Trends Phytochem. Res. 5(2) 2021 71-91

Trends in Phytochemical Research (TPR)

Journal Homepage: http://tpr.iau-shahrood.ac.ir

Original Research Article

Nutritional assessment, antioxidant, anti-inflammatory and antidiabetic potential of traditionally used wild plant, *Berberis tinctoria* Lesch.

ARUMUGAM VIGNESH[1], KUMARASAMY PRADEEPA VEERAKUMARI[2], SUBRAMANIAM SELVAKUMAR[3], RAJAN RAKKIYAPPAN[4] AND KRISHNAN VASANTH[1] ✉*

[1]Department of Botany, Bharathiar University, Coimbatore 641 046, Tamil Nadu, India
[2]Department of Statistics, Bharathiar University, Coimbatore - 641 046, Tamil Nadu, India
[3]Department of Biochemistry, Bharathiar University, Coimbatore 641 046, Tamil Nadu, India
[4]Department of Mathematics, Bharathiar University, Coimbatore - 641 046, Tamil Nadu, India

ABSTRACT

This study divulges a comparative analysis of *Berberis tinctoria* Lesch. leaf, stem, and fruit towards proximate contents assessment, e.g., moisture, crude-ash, crude-fibre, crude-lipid, crude-protein, nitrogen-free extract, energy followed by ICP-MS analysis which revealed that fruits have a higher amount of essential minerals. The primary metabolites were higher in the fruit extract, whereas more secondary metabolites were observed in the methanolic extracts in the hierarchy of leaf and fruit then stem, respectively. Likewise, a higher amount of antioxidant activities were observed in ethyl acetate and methanolic extracts. The fruit ethyl acetate extract showed a maximum of 95.27% anti-inflammatory activity. The fruit methanolic extract exhibited an antidiabetic activity with IC_{50} values of 75.5 ± 6.8 µg/mL and 45.4 ± 11.1 µg/mL for α-amylase and α-glucosidase, respectively. A significantly positive correlation was identified between the secondary metabolites and antioxidant activities. The principal component analysis implies ethyl acetate and methanolic extracts as pivotal extracts of *B. tinctoria*.

ARTICLE HISTORY

Received: 11 November 2020
Revised: 24 April 2021
Accepted: 15 May 2021
ePublished: 08 June 2021

KEYWORDS

Antidiabetic
Anti-inflammatory
Antioxidant
Berberis tinctoria Lesch.
Micronutrients
Proximate analysis

1. Introduction

Plants are significant sources of medicine and their nutritional properties are important for the improvement of the human immune system, even though the natural resources are scanty to meet the increasing demand by the rising world population. Nowadays, an unhealthy lifestyle is unfortunately trending among the people all over the world. Therefore, an intake of junk food habits which contain high fats and cholesterol causes obesity (Gurib-Fakim, 2006). Nutritional deficiencies lead to blindness, anaemia, preterm birth, stillbirth, cretinism and some common chronic systematic diseases like cardiovascular, inflammation, and diabetes (Shenoy et al., 2018). Diabetes is the most common chronic group of metabolic disorders characterized by hyperglycemia and hyperlipidemia expanded throughout the world, a source for serious damage to the heart, eyes, nerves, kidneys and blood vessels (Shenoy et al., 2018). World Health Organization (WHO) recently declared that around 190 million people have diabetes across the globe, where the majority of the cases are noticed in developing countries by the cause of ageing, obesity, population growth, sedentary lifestyles, and unhealthy diets and this would increase up to 642 million by 2040 (Ogurtsova et al., 2017). Type-2 diabetes accounts for more than 90% of diabetes which leads to many typical risk factors. Oxidative stress is induced by reactive oxygen species (ROS) that have turned pancreatic islets to suppress the insulin secretion by β-cell dysfunction which is the pivotal development of type-2 diabetes (Marchetti et al., 2006). The functional defect in these cells was caused by a reduction in expression of glucose transporter-2 (GLUT2) levels and further differentiated by growing β-cells apoptosis (Sampson et al., 2010). Therapies for reducing oxidative stress and improving pancreatic function have become a

✉Corresponding author: Krishnan Vasanth
Tel: 0422-2428309; Fax: 0422-2422387
E-mail address: vasanthlabbu@gmail.com, **doi: 10.30495/TPR.2021.1914719.1186**

