Asian Journal of Research in Botany

Volume 8, Issue 2, Page 10-20, 2022; Article no.AJRIB.93233

Phytochemical Screening, Antioxidant Activity and UHPLC Fingerprinting of Methanolic Extract of the Fruits of *Quercus semiserrata* Roxb.

Larima Sten [a] and Deepu Vijayan [a*]

[a] Botanical Survey of India, Eastern Regional Centre, Lower New Colony, Laitumkhrah, Shillong-793003, Meghalaya, India.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors contributed to the study conception and design. Author LS managed the analyses of the study, performed statistical analysis, wrote the protocol and first draft of the manuscript. Author DV supervised the work, checked and corrected the manuscript. Both authors read and approved the final manuscript.

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/93233

Original Research Article

Received: 03/09/2022
Accepted: 07/11/2022
Published: 02/12/2022

ABSTRACT

Quercus semiserrata Roxb. belongs to the family Fagaceae which is an Asian species of trees in the beech family. It has been found in Northeastern India, Bangladesh, Myanmar, Yunnan, Thailand and Tibet. Proteins, carbohydrates, phenols and tannins, flavonoids, steroids, terpenoids and alkaloids were detected in the fruits studied. The total phenolic content determined using Folin-ciocalteu reagent (FCR) was found to be 155.95 ± 0.18 mg gallic acid equivalents/g dry weight fruit extract and the total flavonoid content by Aluminium chloride colorimetric method was 238.78±0.007 quercetin equivalent/g dry weight fruit extract respectively. DPPH, ABTS radical scavenging activity and Reducing Power Assay were analyzed to study the antioxidant activities. Methanolic extract of *Q. semiserrata* fruit plays a significant role in antioxidant properties where the ABTS assay inhibition concentration (IC_{50}) values for ascorbic acid and *Quercus semiserrata* are 15.88±0.05 µg/ml and 49.46±0.07 µg/ml respectively. DPPH assay IC_{50} values are found to be 13.74±0.09 µg/ml and 50.17±0.009 µg/ml. β-carotene content was found to be 0.002±0.19 mg/100 ml and lycopene

**Corresponding author: E-mail: deepundd@gmail.com;*

content 0.0013±0.32 mg/100ml respectively. A simple method using reverse-phase Ultra High-performance Liquid Chromatography was developed for the detection of ascorbic acid, phenolic acids and flavonoids. It was observed that *Q. semiserrata* plays an important role in antioxidant activity even in low concentration.

Keywords: Phytochemicals; antioxidant activity; Quercus semiserrata Roxb*; fruit extract; UHPLC.*

ABBREVIATIONS

FCR : Folin-Ciocalteu Reagent
DPPH : 2,2-diphenyl-1-picryl-hydrazyl-hydrate
ABTS :2,2'-azinobis-bis (3-ethylbenzothiazoline-6-sulfonic acid)
IC_{50} : Half Maximal Inhibitory Concentration
UHPLC : Ultra High Performance Liquid Chromatography
TPC : Total Phenolic Content
TFC : Total Flavonoid Content
BHT : Butylated Hydroxy Toluene
QS : Quercus semiserrata
Ao : Absorbance of Reaction Control
A_1 : Absorbance of Extracts or Standards
$Abs_{control}$: Absorbance of Control
Abs_{sample} : Absorbance of Sample

1. INTRODUCTION

"Phytochemicals also referred to as phytonutrients are chemical compounds produced by plants to help them resist virus infection, bacteria, and fungi and also for the consumption of insects and other animals. These plant-based foods are complex mixtures of bioactive compounds. Information on the potential health effects of individual phytochemicals is linked to information on the health effects of foods that contain those phytochemicals. Phytochemicals, as plant components with discrete bio activities towards animal biochemistry and metabolism are widely examined for their ability to provide health benefits. Such phytochemicals which include terpenoids, phenolics, alkaloid, fiber and other phytochemicals" [1]. "The secondary metabolites are present in all parts of higher plants (fruits, seeds, roots, stems, leaves, flowers) are involved in several physiological processes" [2]. They also act as a possible clue on the mechanism involved in the prevention of oxidative stress, inflammation and cancer.

"The risk of chronic diseases can be reduced by regular consumption of vegetables and fruits" [3]. Recently, "antioxidants have attracted considerable attention concerning oxidative stress, radicals, therapy, cancer prophylaxis, and longevity" [4]. "The damaging effects of free radicals and toxic products of their metabolism can be prevented by all antioxidants which are working in concert as a team, (the antioxidant system). However, deficiencies in one component impact the efficiency of others. This is how antioxidant acts to control levels of free radical formation as a coordinated system" [5]. "Several degenerative diseases (consumption of fruits reduced the rate of chronic health disorders like cardiac problems, aging, and cancers of the respiratory tract, alimentary canal, lungs, bladder, and breast are well recognized) can be combated with the help of the antioxidant activity of fruits which is assumed to be of greatest importance" [6-7]. "This is credited to the fact that fruits are a rich source of health-beneficial bioactive substances including polyphenolics, minerals and vitamins" [8]. "In order to protect humans from free radicals and improve the antioxidant potential of plasma after the inhibition of atherosclerosis, these compounds may act synergistically or individually by various mechanisms" [9].

