International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 04-05-2021, Accepted: 16-05-2021, Published: 31-05-2021
Volume 6, Issue 3, 2021, Page No. 499-504

Evalution of total phenolic and flavonoid content, free radical scavenging activity and phytochemical screening of fruits of *Raphanus caudatus* l.

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Abstract
This study aimed to evaluate the phytochemical analysis and the total phenol and flavonoid content and antioxidant activity of fruit extract of *Raphanus caudatus* L. from brassicaceae family. The fruits of *Raphanus caudatus* L. were dried and extracted with two different solvents (methanol and chloroform). Secondary metabolites are important for medicinal activity of plants. So in the present study phytochemical screening of fruits of *Raphanus caudatus* L. was carried out. Quantitative analysis of phenols was carried out using folin- ciocalteu method. Gallic acid was used as the standard for assessment of phenols. Evaluation of total flavonoid content was carried out by using quercetin as a standard. The antioxidant activity of extract was determined by DPPH assay. Preliminary analysis of each crude extract shows the presence of alkaloids, carbohydrates, glycosides, phenols, flavonoids, terpenoids, steroids, protein. The crude methanol extract was collected of a remarkable quantity of TPC and TFC 186.66±1.013 mg/GAE/g and 26.83±1.166 mg/QE/g of sample respectively. The IC_{50} value of crude methanolic extract was 612.58±1.41 µg/ml.

Keywords: phytochemistry, quantitative analysis, dpph assay, *Raphanus caudatus* l

Introduction

Medicinal plants continue to play an important role in the lives of a significant proportion of the global population. Herbal medicine plays a dominant role in the healthcare system, which is particularly true in developing countries (Dar *et al.*, 2017) [1]. The world Health Organization reports that about 80% of the world's 5.2 billion people live in much less developed nations, and that about 80% of these people depend almost entirely on conventional medicine for their primary health protection services. Traditional medicines are the "backbone" of conventional medicine, which indicates that over 3.3 billion people in less developing counties use them on a routine basis (Ahvazi *et al.*, 2012) [2]. More than 7000 species of angiosperms are reported to have therapeutic use in traditional and known systems of medicine such as Ayurveda, Unani, siddha, and Homoeopathy, out of the 17000-18000 species of flowering plants (Kala *et al.*,2007) [3]. Medicinal plants have long been used in some type or the other by native medical systems. Full phytochemical studies of Indian medicinal plants should be conducted, as these secondary metabolites are liable for the plant's medicinal function. Secondary metabolites were tested for medicinal benefit in a variety of plants (Savithramma *et al.*,2011) [4].

Medicinal plants are rich source of data on board range of chemical constituents that could be produced as detailed specificity drugs. These are the sources of possibly beneficial chemical molecules that could be used as new leads and hints in current drug development. Alkaloid, tannins, flavonoids, and phenolic molecules are the most essential bioactivity substance of plants. The relationship among phytoconstituents and plant bioactivity is important to understand for the synthesis of substance with unique functions to cure a variety of health problems and chronic diseases (Yadav *et al.*,2014) [5]. Antioxidants are effective free radical scavengers in the cell, while free radicals are awfully reactive chemical compounds like superoxide, hydroxyl radicals, or singlet oxygen that circulate in the body and create cell harm. Free radical injury is among the most likely reasons of debilitating diseases like cardiovascular disease, which can occur as heart attacks, and cancer, which kill many people around the world (Nafiu *et al.*,2013) [6]. This study stated that qualitative and quantitative analysis of phytochemicals and antioxidant activity.

Raphanus caudatus L. common name is Rat tailed radish and mogri. Rat tailed radish is an erect, branched, annual plant growing around 45 cm tall. *Raphanus caudatus* L. is herb type of plant from brassicaceae. This groups of radish do not produce good quality roots of, it is cultivated mainly for the edible young seedpods which are harvested in the particular season. Podding radish have no thickened root. From digital Gujarat flora. It has medicinal properties which are very useful for human.

Materials and Methods

Chemicals Required

Gallic acid, Folin reagent, Sodium carbonate, Quercetin, Aluminium chloride, Potassium acetate, Ascorbic acid, DPPH powder, Mayer's reagent, Wager's reagent, Hager's

reagent, Dragendroff reagent, Molish reagent, Fehling A&B reagent, Barford reagent, Benedict reagent, Ammonium solvent, Acetic acid, Sulfuric acid, Glacial acetic acid, Ferric chloride, Copper sulfate, Ethanol, KOH pellet, Lead acetate, Folin – Ciocalteu reagent, Sodium hydroxide, Copper acetate, pyridine, Sodium nitroprusside.