promising approach to control diabetes. Some natural antioxidant supplementary products like nutritional and functional foods have greater target activity in hyperglycemic stature by reducing oxidative stress (Tan et al., 2018). Moreover, the food is endorsed as potential medicine with the sources of nutrients which perform the basic nutritional role and have been conferred as functional foods (Cencic and Chingwaru, 2010). WHO also outlined that the antioxidant rich fruits are likely to contribute to the protection of cellular membranes, nucleic acids, lipids and proteins from oxidative damage (Paredes-López et al., 2010). Inflammation is a regulated physiological response against impairment stimuli by pathogens and tissue injury which activates immune cells for quick orchestrate resolution and healing damaged tissue structure (Chen et al., 2018). Macrophages are immune effector cells that perform a momentous homeostatic role in the activation of inflammation to the regulation of tissue remodelling during apoptosis. Lipopolysaccharide (LPS) is an endotoxin that can cause a pro-inflammatory stimulus by mobilizing the macrophages and triggering the inflammatory response (Arango Duque and Descoteaux, 2014). Under inflammatory conditions, macrophages fuse with ROS and produce "oxidative burst" which causes cellular damage associated with the histolesivity of inflammation (Ricciotti and FitzGerald, 2011). Natural anti-inflammatory compounds associated with antioxidant properties would be beneficial for various chronic disease therapies including conventional drugs (Vasarri et al., 2020). Distinct plant species have significant sources of antioxidants and anti-inflammatory compounds capable of inflecting the functional phenotype of macrophages and alleviating inflammatory disorders (Vasarri et al., 2020).

In some countries, several wild plants were valued as edible and a source of supplementary nutrition including carbohydrates, protein, fat, vitamins and minerals (Ali and Deokule, 2009). Natural antioxidants have an essential aspect in health care and prevention of some degenerative, e.g. atherosclerosis, cerebral ischemia, neurodegenerative disorders, rheumatic disorder, DNA damage, ageing and chronic disorders like cardiac, carcinogenesis and gestational diabetes (Pham-Huy et al., 2008). Jayasri et al. (2009) suggest that minimal side effects are caused by the use of phytocompounds screening of medicinal plants. Some of the *Berberis spp.* exhibited multiple pharmacological activities, such as anti-inflammatory, antidiabetic, and anticancer effects. As an evergreen shrub, *Berberis tinctoria* belonging to the family Berberidaceae, grows up to 2-3 feet in height and reaches up to 15 feet in the forest. It has a hard stem having long limited branches, with numerous slender leafy twigs (Fyson, 1974). *B. tinctoria* is endemic to the region of Doddabeta, the Nilgiris hills, Western Ghats, India. The Nilgiris hills, at an altitude of 2,634 mean sea level (MSL), and *B. tinctoria* is found in temperate acidic soil also in gneissic rock rich in sesquioxides. Provincially, the plant is called Oosikala by the local ethnic community of Kurumbas, and is medicinally used for stomachache (root paste) (Fyson, 1974). The root mixed with honey acts as an antimicrobial agent against skin diseases. The wood and root bark extracts have been used for menorrhagia, diarrhoea, eye infection, jaundice, and skin diseases (Kirtikar and Basu, 1995). The uncooked leaf and fruit of the plant are predominantly consumed by the tribal and rural people of the Nilgiris and used as medicine for many diseases, which are not scientifically documented. The genus *Berberis* consists of 55 species in India among which the most well-known species with medicinal properties are *Berberis aristata* DC., *Berberis lyceum* Royle., *Berberis pachycantha* Koehne., *Berberis vulgaris* L. and *Berberis coriaria* Royle ex Lindl.. These herbal materials are extensively used as effective remedies for treating gallstones, eye disease, jaundice, rheumatism, diabetes, fever, kidney, vomiting during pregnancy, and varied ailments due to their rich biologically active alkaloids (Srivastava et al., 2015).

Berberis has potential pharmacological activity to be used as polyherbal formulation for the treatment of numerous diseases and disorders. Therefore, the current study was designed to delve into the nutritional properties of fresh leaf, stem and fruit by proximate analysis and macro and micro nutritional composition using inductively coupled plasma mass spectrometry (ICP-MS) technique. The different extracts of *B. tinctoria* leaf, stem, and fruit organs were investigated for the presence of secondary metabolites, antioxidants and anti-inflammatory agents. This study focused on the determination of the metabolites having *in vitro* antidiabetic properties for the inhibition of pancreatic α-amylase and α-glucosidase with improvement in beta cell function. Oxidative stress and inflammation are proved to be a critical factor for the pathogenesis of diabetes mellitus. According to the earlier reports, *B. tinctoria* plant has been recognized as a rich source of alkaloids, particularly berberine (Shigwanb et al., 2013). Based on the results of another study, the interaction between the antidiabetic, antioxidants and anti-inflammation were analyzed. Furthermore, berberine has been characterized with remarkable antioxidant and anti-inflammatory activities and promising efficacy against diabetes mellitus. It has remarkable changes in oxidative stress markers, antioxidant enzymes and proinflammatory cytokines in diabetic animals (Li et al., 2014). The alkaloids inhibited oxidative stress and inflammation in a variety of tissues including liver, adipose tissue, kidney, and pancreas. Mechanisms of the antioxidant and anti-inflammatory activities of berberine were complex, which involved multiple cellular kinases and signalling pathways, such as AMP-activated protein kinase (AMPK), mitogen-activated protein kinases (MAPKs), nuclear factor erythroid-2-related factor-2 (Nrf2) pathway, and nuclear factor-κB (NF-κB) pathway (Li et al., 2014). Therefore, the metabolites of *B. tintoria* were investigated for their biological activity against inflammation and diabetes. The interaction of polyphenols between the antioxidants, antidiabetic and anti-inflammatory were also validated to promote the development of natural products against diabetic disorders.