Quercus semiserrata Roxb. is a large tree of about 16-30 m tall which is widely distributed in Asian countries and the Northeastern part of India. Fruits are about 2-3 cm in diameter with soft hairs and 7-8 lines of cupular bracts. The branches of these trees are spread in nature, leaves are broader and dentated from the base, brownish green colour and greyish white beneath. It is mostly found in moist shady forest and rocky clay loam. In some places, it grows on the hill slopes along with *Rhododendron* species. In Nagaland, it can be found at an altitude of about 2000-2800 m. It is commonly called as sekho, phong-rong-lang-poh and sehop in Assam. There are several species of *Quercus* found in Northeastern part of India. Based on the available literature no work has been done on the phytochemical evaluation and antioxidant activity of this plant. The present study aimed to investigate the presence of bioactive compounds, antioxidant activity and UHPLC analysis of fruits of *Q. semiserrata*.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

The fruits of *Q. semiserrata* were collected from Laitumkhrah, Shillong, East Khasi Hills District Meghalaya in the month of October 2018. Fruits

were thoroughly cleaned and washed with distilled water and tap water. Once all the water molecules had evaporated, these were cut into small pieces and shade dried until they were thoroughly dried and ready to be ground in a mechanical blender. After grinding into a fine powder, ground fruits were transferred into airtight containers with proper labeling for future use.

2.1.1 Preparation of plant extracts

2.1.1.1 Hot water extraction

1 g of dried finely powdered plant material was taken in a beaker and 40 ml of distilled water was added and mixed. The mixture was heated on a hot plate with continuous stirring at 37ºC for 20 minutes. The water extract was then filtered through filter paper and the filtrate was used only for the qualitative phytochemical analysis. The water extract was kept in the refrigerator when not in use.

2.1.1.2 Solvent extraction

Soxhlet extraction was used to create crude plant extract. A thimble was filled evenly with 5 g of powdered plant material, and 250 ml of solvent was used for the extraction. Methanol was employed as the solvent. The extraction process continues for 24 hours or until the solvent in the extractor's syphon tube turns colourless. After that, the extract was moved to a Rotary Evaporator chamber and heated at 40 to 50°C to completely evaporate the solvent. For use in upcoming phytochemical analyses, the dried extract was stored at 4°C in the refrigerator.

2.2 Qualitative Phytochemical Analysis

"The extract was tested for the presence of bioactive compounds by using the following standard methods" [10-12]. Test for proteins, carbohydrates, phenols & tannins, flavonoids, saponin, glycosides, steroid, terpenoid and alkaloid was carried out.

2.3 Quantitative Phytochemical Analysis

2.3.1 Determination of total phenolic content

Total phenolics were determined using Folin-Ciocalteu Reagent (FCR) as described by Velioglu et al. [13]. 100 µl of plant extract dissolved in methanol (1 mg/ml) was mixed with 750µl of FCR (diluted 10-fold) and allowed to stand for 5 min at 22˚C; 750 µl of Na_2CO_3 (60 g/l) solution was then added to the mixture. After 90 min, the absorbance was measured at 725 nm. The tests were performed in triplicates. Phenolic content was determined from the standard curve and results were expressed as gallic acid equivalents.

2.3.2 Determination of total flavonol content

To determine flavonol content, Aluminium chloride colorimetric method was used which was describe by Aiyegroro and Okoh [14]. 1 ml of sample plant extract was mixed with 3 ml of methanol, 0.2 ml of 10% Aluminium chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water and kept at room temperature for 30 minutes. Quercetin was used as standard and the absorbance was measured at 420 nm. All tests were performed in triplicates. Flavonol content were determined from the standard curve and were expressed as quercetin equivalent (µg/mg of extracted compound).

2.4 *In vitro* Antioxidant Activity

2.4.1 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging activity

"DPPH radical scavenging activity was estimated by measuring the decrease in the absorbance of methanolic solution of DPPH" (Brand-Williams et al.) [15]. To 5 ml of DPPH solution (3.3 mg of DPPH in 100 ml methanol), 1 ml of each plant extracts were added and incubated for 30 min in the dark and the absorbance (A_1) was read at 517 nm. The absorbance (A_0) of a reaction control (methanol instead of plant extract) was also recorded at the same wavelength. Ascorbic acid (10-50 µg/ml) was used as a standard. Scavenging ability (%) was calculated by using the equations:

$$\text{DPPH radical scavenging activity (\%)} = [(A_0 - A_1)/A_0] \times 100$$

where A_0 was the absorbance of reaction control and A_1 was the absorbance of extracts or standards. All tests were performed in triplicates.

2.4.2 2,2'-azinobis-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity

"For ABTS assay, the method of Arnao et al. was followed" [16]. The stock solutions included 7 mM ABTS solution and 2.4 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 14h at room temperature in the dark. The solution was then diluted to obtain an absorbance of 0.706 ±

0.01 units at 734 nm using a spectrophotometer. Fresh ABTS solution was prepared for each assay. Plant extracts (200 µl) were allowed to react with 1 ml of the ABTS solution and the absorbance was taken at 734 nm after 7 min using a spectrophotometer. The ABTS scavenging capacity of the extract was compared with ascorbic acid and percentage inhibition calculated as:

$$\text{ABTS radical scavenging activity (\%)} = (Abs_{control} - Abs_{sample}/Abs_{control}) \times 100$$

where $Abs_{control}$ is the absorbance of ABTS radical in methanol; Abs_{sample} is the absorbance of ABTS radical solution mixed with sample extract/standard. All tests were performed in triplicates.