Collection of plant material

The fruits of the *Raphanus caudatus* L. were collected from the Gandhinagar district, Gujarat state in the month January 2021. Then the collected plant fruits were washed with simple tap water and then cut it in 2 to 3 cm. After that dried for 10 to 12 days in room temperature.

Extract preparation method

The dried plant material was crushed with the help of electronic grinder. Then powdered sample was weighed with weighing machine. After that 10-gm powder material was taken and kept in 100 ml solvent like Methanol and chloroform in the conical flask. Cover the conical flask with help of aluminium foil. And then kept it on shaker for 24 hours. Next the solution was filtered with whatman filter paper number 1. Four petri plates were weighed and then the filtered solution was transferred in to the petri plates. And allowed it to evaporate at the room temperature for 24 hours. Then dried extract was ready for weighing. After weighing yield of extract was calculated using below equation.

$$\text{The yield } \% = \frac{\text{Weight of dry extract} \times 100}{\text{Weight of plant powder}}$$

Phytochemical screening of secondary metabolites

Preliminary screening of phytochemicals is an important step, in the observation of the bioactive principles occur in medicinal plants and afterwards may lead to drug exploration and evolution (Rathore *et al.,* and Rasool *et al.*,) [7-8].

Test for alkaloids

Mayer's test: Taken 2 ml filtrate then add side by side 1 ml mayer's reagent, the white creamy precipitates indicate alkaloids present.

Wager's test: Taken 2 ml filtrate then add 2 ml wager's reagent side by side reddish, brown precipitates are seen which shows that alkaloid present.

Hager's test: 2 ml filtrate taken with 2 ml hager's reagent, yellow precipitates shows that alkaloids present.

Dragendroff 's test: 1 ml filtrate with 2 ml dragendroff reagent, orange precipitates show alkaloids present.

Test for carbohydrate

Molish test: 2 ml filtrate add moish reagent's drop side by side if violet ring was seen than carbohydrate present.

Fehling's test: 1 ml filtrate with 1 ml fehling A&B reagent than kept it in water bath for 5 min if Red precipitates seen that carbohydrate present.

Barford test: 1 ml filtrate with 1 ml Barford reagent and boil for 2 min then red precipitates indicates carbohydrate present.

Benedict test: 1 ml filtrate then add 1 ml benedict reagent then boil it for 2 min as a result-coloured precipitates are seen and it indicate that carbohydrate present.

Test for Glycosides

Taken 2 ml filtrate then add 3 ml chloroform after that shake the test tube add 10% ammonium solvent, pink colour seen that indicates glycosides present.

Legal's test: Taken 2 ml filtrate with 2 ml chloroform add 2 ml acetic acid add concentrated sulphuric acid then cooled test tube with ice plate. first violet than blue and green colour indicate that glycosides present.

Keller-kilani test: 1 ml extract add 1 ml glacial acetic acid also add 2 drops of 2% ferric chloride, then concentrated sulphuric acid was added and observed for the formation of two layers; the upper layer was reddish brown and the lower layer was bluish green indicates that glycosides present.

Test for protein

Millon's test: 2 ml filtrate was treated with 1-2 ml millon reagent; white precipitates indicate presence of protein.

Biuret test: 2 ml filtrate was treated with 0.5 ml of 2% copper sulfate and add 1 ml ethanol then add one KOH pellet; observed pink coloured of ehanol layer indicates presence of protein.

Test for phenols

Ferric chloride test: 2 ml filtrate taken with 1-2 drops of 5% ferric chloride; dark green colour shows presence of phenols.

Lead acetate test: 2 ml filtrate with 0.5 ml of lead acetate; white precipitates indicate presence of phenols.

Folin- cioculten test: 0.5 ml extract with 1 ml folin ciocalteu reagent; bluish green colour shows presence of phenols.

Test for flavonoids

Alkaline Test: extract with 3 ml 2% sodium hydroxide; yellow colour was seen then add diluted sulphuric acid, yellow colour disappear indicates presence of flavonoids.

Lead acetate test: extract was treated with few drops of 10% lead acetate; if yellow precipitates was seen then flavonoids was present.

Test for saponin

Forth test: few drops extract with 20 ml DH2O then shake for 1 min; presence of foam indicates positive test for saponin.

Test for fixed oils& fats

Small amount of extract pressed between filter paper, leave oil stain on filter paper indicates presence of fixed oil.