2. Experimental

2.1. Collection and identification of plant material

The plant *B. tinctoria* was collected from Doddabetta, (11°24'08.7"N 76°44'12.2"E) at an altitude of 2623 Mean Sea Level (MSL) in the Nilgiri biosphere reserve, Tamil Nadu, India. The fresh leaves, stems, and fruits were collected from December 2018 to May 2019 and shown in Fig. 1. The taxonomic identity of the plant was confirmed and certified by the Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamil Nadu, India, Ref no: (BSIS/RC/5/23/2016/Tech./164) through comparing with the type specimen depository in the herbarium and also by characteristic identification. The plant parts were washed with distilled water and dried in shade at room temperature (26 ± 2 °C). The dried plant parts were ground well and subjected for the extraction process and stored at -20 °C for further analysis.

2.2. Chemicals and reagents

Alkaline copper solution, anthrone (97%), bovine serum albumin (98%), di-nitrosalicyclic acid reagent (98%), rutin (94%), Folin-Ciocalteu phenol reagent, polyvinyl polypyrolidone (PVPP), 2-diphenyl-1-picryl-hydrazyl (DPPH), potassium persulfate (99%), 2,2′-azinobis (3-ethyl-benzothiozoline)-6-sulfonic acid disodium salt (ABTS) (98%), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) (97%), butylated hydroxy

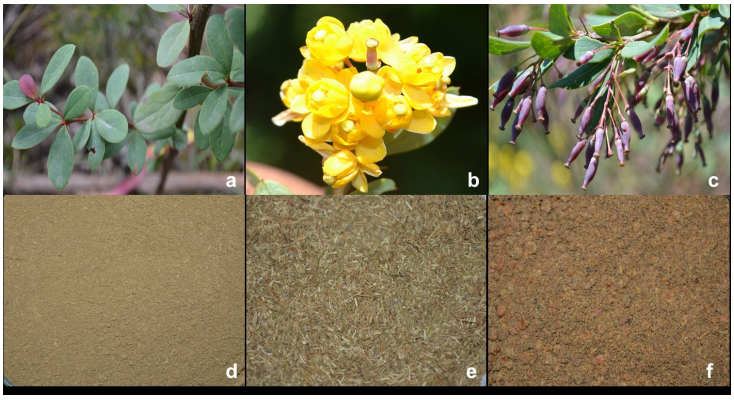

Fig. 1. Habit of *Berberis tinctoria*. (**a**. Leaf, **b**. Flower, **C**. Fruit, **d**. Leaf powder, **e**. Stem powder and **f**. Fruit powder)

toluene (BHT) (99%), gallic acid (97.5%), ferrous chloride (98%), ferric chloride, hydrogen peroxide (36.5%), ferrous ammonium sulfate (99%), ethylene diamine tetra-acetic acid (edta) disodium salt (99%), *N*-(1-naphthyl) ethylene diamine dihydrochloride (98%), sodium nitroprusside, riboflavin, acetyl salicylate (99%), pentazocine (98%), indomethacin (98.5%), carrageenan, carboxy methyl cellulose, petroleum ether (95%), chloroform (99.5%), ethyl acetate (99.5%) and methanol (99.8%) were procured from Sigma Aldrich, Bengaluru and Himedia, Mumbai. All the chemicals and solvents used were of analytical grade.

2.3. Proximate analysis

The moisture content of the leaf, stem and fruit samples were analyzed by weighing the fresh and dried materials (AOAC, 2000). The fresh samples were weighed, chopped into small pieces and kept in a hot air oven at 60 °C for 2 days. Then, the dried samples were weighed and moisture content was calculated by using the formula (Eqn.1):

Moisture (%) = (Fresh weight of sample - Dried weight sample)/(Fresh weight sample)×100 (Eqn.1)

The crude fibre of leaf, stem and fruit samples was estimated according to a previously reported paper (Sadasivam and Manikam, 2008) by dissolving 2 g of samples in petroleum ether (1:10) ratio. The resulting solution was kept in a mechanical shaker for 24 h to remove fats. Then, the air-dried samples were boiled with 200 mL of H_2SO_4 (0.255 N) for 30 min, then filtered and washed with distilled water. Again, the contents were boiled with 200 mL of NaOH (0.313 N) for 30 min and then filtered. Then, the contents were washed with 25 mL of boiling H_2SO_4 (1.25%), 50 mL of water and 25 mL of ethanol. The residue was transferred to ash crucible and dried for 2 h at 130 ± 2 °C, after that the crucible was weighed and the contents were ignited for 30 min at 600 ± 5 °C and reweighted again. The crude fibre value was calculated by using the formula (Eqn. 2):

Crude fibre value (g⁄100 g) = (Loss in weight on ignition (W_2-W_1)-(W_3-W_1))/(Weight of the sample (g))×100 (Eqn.2)