2.4.3 Reducing power assay

"The reducing power ability of the extracts was evaluated by following the method of Oyaizu" [17]. The reaction mixture contained 1.0 ml of various concentrations of extracts (200-1000 µg/ml), 2.5 ml of 1% potassium ferricyanide and 2.5 ml of 0.2 mol/L sodium phosphate buffer. The mixture was incubated at 50°C for 30 min and the reaction was terminated by the addition of 2.5 ml of 10% TCA, followed by centrifugation at 3000 rpm for 10 min. 2.5 ml of the upper layer was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride. The absorbance was measured at 700 nm against the blank. The reducing power ability of the sample is determined by increase in absorbance of the sample. BHT was used as the standard for comparison.

2.5 Estimation of Carotenoids

"To determine the content of β- carotene and lycopene content, 0.1 g of samples were taken and shaken with 10 ml of acetone-hexane mixture in the ratio of 4:6 for one minute and filtered through Whatman No. 1 filter paper. Absorbance was measured at 453,505 and 663nm using UV-VIS spectroscopy" [18].

Content of β-carotene and lycopene was calculated according to the following equations:

$$\beta\text{-Carotene (mg/100ml)} = 0.216A_{663} - 0.304A_{505} + 0.452A_{453}$$

$$\text{Lycopene (mg/100mL)} = -0.0458A_{663} + 0.372A_{505} - 0.0806A_{453}$$

2.6 Ultra-High Performance Liquid Chromatographic (UHPLC) Analysis

2.6.1 Standard preparation

1 mg/ml of each standard was dissolved in methanol and diluted to different concentration of all the standard in methanol. The standard solutions were stored in the refrigerator at 4°C whereas the working solution was filtered through Whatman Syringe Filter-Sterile 0.45-µm RC before UHPLC injection.

2.6.2 Sample preparation

Plant materials were cleaned and the edible parts were rinsed thoroughly with distilled water. This was then dried and ground into powder form. The crude plant extract was prepared by the Soxhlet extraction method. About 5 g of powdered plant material was uniformly packed into a thimble and extracted with 250 ml of solvent. The solvent used was methanol. The process of extraction continues for 24 hours or till the solvent in the siphon tube of an extract becomes colorless. The extract was evaporated to dryness. 1 mg/ml of the crude extract (sample) was dissolved in methanol and was filtered through Whatman Syringe Filter-Sterile 0.45-µm RC prior to UHPLC injection.

"Compounds were determined by a UHPLC method using a Dionex Ultimate 3000 liquid Chromatograph (Thermo Scientific) equipped with a UV/DAD detector. Chromatographic separation was performed on a Hypersil Gold aQ (250 mm × 4.6 mm) column. The solvent system had a constant flow rate of 1.2 mL/min. The mobile phase was 100% Acetonitrile (solvent A), 0.1 % acetic acid (solvent B) and 100% HPLC water (solvent D). The gradient was as follows: 0–2 min, 1.5 %B, 92% D; 2-11min, 1.5-4% B, 80%D; 11–15 min, 4%B ,80%D; 15–20 min, 4%B, 30%,5%D; 20–30 min, 4%B, 5-92%D; 30-35 min and 35- 38 min, 1.5%B, 92% D. The injection volume was 20µL, and the temperature was kept constant at 28°C. Detection wavelengths were chosen considering the absorption maximums of UV spectra of the selected compounds (Seal) [19] with modifications".

3. RESULTS AND DICUSSION

3.1 Qualitative Phytochemical Analysis

The results of qualitative phytochemical analysis *Q. semiserrata* fruit is furnished in Table 1. Proteins are important essential building blocks

of life and carbohydrates are the main source of energy in living things. Phenols play a vital role in antioxidant activity. Flavonoids promote cellular health, antioxidant activity, normal tissue growth and renewal throughout the body [20]. Terpenoids possess antimalarial effects, anti-inflammatory, antitumor, antiviral, antibacterial, promote transdermal absorption, have hypoglycemic activities, prevent and treat cardiovascular diseases. Previous studies have also found that terpenoids have many potential applications, such as insect resistance, neuroprotection, immunoregulation, antiaging and antioxidation [21]. "Neuroactive steroids also help in the control of central nervous system functions during pathological and physiological conditions that they may represent good candidates for the development of neuroprotective strategies for neurodegenerative and psychiatric disorders" [22]. Alkaloids of plant origin plays a potential role as antimicrobials against Antibiotic-Resistant Infections [23] and antidiabetic properties of alkaloids of different plants was also studied [24].