Test for terpenoids

Salkowski test: extract with 2 ml of chloroform then add 3 ml concentrated sulphuric acid; formation of Reddish-brown colour ring was presence of terpenoids.

Copper acetate test: 1 ml filtrate taken with 1-2 drops of acetate solution; if green colour precipitates was seen then terpenoids present.

Test for Cardiac glycoside

Legal test: 2 ml filtrate then add 1 ml pyridine after that add 1 ml 20% of sodium nitroprusside; pink or red colour indicates presence of cardiac glycoside.

Test for Steroids

Liebermann Burchard' s test: 1 ml filtrate with 2-3 ml acetic anhydride solution and then add 2 ml sulphuric acid; violet or green colour shows presence of steroids.

Salkowaski's Test: 2 ml filtrate shaken with chloroform add sulphuric acid dropwise side by side; red colour indicates presence of steroids.

Quantitative Analysis

Total phenolic content

The total phenolic content in plant extracts was determined by using Folin-Ciocalteu colorimetric method based on oxidation and reduction reaction. TPC activity is the main process to assess the amount of phenolic content in the samples. Phenolic compounds that encompass in the plants has redox properties, and these properties allow them as antioxidant. For total phenolic content estimation, 1 ml folin-ciocalteu reagent was added to the 1 ml of methanolic extract having concentration 0.2 mg/ml. Then 10 ml distilled water and 20 % 4 ml sodium carbonate was added to mixture. At last, total volume was made up to 25 ml with addition of distilled water. After that, test-tubes were kept in dark room for 30 min for incubation. Then, O.D. was recorded in spectrophotometer at 765 nm. For the standard reference gallic acid was used and similar procedure was followed for the series of gallic acid with concentration 20 µg/ml to 200 µg/ml. The results were presented as Gallic acid equivalent per gram of sample (GAE/g of sample) calculated using the below equation (Madhu *et al*., 2016) [9].

$$GAE = C \times V/M$$

Where, C = Concentration of gallic acid established from the calibration curve in mg/ml
V = Volume of the extract solution in ml
M = Weight of the extract in g.

Total flavonoid content

The elemental proposition of total flavonoid content method is that aluminium chloride forms acid secure compound with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. 1 ml methanolic extract of sample was taken with concentration 0.2 mg/ml and mixed with 100 µL of 10% of aluminium chloride. After that, 100 µL of 1 M potassium acetate was added. Then 4.8 ml distil water was added and test-tubes were shaken well. Incubation of 30 minutes in dark was given. Finally, O.D. was recorded at 415 nm in spectrophotometer. Similar procedure was followed for the standard quercetin with concentration range of 20 µg/ml – 200 µg/ml to obtain reference calibration equation. The results were presented as Quercetin equivalent per gram of sample (QE/g of sample) calculated using the below equation. Following protocol from same as total phenolic content.

$$QAE = C \times V/M$$

Where, C = Concentration of quercetin established from the calibration curve in mg/ml
V = Volume of the extract solution in ml
M = Weight of the extract in g.

Measurement of antioxidant activity (DPPH radical scavenging assay)

The DPPH assay is focused on an antioxidant's ability to contribute a hydrogen or an electron to the DPPH radical, a stable free radical with a deep violet shade. DPPH radicals are converted to equivalent hydrazine, when an unusual electron is coupled in the existence of a free radical scavenger such as an antioxidant agent, and the mixture is decolorized from its original deep violet to light yellow colour. DPPH is a common abbreviation for the organic chemical compound 2,2- diphenyl-1-picrylhydrazyl. It is a dark coloured crystalline powder composed of stable free radical molecules. For the estimation of antioxidant activity stock solution of DPPH was prepared with concentration 0.04 mg/ml. 1 ml of methanolic extract with concentration range from 20 µg/ml to 200 µg/ml was taken in test-tubes covered with aluminium foil. Then, 2 ml of DPPH was added to the extract solutions. Incubation was given for 20-30 minutes in complete dark at room temperature. The O.D. was recorded after incubation at 517 nm in spectrophotometer. The methanol was used as negative control and Ascorbic acid with similar concentration range i.e., 20 µg/ml to 200 µg/ml used as positive control. The results were presented as IC_{50} value, that was calibrated from the graph of concentration vs percentage inhibition. Percentage inhibition was calculated from the following formula [10].

$$\%\ \text{Inhibition Activity} = \frac{\text{Absorbance of the blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

Results

Percentage of yield

The percentage yield for methanolic extract was 30.46% and the percentage yield of chloroform extract was 1.64%.