W_1 - The weight of the crucible, W_2- Weight of the crucible with sample (130 ± 2 °C), W_3 – The weight of the crucible with ash (600 ± 15 °C). The crude ash content of the samples was estimated by the method AOAC, (2000). 2 g of each sample leaf, stem and fruit was placed in a weighed crucible and incinerated at 550 ± 5 °C for 8 h in a muffle furnace (KTS 014, Mumbai, India). The incinerated samples were cooled and weighed to calculate the ash value (Eqn. 3):

Crude ash value (g⁄100 g) =(C-A)/(B-A)×100 (Eqn.3)

A - Weight of the crucible (g), B - Weight of the crucible

with sample (g) and C - Weight of crucible with ash (g). The total nitrogen was determined by the Micro-Kjeldahl method and nitrogen protein conversion factors were used for the determination of crude protein (Nx6.25). The carbohydrate or nitrogen-free extracts (NFE) content was estimated by the difference between the total nitrogen and nitrogen protein. The final results of proximate composition were expressed in gram per hundred gram of dry matter (g/100 g DM). The gross energy (KJ) was calculated by multiplying the percentage of crude protein, crude lipid, and NFE by 16.7, 37.7, and 16.7, respectively (Siddhuraju et al., 1996).

2.4. Minerals analysis

200 mg samples of the leaf, stem and fruit were digested with 10 mL of triacid (9:2:1 nitric acid: sulphuric acid: perchloric acid) at 80 °C in a fume hood. After digestion, the samples were made up to 100 mL with distilled water. 3-4 mL of samples were used to analyze the minerals using ICP-MS (NeX Ion 300 X, Perkin Elmer, USA). The operating manual of the ICP-MS instrument were spray chamber- glass cyclonic, RF power- 1600 W, gas flow rate (plasma gas flow-16.0 L/min, auxiliary-1.2 L/min, nebulizes-1.00-1.05 L/min), sample uptake rate- 250 µL/min and dwell time- 500-1500 ms. The 16 mineral isotopes monitored were ^{9}Be (beryllium), ^{23}Na (sodium), ^{24}Mg (magnesium), ^{27}Al (aluminium), ^{39}K (potassium), ^{52}Cr (chromium), ^{55}Mn (manganese), ^{57}Fe (iron), ^{60}Ni (nickel), ^{63}Cu (copper), ^{66}Zn (zinc), ^{75}As (arsenic), ^{82}Se (selenium), ^{98}Mo (molybdenum), ^{111}Cd (cadmium) and ^{208}Pb (lead).

2.5. Successive solvent extraction of plant parts

The dried and powdered leaf, stem and fruit samples (50 g) were extracted in a Soxhlet extractor successively using 300 mL of non-polar to polar solvents like petroleum ether, chloroform, ethyl acetate, methanol, and water until content becomes colorless. Before extracting with the solvent, each time the material was dried in a hot air oven below 40 °C. The rotary vacuum evaporator (Equitron Ev11-ABS.051) was used to concentrate the different solvent extracts at 40-100 °C depending on the boiling point of the solvents. The dried extract was collected, weighed and finally stored at -20 °C.

2.6. Primary metabolites

2.6. 1. Estimation of protein

The protein was estimated as described by Lowry et al. (1951) with some modifications using the standard bovine serum albumin (10-50 µg/mL). 100 mg of powdered leaf, stem and fruit samples was ground using 10 mL of phosphate buffer (0.2 M pH 7.2) in mortar and pestle and centrifuged at 3000 x g for 10 minutes at room temperature. The supernatant was made up to 10 mL with distilled water. 1 mL of the supernatant was diluted to 10 mL with distilled water and used for estimation. About 500 µL of the extract were added into the test tube and made up to 1 mL with distilled water. Then, 5 mL of reagent C [(reagent A: sodium carbonate (2%) in 0.1 N sodium hydroxide; reagent B: copper sulphate (0.5%) in potassium sodium tartrate (1%); reagent C: 175 mL of reagent A being added to 3.5 mL of the reagent B and was mixed before use)] was added. To this solution, 0.5 mL of Folin-Ciocalteau's reagent (1 N) was added and allowed to incubate in dark for 30 min. The absorbance was then determined at 660 nm using a spectrophotometer and expressed in mg bovine serum albumin equivalents per g extract.