3.2 Quantitative Phytochemical Analysis

"Fig. 1 is the standard curve of total phenolic content using gallic acid as a standard and Fig. 2 is the standard curve of flavonols using quercetin as standard. The total phenolic content was 155.95±0.18 mg gallic acid equivalents/mg dry weight plant extract and total flavonol content was 238.78±0.007 quercetin equivalent/mg dry weight plant extract (Table 2). *Q. semiserrata* shows high content of phenolics and flavonols which shows good antioxidant activity. Phenolic compounds possess biological properties such as antiinflammation, antiatherosclerosis, antiapoptosis, antiaging, anticarcinogen, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities [25]. Flavonoids are known to synthesized by plants in response to microbial infection and have been found to possess antimicrobial, antioxidant and also show strong anticancer activities" [26-28].

Table 1. Phytochemical constituents of *Q. semiserrata* fruit

Plants	Proteins	Carbohydrates	Phenols/ Tannins	Flavonoids	Saponins	Glycosides	Steroids	Terpenoids	Alkaloids
Quercus semiserrata (fruit)	+	+	+	+	-	-	+	+	+

(+) indicates present, (-) indicates absent

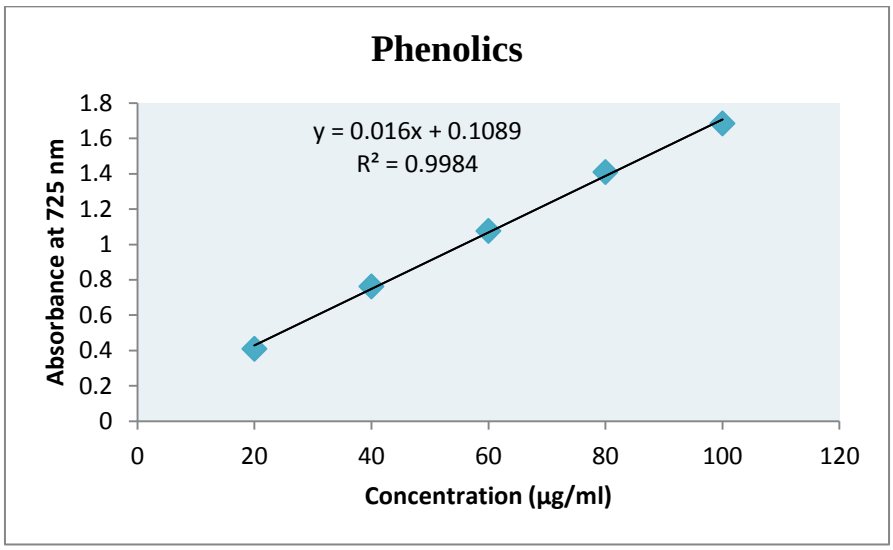

Fig. 1. Calibration graph for total phenolic content

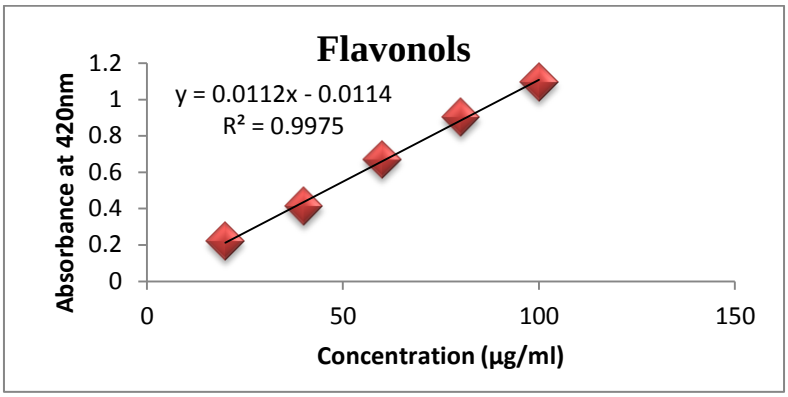

Fig. 2. Standard curve of flavonols

Table 2. Total phenolic content (TPC), total flavonoid content (TFC), ABTS, DPPH activity and carotenoids

TPC (mg/g)	TFC (mg/g)	ABTS (IC_{50}) µg/ml (Ascorbic acid)	ABTS (IC_{50}) µg/ml *Q. semiserrata*	DPPH (IC_{50}) µg/ml (Ascorbic acid)	DPPH (IC_{50}) µg/ml *Q. semiserrata*	Carotenoids	
						β-carotene (mg/100 ml)	Lycopene (mg/100 ml)
155.95±0.18	238.78±0.007	15.88±0.05	49.46±0.07	13.74±0.09	50.17±0.09	0.002±0.19	0.0013±0.32

3.3 *In vitro* Antioxidant Activity

This method measures the relative antioxidant activity of fruits to scavenge the radical ABTS and DPPH in the aqueous phase compared with a standard amount of ascorbic acid. DPPH radical scavenging activity of ascorbic acid and *Q. semiserrata* is shown in Fig. 3 and Fig. 4 and ABTS radical scavenging activity of ascorbic acid and *Q. semiserrata* is shown in Fig. 5 and Fig. 6 respectively. The IC_{50} value or Inhibition concentration of Ascorbic acid for DPPH was 13.74±0.09 µg/ml and 15.88±0.05 µg/ml for ABTS (Table 2). Whereas, IC_{50} value of *Q. semiserrata* was 50.17±0.009 µg/ml for DPPH and 49.46±0.07 µg/ml for ABTS expressed in mean and standard deviation (Table 2). "$ABTS^{\bullet+}$ radical cation-based assays are among the most abundant antioxidant capacity assays, together with DPPH radical-based assays according to the Scopus citation rates" [29]. "DPPH can accept an electron of hydrogen radical to become a stable diamagnetic molecule, which is widely used to investigate radical scavenging activity. The discoloration of the reaction mixture can be quantified by measuring the absorbance at 517 nm which indicates the radical scavenging ability of the antioxidant" [30].