Phytochemical analysis result

Phytochemical analysis indicated the presence of alkaloids, carbohydrates, glycosides, protein, phenols, flavonoids, terpenoids, cardiac glycosides, and steroids.

Table 1: Showing phytochemical screening of *Raphanus caudatus* L. fruit extract (+) indicates presence and (-) indicates absence

Sr. No	Phytochemicals	Test	Results	
			Methanol	Chloroform
1	Alkaloids	Mayer's test	+	+

		Wager's test	+	+
		Hager's test	-	+
		Dragendroff's test	+	+
2	Carbohydrate	Molish test	-	-
		Fehling test	-	+
		Barford test	-	-
		Benedict test	-	+
3	Glycosides	Borntrager's test	+	-
		Legal's test	-	+
		Keller-killani test	-	+
4	Protein	Millon's test	+	+
		Biuret test	-	-
5	Phenols	Ferric chloride test	-	-
		Lead acetate	+	+
		Folin – ciocalteu test	-	+
6	Flavonoids	Alkaline test	-	-
		Lead acetate test	+	+
7	Saponin	Forth test	-	-
8	Fixed oils & fats		-	-
9	Terpenoids	Salkowski test	+	-
		Copper acetate	-	+
10	Cardiac glycoside	Legal test	+	+
11	Steroids	Libermann burchard's test	-	+
		Salkowaski's test	+	-

Total phenolic content

The total phenolic content in methanolic extracts of fruits of *Raphanus caudatus* L. was determined by folin-Ciocalteu method using gallic acid as the standard. The phenolic content of extracts in methanol was calculated using the standard curve potted for gallic acid. That are showed Spectro photo-metrically at 760 nm. The total phenolic content of the extracts was calculated from the regression equation of calibration curve (Y= 0.0049x-0.0971 =R^2=0.9892). The total phenolic content of fruit extracts contained (186.66±1.013 mg/ GAE/ g of sample).

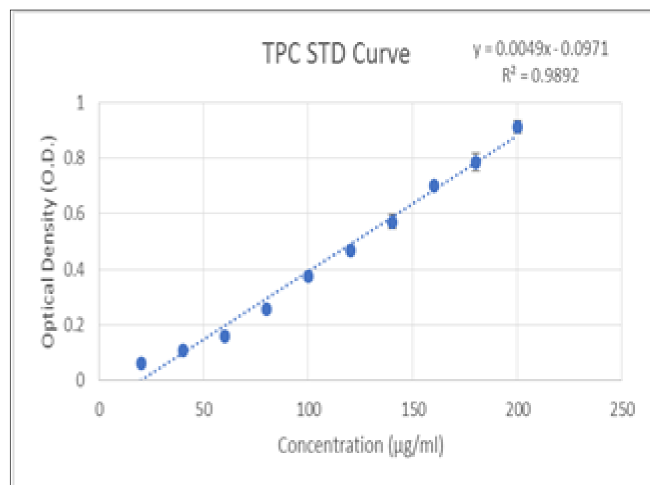

Fig 1: Standard curve of Gallic acid for total phenol content

Table 2: Total phenol content of sample (Methanolic fruit extract)

Sr. No.	GAE/g of sample	Average
1	185	186.6667 ± 1.013
2	188.5	
3	186.5	

Total flavonoid content

The flavonoid content in methanolic extracts of fruits was determined by using quercetin as standard. The flavonoid content of extracts in methanol was calculated using the standard curve potted for quercetin. That are showed Spectro-photometrically at 415 nm. The total flavonoid content of the extracts was calculated from the regression equation of calibration curve (Y= 0.0128x+0.1165 R^2 = 0.9915). The total flavonoid content of fruit extracts contained (26.83±1.166 mg/QE/g of sample).

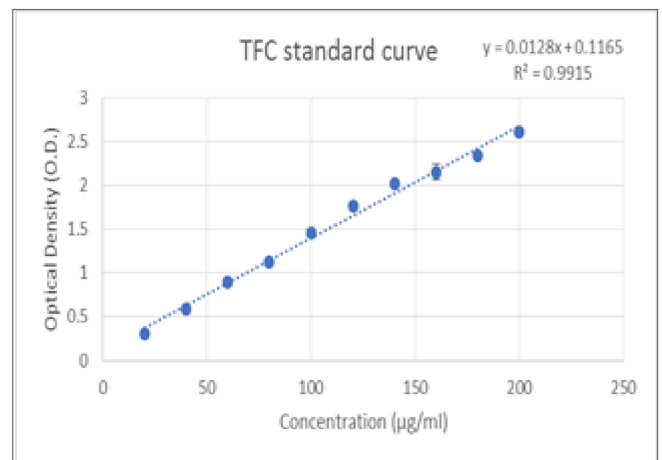

Fig 2: Standard curve of Quercetin for total flavonoid content

Table 3: Total flavonoid content of sample (Methenolic fruit extract)