2.6.2. Estimation of carbohydrates and starch

Carbohydrates and starch were estimated by the protocol of Hodge (1962). For carbohydrate estimation, 100 mg of leaf, stem and fruit dried extracts were added to 5 mL of HCl (2.5 N) and placed in a water bath for 3 h. After the mixture reached the room temperature, sodium carbonate was added to balance the mixture till the effervescence ceased. In the next step, the mixture was diluted with 100 mL distilled water then centrifuged for 10 min at 3000 rpm. To that, 1 mL of supernatant and 4 mL of anthrone reagent (200 mg of anthrone in 100 mL of ice-cold 95% sulphuric acid (H_2SO_4)) were added and boiled for 8 min in the water bath. After it reached the room temperature, the absorbance of green color was read at 630 nm against the reagent blank. For starch estimation, 1 g of dried leaf, stem and fruit extracts were weighed and ground with 10 mL of ethanol (80%) and centrifuged at 3000 rpm for 10 min. The procedure was repeated two times for the complete removal of simple sugars. After centrifugation, the residues were collected and dried well, then perchloric acid (52%) was added to the residues and centrifuged at 3000 rpm for 10 min. 1 mL of supernatant was collected and 4 mL of anthrone reagent consisting of 200 mg of anthrone in 100 mL of ice-cold 95% sulphuric acid was added and then kept in a boiling water bath for 8 min. After incubation, the mixture was cooled rapidly and the absorbance of green color was read at 630 nm against the reagent blank. The carbohydrates and starch content were determined by using the standard graph plotted against glucose aliquots (10-50 µg/mL). The results were shown in mg of glucose equivalents present in a gram of sample extract.

2.6.3. Estimation of amino acids

The amino acid present in the samples was estimated by Moore and Stein (1948). Under the experimental conditions, 500 mg of dried leaf, stem and fruit extracts were ground well separately with the addition of 5 to 10 mL of ethanol (80%) and centrifuged for 10 min at 3000 rpm. To the supernatant and the aliquots of leucine (10-50 µg/mL), 0.1 mL of ninhydrin solution, a mixture of 0.2 g stannous chloride in 125 mL of 0.2 M citrate buffer (pH 0.5) and 5 g of ninhydrin in 125 mL of methyl cellosolve, was added and made up 2 mL by distilled water. The mixture was heated for 20 min in a boiling water bath then 5 mL of diluent solvent was added (equal volume of water and *n*-propanol) and left for 15 min at room

temperature. All the test tubes were vortexed well and the development of purple color by the sample was measured against the reagent blank at 570 nm. The amino acid present in the extract was determined by using the standard plotted against leucine. The results were expressed in milligrams of leucine equivalents present in one gram of sample extract (mg/g).

2.6.4. Estimation of reducing sugars

The reducing sugar content in leaf, stem, and fruit extracts was estimated by the procedure reported by Miller (1959). Briefly, 100 mg of sample was dissolved in 5 mL of hot ethanol (80%) and evaporated in a water bath (80 °C). Afterwards, 10 mL of distilled water was added to dissolve the residue. Aliquots of 0.5-3 mL of sample extract and standard glucose (10-50 µg/mL) were taken in separate test tubes and made up the volume to 3 mL with water in all the tubes. 3 mL of di-nitrosalicyclic acid reagent (DNS, 1 g) and 200 mg crystalline phenol were immediately added. Thereafter, 50 mg of sodium sulphate was dissolved in 100 mL of NaOH (1%), and the test tubes were kept in a boiling water bath for 5 min. 1 mL of Rochelle salt solution (40%) was added and dissolved by heating. After cooling the mixture, the intensity of dark red color was read at 510 nm using a spectrophotometer. The reducing sugars present in the extract were determined by a standard graph plotted against glucose and expressed in mg with glucose equivalents (mg GE/g) per gram sample extract.

2.6.5. Estimation of vitamin C

Vitamin C contents of the leaf, stem and fruit extracts were estimated according to the protocol developed by Benderitter et al. (1998). In this sense, 75 µL of dinitrophenyl hydrazine (DNPH) solution (2 g of dinitrophenyl hydrazine), 230 mg of thiourea, and 270 mg of copper sulphate pentahydrate ($CuSO_4.5H_2O$) in 100 mL of H_2SO_4 (5 M) were added to 500 µL of reaction mixtures composed of 300 µL of the aqueous leaf, stem and fruit extracts, 100 µL of 13.3% trichloroacetic acid (TCA) and the aliquots of ascorbic acid (10-50 µg/mL). The reaction mixture was finally incubated at 37 °C for 3 h. Then, 0.5 mL of H_2SO_4 (65% v/v) was added to the mixture, and the color absorbance was read at 520 nm. The results were expressed in milligrams of ascorbic acid equivalents present in one gram of sample extracts (mg/g).

2.7. Secondary metabolites

2.7.1. Quantification of total phenolic and tannin content

From the extracted stock solution, 100 µL of the leaf, stem and fruit extracts along with aliquots of (10-50 µg/mL) gallic acid were taken in an array of test tubes and made up to 1 mL with distilled water. A test tube with 1 mL distilled water served as blank. Then, 500 µL of Folin-Ciocalteau reagent (1 N) was added to the sample as well as to blank and kept for 5 min. Then, 2.5 mL of sodium carbonate solution (20%) was added to all the test tubes, vortexed well, and incubated in dark at room temperature for 40 min, and the absorbance was read at 725 nm using the spectrophotometer (UV-1800, Shimadzu, Japan) against the reagent blank. The total phenolic content was quantified by using the standard calibration curve obtained from gallic acid, and the results were expressed in milligrams of gallic acid equivalents present in one gram of extract (mg GAE/g) (Makkar, 2003).