Reducing Power Assay: Reducing power assay is a convenient and rapid screening method for measuring the antioxidant potential. The reduction potential (Fe^{3+} to Fe^{2+} transformation in terms of increasing absorbance) was found to increase with rising concentrations. *Q. semiserrata* (0.59, 0.80, 0.97, 1.04 and 1.19) showed good reducing power capacity which can be compared with standard BHT (0.275, 0.489, 0.651, 0.729 and 0.872) in a concentration of 20-100µg/ml (Fig. 7). The test solution's initial yellow hue transforms into various green hues. This shows that the reducers necessary for the conversion of the Fe3+ or ferricyanide complex used in this method are present. There is a strong correlation between the total antioxidant capacity assayed by DPPH, ABTS, RPA methods and phenolic content which indicate that the phenolic compounds largely contribute to the antioxidant activities of *Q. semiserrata* and therefore could play an important role in the beneficial effects of this important medicinal plants. "The results were in

accordance with other researches, several studies have found that phenolic compounds are major antioxidant constituents in selected plants, and there are direct relationships between their antioxidant activity and total phenolic content" [31-34].

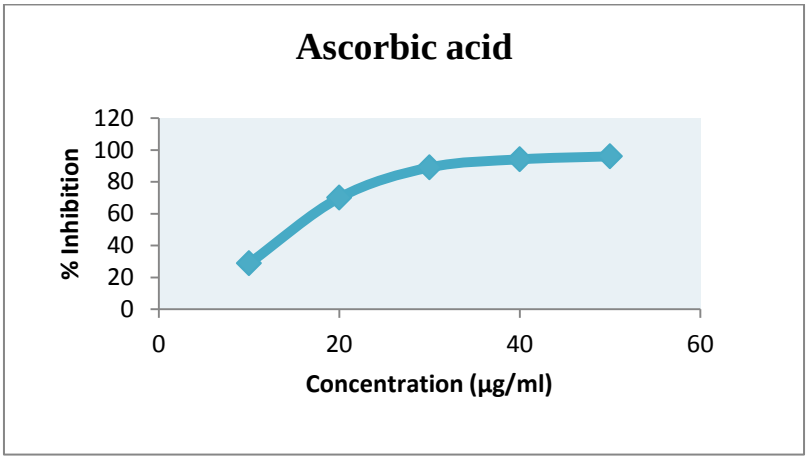

Fig. 3. DPPH percentage inhibition of standard ascorbic acid

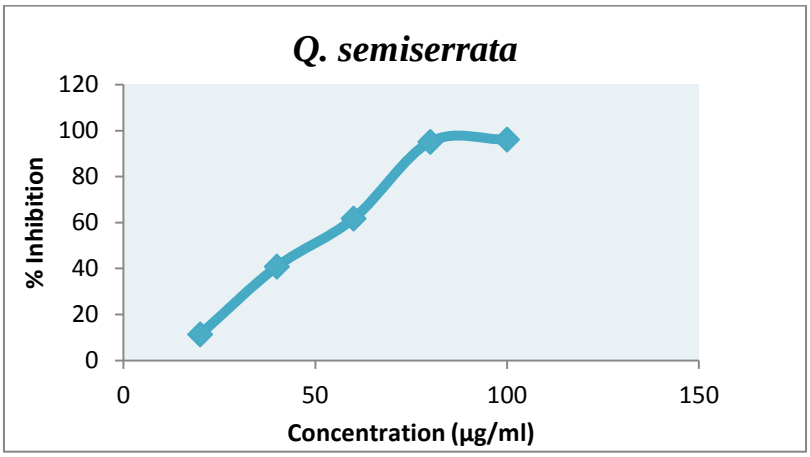

Fig. 4. DPPH radical scavenging activity of *Q. semiserrata*

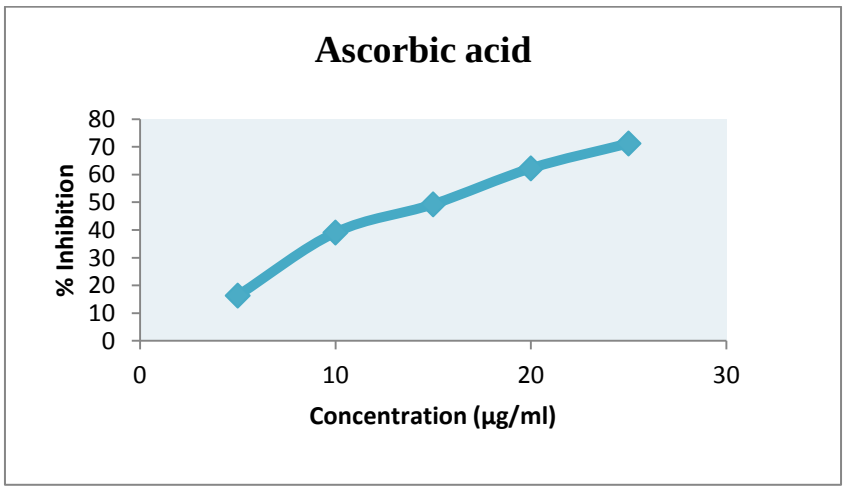

Fig. 5. ABTS radical scavenging activity of ascorbic acid

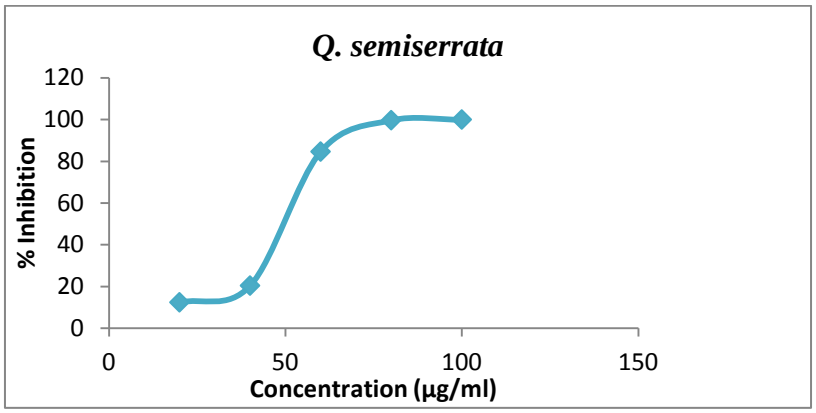

Fig. 6. ABTS radical scavenging activity of *Q. semiserrata*

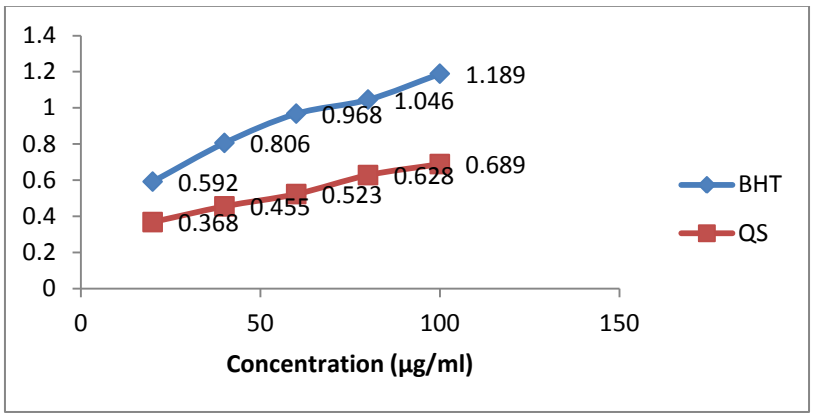

Fig. 7. Reducing power assay of BHT (Butylated Hydroxy Toluene) and *Q. semiserrata* (QS)