Sr. No.	QE/g of sample	Average
1	26.5	26.833 ± 1.166
2	25	
3	29	

DPPH radical scavenging assay:

The ability to scavenge free radicals was assessed using the measuring the reduction of DPPH scavenging operation 517 nm absorbance (Peteros and Uy., 2010) [11]. This assay assesses the ability of the extracts under investigation to scavenge free radicals. A molecule carrying balanced free radical is known as DPPH. The purple colour of the free

DPPH radical decays in the presence of an antioxidant that can donate an electron to it. This test determines a compound's capacity to contribute a hydrogen atom, the number of neutrons given molecules may donate, and the antioxidant action mechanism (Mothana *et al*., 2009) [12]. The methanolic extract of *Raphanus caudatus* L. fruits exhibited a maximum DPPH scavenging activity. Findings were showed as 50% inhibition concentration (IC_{50}) calculated from the regression equation get through a graph of concentration against the percentage of inhibition. In this study, the **IC_{50}** value of methanolic fruit extract and standard was **612.58±1.41** µg/ml and **133.62±0.41** µg/ml respectively.

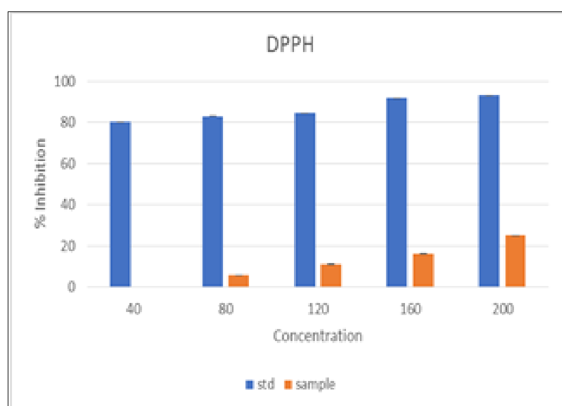

Fig 3: DPPH radical scavenging activity of standard Ascorbic acid and fruit extract of *Raphanus Caudatus* L.

Table 4: DPPH assay for sample (Methanolic fruit extract)

Sr. No.	Concentration(µg/ml)	Sample (µl)	Solvent (µl)	O.D. 1	O.D. 2	O.D. 3	Average
1	40	200	800	1.33	1.33	1.33	0.075 ± 0.000
2	80	400	600	1.254	1.255	1.254	5.760 ± 0.025
3	120	600	400	1.181	1.183	1.184	11.144 ± 0.066
4	160	800	200	1.114	1.115	1.116	16.228 ± 0.043
5	200	1000	-	0.997	0.996	0.997	25.118 ± 0.025
6	Blank	-	1000	1.331			

Discussion

The aim of this study was to identify secondary metabolites which present in given plant material, as well as total phenolic and flavonoid content. Antioxidant activity was also tested in this research work. Plants which produce phytochemicals are very useful to mankind. They are extremely significant in terms of medicine. Phytochemicals shows various pharmacognosy activities. So many phytochemicals are present in plants such as alkaloids, glycosides, protein, phenols, flavonoids, steroids, terpenoids etc. In this research the phytochemical analysis showed that the plant extract contains so many secondary metabolites. In both methanolic and chloroform fruit extracts indicates the positive result of Alkaloids, Glycosides, protein, phenols, flavonoids, terpenoids, cardiac glycosides, and steroids. Carbohydrates only present in chloroform extract. Saponin and fixed oil & fats are absent in both extracts.