The total phenolics contain both tannin and non-tannin phenolics. The total amount of tannins was calculated by subtracting the non-tannin phenolics from total phenolics. For each 500 µL of the leaf, stem and fruit extract, 500 µL of distilled water and 100 mg of PVPP were added. The content was vortexed and kept in an Eppendorf tube at 4 °C for 4 h. After incubation, the sample was centrifuged at 4000 rpm for 10 min at 4 °C and the supernatant was collected. This supernatant has only non-tannin phenolics (where the tannins would have been precipitated along with the PVPP). The non-tannin phenolic in the supernatant was determined by the same method described for the quantification of total phenolics (Makkar, 2003). The results were expressed with gallic acid equivalent (10-50 µg/mL) and the tannin content was calculated as follows (Eqn. 4):

Tannin(%) = Total phenolics(%) - Non tannin phenolics(%) (Eqn. 4)

2.7.2. Total flavonoid

Total flavonoid was estimated by Zhuang et al. (1992). Accurately, 100 µL of the leaf, stem and fruit extracts were taken and aliquots of quercetin (10-50 µg/mL) in different tubes were made up to 2 mL using distilled water while considering a test tube containing 2 mL of distilled water as blank. Then, 150 µL of sodium nitrite ($NaNO_2$, 5%) was added and incubated at room temperature for 6 min. After incubation, 150 µL of aluminium chloride ($AlCl_3$, 10%) was added to all the test tubes and incubated for 6 min at room temperature. Then, 2 mL of sodium hydroxide (NaOH, 4%) was added and made up to 5 mL using distilled water. The contents of all the test tubes were vortexed well and allowed to stand for 15 min at room temperature. The pink color developed denoted the presence of flavonoids and was read at 510 nm against the reagent blank. The standard quercetin was used for the quantification of flavonoids and expressed in milligram of quercetin equivalents (QE) in one gram of sample extract (mg QE/g extract).

2.7.3. Total alkaloids

The determination of total alkaloids was done following a method given by Singh et al. (2004). Accordingly, the reaction mixture contained 100 µL of leaf, stem and fruit extracts in separate test tubes to which 1 mL of ferric(III) chloride ($FeCl_3$, 0.025 M) in hydrochloric acid (HCl 0.5 M) and 1 mL of 1,10-phenanthroline (0.05 M) in

ethanol were added. Then, the tubes were incubated for 30 min in the water bath with a maintained temperature of 70 ± 2 °C. The absorbance of the red color complex was measured at 510 nm against the reagent blank. The alkaloid contents were calculated by using the standard curve of quinine aliquots of 10-50 µg/mL. The values were expressed in milligram (mg) of quinine equivalents (QE) in one gram of sample extract (mg QE/g extract).

2.7.4. Determination of saponin

The saponin content in leaf, stem and fruit extract was determined by Makkar et al. (2007). About 100 µL triplicates of the plant extracts at various concentrations (10-50 µg) of diosgenin were taken and made up to 300 µL with distilled water. 0.25 mL of vanillin reagent (8% v/v in ethanol) and 2.5 mL of aqueous H_2SO_4 (72%) were added and heated in a water bath for 10 min at 60 °C. After boiling, the tubes were cooled for 4 min in ice and allowed to reach the room temperature. At last, the absorbance was measured in a UV/Vis. spectrophotometer at 544 nm. The results obtained were expressed in milligram diosgenin equivalent per gram of sample extract (mg/g).

2.8. Antioxidant activity analysis

2.8.1. DPPH scavenging activity

The hydrogen donating or radical scavenging ability (antioxidant activity) of the sample was determined in terms of using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) following the method of Braca et al. (2001). The leaf stem and fruit extracts of *B. tinctoria* were taken in different concentrations and the final volume was made to 100 µL with methanol. About 3 mL of a DPPH (0.1 mM) solution was added to the aliquots of samples. Then, 3 mL DPPH methanolic solution with 100 µL methanol was prepared as the negative control, different concentrations of rutin and BHT are prepared as a positive control. The absorbance was recorded at 517 nm against the blank after 30 min in dark incubation at room temperature. The DPPH radical quenching results were expressed as IC_{50} value.

2.8.2. $ABTS^{\bullet+}$ scavenging activity

The total antioxidant activity of the leaf, stem and fruit extracts was measured by 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid ($ABTS^{\bullet+}$) radical cation decolorization assay according to the method developed by Re et al. (1999). $ABTS^{\bullet+}$ solution was prepared by ABTS (7 mM) aqueous solution with potassium persulfate (2.4 mM) reacting in the dark for 12-16 h at room temperature. Then, the solution was diluted in ethanol (about 1:89 v/v) and equilibrated at 25 °C to give an absorbance of 0.700 ± 0.02 at 734 nm. 1 mL of diluted $ABTS^{\bullet+}$ solution was added to triplicate of the sample extracts and standard trolox over the concentration range of 0-15 µM/mL. The extraction mixture was incubated at 30 °C exactly for 30 min. After incubation, the absorbance of samples and standard were measured at 734 nm against the ethanol blank. The results were expressed in micromolar trolox equivalent antioxidant capacity (TEAC) per sample extracts (µM/g).

