid, tereponoid and phenol. Previous reports also shown that the preliminary phytochemical analysis of *Spinach oleraceae* revealed that different active constituent present in different extracts such as carbohydrates, proteins, aminoacids, fats, oils, steroids, terepenoids. Glycosides, alkaloids, tannins and other phenolic compound [11].

Table 1: Phytochemical analysis

Parameters	*Spinach oleracea*		
	Petrolem ether	Ethyl acetate	Methanol
Carbohydrate	+	+	+
Tannins	+	+	+
Saponins	-	-	-
Flavonoids	+	+	+
Alkaloids	-	+	-
Quinones	-	-	-
Glycosides	-	+	-
Cardiac Glycosides	-	-	-
Terpenoids	+	+	+
Coumarins	-	-	-
Phytosteroids	-	-	-
Phlobatannines	-	-	-
Anthroquiones	-	-	-
Phenol	-	+	+

(+) =Indicates presence
(-) =indicates absence

Quantification of total flavnoids and Phenol content

Total flavonoid concentration was quantified by using Quercetin as the standard and measured OD at 510 nm. Using Methanol as an extraction results in the maximum flavonoid extraction with 11.41mg/g of quercetin equivalent of extract followed by petroleum ether and ethylacetate with 4.21mg/g, and 3.37 mg/g of quercetin equivalent of extract (Table 2).

Total phenol content in the plant was estimated using gallic acid as a standard. The OD was taken at 760 nm. Maximum phenol concentration was observed when ethylacetae 4.33(mg/g) and Methanol 2.06(mg/g). the ethyl acetate extracts were registered the high amount of phenol content. (Table 3)

A Quantitative study was further conducted to detect the amount of the total flavonoids and total phenols in these plant extracts for direct comparison and have demonstrated that methanolic extract contains the maximum amount of flavonoids and phenols. The quantification of phytochemicals of *S. oleraceae* showed the presence of the highest amount of phenols and flavonoids justified the potent antioxidant nature of the plant. The study also revealed that flavonoids represent the main group of phenolic compounds in *S. oleraceae* [12].

Table 2: Total flavonoid content

Extracts	Sample OD at 510 nm (µg)	Total flavonoid content (mg/g) equivalent of Quercetin Content
Petroleum ether	-	2.05
Ethyl acetate	3.37	3.37
Methanol	3.08	11.41

Table 3: Total Phenolic content

Extracts	Sample OD at 700 nm (µg)	Total Tannin content (mg/g) Equivalent of Quecetin Content
Petroleum ether	-	-
Ethyl acetate	0.4333	4.33
Methanol	0.2066	2.06

Antioxidant activity

The ability of *Spinach oleracea extracts to* scavenging the DPPH free radical assay by petroleum ether, ethyl acetate and methanol extract The samples are added to the mixture and incubated for 30minutes in dark. On completion of 30minutes of incubation, colour change is observed in the mixture. The colour changed from violet to pale yellow. The readings are recorded at 517nm. Colour change represents the scavenging activity of the mixture. The antioxidant activities of particles were compared with the standard in graphical representation. The mean IC_{50} values of the leaves and Methanol extract were lower which showed the radical scavenging activity was shown to be effective in this solvent extracts19.74mg/g then the ethyl acetate extract 43.42mg/ml and Petroleum ether extract 49.21mg/ml. The IC_{50} value and scavenging activity of Petroleum ether, Ethyl acetatae, methanol extract of *Spinach oleracea* were 24.31µg/ml, 23.43µg/ml, 10.63µg/ml and scavengimg effect of 55.07% inhibition effect (Table 4).

Spinach ranks high among vegetables regarding antioxidant capacity because of an abundance of phenolic compounds [13, 14], suggesting that spinach consumption may afford protection against oxidative stress mitigated by free-radical species. Spinach phenolic compounds exhibit a wide range of biological effects including antioxidative [15]. Flavonoids are the most abundant polyphenols in our diet. The free radicals produced in the body are neutralized by flavonoids which are well known for their antioxidant properties. The results from these studies which highlights that methanolic *Spinach oleraceae* plant leaves extract possess the maximum concentration of flavonoids, phenols and s also possess the maximum antioxidant activities.