3.4 Carotenoids

"It was observed that *Q. semiserrata* contain more β-carotene content than lycopene (Table 2). Many epidemiological studies have shown an association between diets rich in carotenoids and a reduced incidence of many forms of cancer, and it has been suggested that the antioxidant properties [35] of these compounds are a causative factor. Attention has focused on the potential role of one specific carotenoid, β-carotene, in preventing cancer, chronic diseases, gastric cancer and numerous publications have described *in vitro* experiments and animal studies which suggest that not only can this carotenoid protect against the development of cancer, but also several other chronic diseases" [36].

3.5 UHPLC Analysis

Chromatogram of ascorbic acid, phenolics and flavonoids is shown in Fig. 8. The chromatogram of ascorbic acid, gallic acid, catechin gallic acid, vanillin, þ-coumaric acid, rutin, quercetin, kaempferol, tannic acid and BHT was observed at a wavelength of 276 nm. The chromatogram of *Q. semiserrata* is shown in Fig. 9. Kaempferol and tannic acid was observed at a retention time of 22.12 and 24.86 minutes at a wavelength of 276 nm where kaempferol was the major peak present and tannic acid as a minor peak. Kaempferol (3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H chromen-4-one) has been demonstrated to have beneficial effects on chronic inflammatory diseases and anti-cancer properties [37]. "The antioxidant properties of tannins are of great importance in food applications due to their ability to prevent disorders related to oxidative stress, such as cancer and cardiovascular diseases" [38-39] and are also characterized to be more environment-friendly option than those based on chemical products.