2.8.3. Phosphomolybdenum assay

The antioxidant activity of leaf, stem and fruit extracts was evaluated by the phosphomolybdenum method followed by Prieto et al. (1999). Aliquots of 100 µL samples and standards (BHT and rutin) were taken into a series of test tubes and were made up to 300 µL with methanol. About 300 µL of methanol taken in a test tube was considered as the blank. 1 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) were added to all the test tubes and incubated in a water bath at 95 °C for 90 min. After reached to room temperature, the absorbance of the mixture was measured at 695 nm against a reagent blank. The results were reported in milligram ascorbic acid equivalents per gram sample extract (mg/g).

2.8.4. Ferric reducing antioxidant power (FRAP) assay

The ability to reduce TPTZ-Fe(III) complex to TPTZ-Fe(II) complex by the antioxidant capacity of different extracts of samples was estimated according to the procedure described by Pulido et al. (2000). Freshly prepared FRAP reagent (900 µL) (2.5 mL of 20 mM/L TPTZ (2,4,6-tripyridyl-S-triazine)) solution in 40 mM/L HCl and 2.5 mL of 20 mM/L, $FeCl_3.6H_2O$ and 25 mL of 0.3 M/L acetate buffer (pH 3.6) was added to the leaf, stem and fruit extracts, also for the aliquots of standards (BHT and rutin) and methanol (blank). All these tubes were incubated at 37 °C for 30 min. The absorbance of the blue color was read against reagent blank at 593 nm. The parameter 'Equivalent Concentration' was expressed as the concentration of antioxidant having a ferric-TPTZ reducing ability equivalent to that of $FeSO_4.7H_2O$ (1 mM) and expressed as mM Fe (II)/mg of sample.

2.8.5. Metal chelating activity

The chelation of ferrous ions by leaf, stem and fruit extracts was estimated by Dinis et al. (1994). 100 µL solution of ferrous chloride (2 mM) was added to 500 µg of sample extracts. The reaction was started by the addition of 400 µL of ferrozine (5 mM) and incubated at room temperature for 10 min. The reaction tube containing deionized water in place of the sample was considered as a negative control. The absorbance of the solution was measured spectrophotometrically at 562 nm against the blank. Ethylenediaminetetraacetic acid EDTA (10-50 µg/mL) was used as the standard metal chelating agent and the results were expressed as milligram EDTA equivalents per gram sample extracts.

2.9. Anti-inflammatory activity

Inhibition of bovine serum albumin (BSA) denaturation method (Kaur et al., 2018) was followed with some

modifications. In this regard, the reaction mixture consisted of a test sample (500 µg/mL) and an aqueous solution of bovine albumin fraction (1%). The pH of the reaction mixture was adjusted to 6.3. The samples were incubated at 37 °C for 20 min and then heated at 51 °C for 20 min. After cooling, the turbidity was measured spectrophotometrically at 660 nm. Aspirin was taken as a standard (100 µg/mL) and the experiment was performed in triplicates. Percentage inhibition of protein denaturation was calculated as follows (Eqn. 5).

Inhibition% = (Absorbance of control - Absorbance of sample)/(Absorbance of control)×100 (Eqn. 5)

2.10. *In vitro* α-amylase and α-glucosidase inhibitor assay

In vitro antidiabetic activities of the leaf, stem and fruit extracts of *B. tinctoria* were estimated by α-amylase inhibition (Ali et al., 2006) with some slight modifications. Accordingly, 500 µL of 0.20 mM phosphate buffer (pH 6.9) with α-amylase (0.5 mg/mL) solution were added to 500 µL of plant samples and standard drug acarbose (10-50 µg/mL) then incubated at 25 °C for 10 min. In the next step, 500 µL of a starch solution (1%) in sodium phosphate buffer (pH 6.9, 0.02 M) was added to all test tubes and kept at 25 °C for 10 min. The reaction was stopped when adding 1 mL of 3,5-dinitro-salicylic acid color reagent. Afterwards, test tubes were incubated in a boiling water bath for 5 min and cooled to room temperature. Then, 10 mL of distilled water was added for diluting the samples and absorbance was measured at 540 nm. The α-glucosidase activity of the plant extracts was determined by the general method given by Kim et al. (2009) with some modifications. Accordingly, 500 µL of plant samples and standard drug acarbose (10-50 µg/mL) was added with pre-incubated 100 µL of α-glucosidase solution for 10 min at 25 °C. In addition, α-glucosidase was pre-incubated with 50 µL of phosphate buffer (pH 6.9) in another set of tubes. To initiate the reaction, 50 µL of *p*-nitrophenyl-a-D-glucopyranoside at increasing concentrations (0.63-2.0 mg/mL) was added to both sets of reaction mixtures. Mixtures without enzyme, sample and acarbose served as blank. The mixture was incubated for 10 min at 25 °C and then 500 µL of sodium carbonate was added to stop the reaction. The α-glucosidase activity was determined by yellow-colored para-nitrophenol released from *p*-nitrophenyl-a-D-glucopyranoside at 405 nm and results were expressed as IC_{50} value.