Table 4: Antioxidant activity of *Spinach oleracea*

Extract	Concentration (µg/Ml)	Control	Test Sample	% of Inhibition
Petroleum ether	200	0.205	0.1536	25.07
	400	0.205	0.1463	28.63
	600	0.205	0.1461	28.73
	800	0.205	0.143	30.24
	1000	0.205	0.1428	30.34
Ethyl acetate	20	0.205	0.1528	25.46
	40	0.205	0.1388	32.29
	60	0.205	0.1341	34.59
	80	0.205	0.1337	34.78
	100	0.205	0.1341	34.59
Methanol	20	0.205	0.1273	37.90
	40	0.205	0.1022	50.15
	60	0.205	0.099	54.15
	80	0.205	0.0934	54.44
	100	0.205	0.0921	55.07

Conclusion

The phytochemical analysis of *Spinach oleraceae* revealed the presence of phytochemicals such as Carbohydrate, Flavonoid, tannins, terponoids and phenol in three different extracts. Furthermore, the quantification of phytochemicals showed the leaves of *Spinach oleraceae* Flavonoid and Phenolic compound. Hence the presence of these beneficial secondary metabolites imparts antioxidant potential to *Spinach oleraceae.* By this present phytochemical scrrening and quantification, we suggest the *Spinach oleraceae* is a good nutrient rich leafy vegetable with an antioxidant value that can be used as a therapeutic medicine.

Reference

1. Farah K, Abdul-Wahab, Thukaa Z, Abdul Jalil. Study of Iraqi Spinach leaves Phytochemical and protective effects against induced hepatotoxicity in rats, Iraqi J Pharm Sci. 2012; 21(2):8-17.
2. Subhash GP, Virbhadrapp SR, Vasant K. *Spinach oleracea* Linn: A pharmacognostic and pharmacological overview. IJRAP. 2010; 1:78-84.
3. Fallah HSM, Alavian HR, Heydari MR, Abolmaali K. The efficacy of liv-52 on liver cirrhotic patient: A randomized, double blind, placebo-controlled first approach. Phytomedicine 2005. 9:619-624.
4. Boivin D, Lamy S, Lord-Dufour S, Jackson J, Beaulieu E, Cote M *et al.* Food chemistry, 2009.112:374-80.
5. Hait-Darshan R, Grossman S, Bergman M, Deutsch M, Zurgil N. Food Res. Int. 2009; 42:246-53.
6. Miller HE, Gigelhof F, Marquart L, Prakash A, Kanter M. Whole grain products and antioxidants. Cereal Foods world, 2000; 45:59-63.
7. Sofowora A. Screening plants for bioactive agents in medicinal plants and traditional medicinal in Africa. 2nd Ed. Spectrum Books ltd, Sunshine house, Ibadan, Nigeria,1993, 134-156.
8. Lin JY, tang CY. Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. Food Chem. 2007; 101:140-147.
9. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology and Viticulture, 1965; 16:144-158.
10. Choi HY, Jhun EJ, Lim BO. Application of flow injection- chemiluminescence to the study of radical scavenging activity in plants, 2000; 14:250-253.
11. Prieto P, Pineda M, Anguilar M. Spectro photometric quantitation of antioxidant capacity through the formation of Phosphomolybdenum Complex: specific application of vitamin E. Anal. Biochem, 1999; 269:337-41.
12. Shivaranjani VL, Poornima H, Umamaheswari J, Devi KL. Preliminary phytochemical screening and quantification of bioactive compounds in the leaves of Spinach (*Spinach oleraceae* L). Int. J. Pharmacognosy, 2104, 465-71.
13. Cao G, Sofic E, Prior RL. Antioxidant capacity of tea and common vegetables. J. Agric. Food Chem. 1996; 44:3426-3431.
14. Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, Prior RL. Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. J. Agric Food Chem. 2004.52:4026-4037.
15. Bergman M, Varshavsky L, Gottlieb HE, Grossman S. The antioxidant activity of aqueous Spinach extract chemical identification of active fractions. Phytochemistry. 2001; 58:143-152.