The phytochemical screening which performed by khamees [13] shows that Brassicaceae members are high in phytochemicals and may have medicinal properties such as antimicrobial, antifungal, antimutagenic, antioxidant, and antitumor. In Raphanus seeds extract, phytochemical research revealed the existence of alkaloids, flavonoids, saponins, anthraquinones, tannins, hormones, steroids, terpenoids, and cardiac glycosides. Sharifi [14] has reported secondary metabolites such as terpenoids, coumarins, flavonoids, anthocyanins, isothiocyanates, saponins, alkaloids, 3 ascorbic acids, and potassium 4 have been found in various parts of *Raphanus sativus* L., indicating its medical properties. Furthermore, the plant's leaf extract has been found to have low levels of saturated fat and cholesterol. In our research, utilized the folin-ciocalteu strategy to evaluate the complete phenolic substance of plant extricates from the methanolic fruit extract. This mix was then given a portion of 24 sodium carbonate. The absorbance was estimated at 760 nm utilizing a spectrometer. After 30 min in dim hatching. The absolute phenolic substance of *Raphanus caudatus* L. was seen to be 186.66±1.013 mg/GAE/g of sample.

Kim *et al*., study was reported that kaur *et al*., used the Folin-Ciocalteu method to assess the total phenolic content of plant extracts from the radish genus. This combination was then given a dose of 24 sodium carbonate. The absorbance was measured at 760 nm using a spectrometer after 30 min in dark incubation. The total phenolic contents in radish roots have previously been identified in many reports. The total phenolic content of radish was observed to be 240 mg GAE/100 g d.m. the total phenolic content of radish plant extract was determined using the Folin-Ciocalteu reagent and expressed in gallic acid equivalents in this analysis. The total phenol concentrations were measured in miligrames of GA per gram of extract. The total phenolic content of radish extracts was larger [15].

The complete flavonoid concentrates in our study, quercetin is a flavonoid present in restorative plants that is created by plant digestion. Anticancer, cancer prevention agent. Neuroprotective, antitumor 16, antiviral17, and calming 18 cycles have likewise been accounted for quercetin. The flavonoid content in methanolic extracts of fruits of *Raphanus caudatus* L. was performed by quercetin as a standard. The total flavonoid content of fruit extracts contained 26.83±1.166 mg/QE/g of sample.

The total flavonoid study of sharifi *et al*., [16] shows that quercetin is a flavonoid present in medicinal plants that is produced by plant metabolism. In a 5 ml microcentrifuge tube, an aliquot of methanolic extract (0.1 mL) was combined with 2.4 mL deionized water, 0.15 mL NaNo2 (50 mg/mL) was added, and the mixture was allowed to respond for 5 minutes. After that, 0.15 mL AlCl3 (100 mg/mL) was added and the mixture was left to sit for another 6 minutes. Finally, the reaction mixture was mixed with 1.0 mL 1 mol/L NaOH and 1.2 mL deionized water. Total flavonoid content was measured using a calibration curve and represented as mg quercetin/100 g d.m.

In our study antioxidant activity was performed by DPPH assay. In which ascorbic acid was used as standard. The absorbance was measured at 760 nm using a spectrometer after 30 min in dark incubation. In this study, the IC_{50} value of methanolic fruit extract and standard was 612.58±1.41 µg/ml and 133.62±0.41 µg/ml respectively.

Antioxidant activity by DPPH radical scavenging assay was examined by Reddy *et al.*, [17] the antioxidant elements, function, and stability of Raphanus leaves were investigated in vitro in this analysis. The DPPH radical scavenging assay has been commonly used to assess the ability of different natural products to scavenge free radicals and has been recognized as a model compound for free radicals originating in lipids. The DPPH radical scavenging behaviour of EE was significantly higher ($p = 0.05$) than that of WE and of antioxidant compounds and have potent antioxidant properties. The research also sheds light on the best solvent for separation and the factors that influence their sustainability. However, further research is required to confirm its antioxidant function in food and biological systems.

Charoonratana *et al.*, [18] analysis the antioxidant activity revealed that the IC50 value, which can be determined from the linear regression of DPPH scavenging activity versus extract concentrations, is inversely related to the antioxidant activity of the extracts. Both extracts had IC50 values greater than 1,000 mg/ml in the DPPH assay, suggesting that they had no antioxidant activity.

Conclusion

The principal objective of the study is to recognize the presence of many secondary metabolites, and their useful groups and antioxidant activity of fruit extract of *Raphanus caudatus* L. The existence of secondary metabolites suggested some medicinal applications. It shows high phenolic and some amount of flavonoid content. The extract of fruits of *Raphanus caudatus* L. indicates DPPH radical scavenging activity. Using of pharmacological properties involves more analysis of these effective additive by implementation techniques of extraction, purification, separation and identification. The study propose that crude extract acquire antioxidant activity, thus organic antioxidant can be provoked for possible therapeutic applications. In vitro study gives role model for screening of drugs and so helps in further evaluations of activities of drugs. So, this study is helpful for further phytochemicals and further medicinal investigation.

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