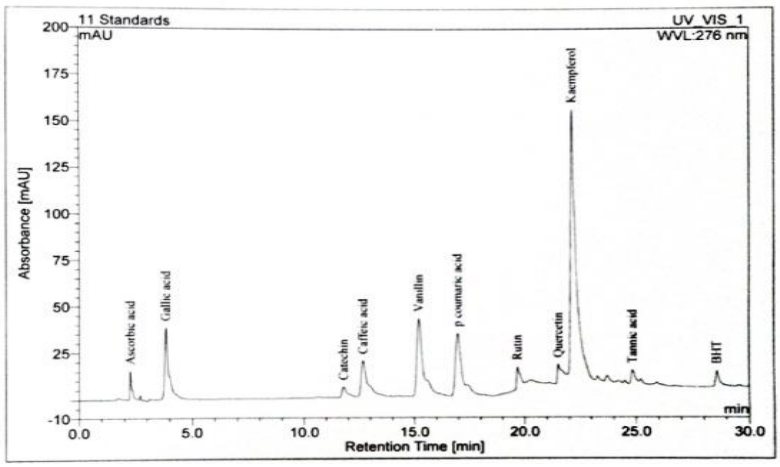

Fig. 8. Chromatogram of ascorbic acid, phenolics and flavonoids

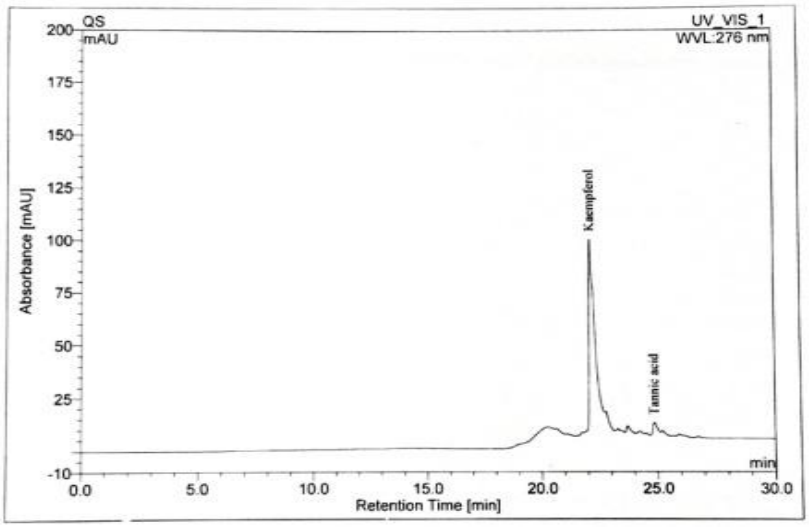

Fig. 9. Chromatogram of *Q. semiserrata* (fruit)

4. CONCLUSION

Quercus semiserrata contains high amount of phenolics and flavonols and less content of carotenoids and act as a potential source of reactive oxygen species inhibiting compound which plays an important role as antioxidant activities. Kaempferol and tannic acid is also found to be present in this fruit which is found to have anti-inflammatory effect and can prevent oxidative stress such as cancer and cardiovascular diseases. The present study focused only on qualitative, quantitative, antioxidant properties, carotenoids and UHPLC analysis. Therefore, further studies may also be carried out to investigate more on the nutritional, mineral analysis and proximate composition of this interesting fruit of Northeast India.

ACKNOWLEGEMENT

The authors are thankful to the Director, Botanical Survey of India for the encouragement and support. The authors also thank the Head of Office, ERC, BSI, Shillong for providing the necessary facilities. The first author is thankful to Dr. Dilip Kumar Roy for identification of plant sample and her lab mates for their help and support during the period of study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Dillard CJ, German JB. Phytochemicals: nutraceuticals and human health. J Sci Food Agric. 2000;80:1744-1756.

2. Awa KA, Badji KD, Sagna MB, Guissé A, Bassène E. Phytochemical screening and antioxidant activity of the fruits of *Boscia senegalensis* (Pers.) Lam. e.g. Pear. (Capparaceae). Pharmacogn J. 2020;12(5):1042-1049.
3. Dembinska-Kiec A, Mykkanen O, Kiec-Wilk B, Mykkanene H. Antioxidant phyto-chemicals against Type 2 Diabetes. British J. Nutri. 2008;99:109-117.
4. Kalcher K, Svancara I, Buzuk M, Vytras K, Walcarius A. Electrochemical sensors and biosensors based on heterogeneous carbon materials. Monatsh Chem. 2009; 140:861-889.
5. Peter FS. Natural antioxidants in poultry nutrition: New developments. 16th European Symposium on Poultry Nutrition. 2007;669-676.
6. Wargovich MJ. Anticancer properties of fruits and vegetables. Hort Science. 2000; 35(4):573–575.
7. Prior RL, Cao G. Antioxidant phytochemicals in fruits and vegetables: Diet and health implications. HortScience. 2000;35(4):588–592.
8. Karakaya S, Kavas A. Antimutagenic activities of some foods. Journal of the Science of Food & Agriculture. 1999;79(2):237–242.
9. Cao SG, Sim KY, Pereira J, Goh SH. Coumarins from *Calophyllum teysmannii* (Guttiferae). Phytochemistry. 1998;47(6): 1051–1055.
10. Sofowra A. Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria. 1993;191-289.
11. Trease GE, Evans WC. Pharmacognosy, 11th Edn., Bailliere Tindall, London. 1989;45-50.
12. Harborne JB. Phytochemicals methods. chapman and hall ltd., London. 1973; 49-188.
13. Velioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. J. Agric. Food Chem. 1998;46: 4113–4117.
14. Aiyegroro OA, Okoh AI. Preliminary phytochemical screening and *In vitro* antioxidant activities of aqueous extract of *Helichrysum longifolium* DC. BMC compl. And Alt. Med. 2010;10: 21.
15. Brand-Williams W, Cuvelier M, Berset C. Use of a free radical method to evaluate antioxidant activity. Lebensm Wiss Technology. 1995;28:25-30. Available:https://doi.org/10.1016/S0023-6438(95)80008-5
16. Arnao MB, Cano A, Acosta M. The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chem 20001;73:239–44.
17. Oyaizu M. Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn J Nutr. 1986;44: 307-315.
18. Bhusal S, Pant DR, Joshi GP, Adhikari M, Raut JK, Pandey MR, Bhatt LR. Antioxidant activity and nutraceutical potential of selected nepalese wild edible fruits. Scientific World, 2020;13(13).
19. Seal P. Quantitative HPLC analysis of phenolic acids, flavonoids and ascorbic acid in four different solvent extracts of two wild edible leaves, *Sonchus arvensis* and *Oenanthe linearis* of North-Eastern region in India. Journal of Applied Pharmaceutical Science; 2016.
20. Kumar S. The Importance of antioxidant and their role in pharmaceutical science. Asian Journal of Research in Chemistry and Pharmaceutical Sciences. 2014; 1(1):27-44.
21. Yang W, Chen X, Li Y, Guo S, Wang Z, Yu X. Advances in pharmacological activities of terpenoids. natural product communications; 2020. DOI: 10.1177/1934578X20903555
22. Melcangi RC, Panzicab G, Garcia-segura LM. Neuroactive steroids: Focus on Human Brain. Neuroscience. 2011;191: 1–5. DOI: 10.1016/j.neuroscience.2011.06.02
23. Casciaro B, Mangiardi L, Cappiello F, Romeo I, Loffredo MR, Iazzetti A, Calcaterra A, Goggiamani A, Ghirga A, Mangoni ML, Botta B, Quaglio D. Naturally-occurring alkaloids of plant origin as potential antimicrobials against antibiotic-resistant infections. Molecules. 2020;25:3619. DOI: 10.3390/molecules25163619
24. Rasouli H, Yarani R, Pociot F, Popović-Djordjević J. Anti-diabetic potential of plant alkaloids: Revisiting current findings and future perspectives. Journal Pre-proof; 2020. Available:https://doi.org/10.1016/j.phrs.2020.104723
25. Han X, Shen T, Lou H. Dietry polyphenols and their biological significance. Int. J. Mol. Sci. 2007;950-988.

26. Marjorie C. Plant products as antimicrobial agents. Clincal Microbiol. Rev. 1996;12: 564-582.
27. Salah N, Miller NJ, Pagange G, Tijburg L, Bolwell GP, Rice E, Evans C. Polyphenolic flavonoids as scavenger of aqueous phase radicals as chai breaking antioxidant. Arc. Biochem. Broph. 1995;2:339-346.
28. Del-Rio A, Obdululio BG, Casfillo J, Main FG, Ortuno A. Uses and properties of citrus flavonoids. J. Agric. Food Chem. 1997;45:4505-4515.
29. Ilyasov IR, Beloborodov VL, Selivanova IA, Terekhov RP. doi: ABTS/PP Decolorization Assay of Antioxidant Capacity Reaction Pathways. Int. J. Mol. Sci. 2020;21:1131. DOI: 10.3390/ijms21031131
30 Choi YJ, Hwang KH. Radical scavenging activities of fruits of *Crataegus pinnatifida* BUNGE Major. from Korea. Natural Product Sciences. 2013;19(2):186-191.
31 Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. Journal of Nutritional Biochemistry. 2002;13(10):572–584.
32 Soong YY, Barlow PJ. Antioxidant activity and phenolic content of selected fruit seeds," Food Chemistry. 2004;88(3): 411–417.
33 Balasundram N, Sundram K, Samman S. Phenolic compounds in plants and agri-industrial by-products: antioxidant activity, occurrence, and potential uses. Food Chemistry. 2006;99(1):191–203.
34 Loizzo MR, Tundis R, Bonesi M. Radical scavenging, antioxidant and metal chelating activities of *Annona cherimola* Mill. (cherimoya) peel and pulp in relation to their total phenolic and total flavonoid contents. Journal of Food Composition and Analysis. 2012;25(2):179–184.
35 Burton GW, William TM, Riley W, Nickerson JG. β-Carotene oxidation products - Function and safety. Food and Chemical Toxicology. 2021;152:112207.
36 Chen QH, Wu BK, Pan D, Sang LX, Chang B. Beta-carotene and its protective effect on gastric cancer. World J clin cases Aug 2021; 16;9(23):6591–6607.
37 Ren J, Lu Y, Qian Y, Chen B, Tao Wu, Ji G. Recent progress regarding kaempferol for the treatment of various diseases (Review). Experimental and Therapeutic Medicine. 2019;18:2759-2776. DOI: 10.3892/etm.2019.7886
38 Hollman PCH, Katan MB. Dietary flavonoids: Intake, health effects and bioavailability. Food Chem. Toxicol. 1999; 37:937–942.
39 Scalbert A, Manach C, Morand C, Rémésy C, Jiménez L. Dietary polyphenols and the prevention of diseases. Crit. Rev. Food Sci. Nutr. 2005;45:287–306.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/93233