2.11. Statistical analysis

All the *in vitro* assays were performed in triplicates and the final results were expressed in mean ± standard deviation (SD). Means were compared with Duncan's multiple range test (DMRT) at a significance threshold of $P < 0.05$. Relationships between variables were quantified using Pearson's correlation and PCA. All calculations were performed using SPSS, version 20.0 (IBM SPSS, IBM Corp., Armonk, New York and XLSTAT 2018, USA).

3. Results and Discussion

3.1. Proximate analysis

The physico-chemical parameters of *B. tinctoria* leaf, stem and fruit were analyzed for moisture contents, crude ash, crude fibre, crude lipid, crude protein, nitrogen-free extracts (NFE), and energy. These parameters were determined based on fresh weight and the results were shown in Table 1. The observed moisture contents in *B. tinctoria* fruit 88.9 ± 2.6%, leaf 71.5 ± 2.9% and stem 56.5 ± 1.9% and the results were expressed in percentage.

Table 1
Proximate analysis of *Berberis tinctoria* leaf, stem and fruit.

Proximate analysis	Proximate analysis of *Berberis tinctoria*			F=df (2,8)
	Leaf	Stem	Fruit	
Moisture%	71.5 ± 2.9^{a}	56.5 ± 1.9^{b}	88.9 ± 2.6^{b}	298.8**
Crude ASH (g/100g)	3.4 ± 0.1^{a}	2.8 ± 0.6^{b}	3.8 ± 0.1^{a}	621.8**
CrudeFiber (g/100g)	2.6 ± 0.7^{a}	2.9 ± 0.2^{c}	2.4 ± 0.1^{b}	256.9**
Crude Lipid (g/100g)	10.1 ± 0.1^{c}	4.2 ± 0.2^{b}	15.8 ± 1.1^{b}	394.7*
Crude Protein (g/100g)	2.1 ± 0.3^{a}	1.5 ± 0.6^{c}	5.6 ± 1.2^{c}	265.9*
NFE (g/100g)	73.8 ± 0.2^{b}	65.8 ± 0.2^{c}	77.9 ± 0.4^{a}	96.9***
Energy (KJ/100g)	1715.8 ± 4.5^{a}	1115.1 ± 3.5^{c}	1827.2 ± 35.2^{b}	967.5***

The proximate analysis between the leaf, stem and fruit were analyzed by DMRT. Mean ± SE followed by same letter (s) are not significantly ($p > 0.05$) different according to DMRT. *** Significant at 0.1% level, **Significant at 1% level, *Significant at 5% level. **NFE**- Nitrogen Free Extractives; **KJ**-Kilo Joules.

While *B. tinctoria* has an ash content of 3.4 ± 0.1 g/100 g, 2.8 ± 0.6 g/100 g and 3.8 ± 0.1 g/100 g, for leaf, stem and fruit, respectively. The maximal ash was observed in fruit because of the anoxic bioflocs which may be the reasons for high mineral contents and acid-insoluble oxides (Tacon and Ferns, 1979). *B. tinctoria* plant has crude fibre and crude lipid of leaf: 2.6 ± 0.7 g/100 g and 10.1 ± 0.1 g/100 g, stem: 2.9 ± 0.2 g/100 g and 4.2 ± 0.2 g/100 g, as well as fruit: 2.4 ± 0.1 g/100 g and 15.8 ± 1.1 g/100 g, respectively. The total nitrogen was observed 0.3% in leaf, 0.1% in stem and 0.9% in fruit samples of *B. tinctoria*. The nitrogen protein conversion factors were calculated using crude protein (Nx 6.25) which depends on the nitrogen content of the sample. The recorded crude protein level was 73.8 ± 0.2 g/100 g in *B. tinctoria* leaf, stem and fruits had 65.8 ± 0.2 g/100 g and 77.9 ± 0.4 g/100 g, respectively. The nitrogen-free extracts (NFE) which are also called total carbohydrate were calculated by adding crude fibre, moisture, crude lipid, crude ash, and crude protein of the sample (Siddhuraju et al., 1996).

The NFE of *B. tinctoria* sample was estimated as 73.8 ± 0.2 g/100 g (leaf), 65.8 ± 0.2 g/100 g (stem) and 77.9 ± 0.4 g/100 g (fruit). The gross energy (KJ) was determined by multiplying the percentage of crude protein, crude lipid and NFE contents defending the energy level reported by Siddhuraju et al. (1996). The *B. tinctoria* sample has respective gross energy of 1715.8 ± 4.5 KJ/100 g in leaf, 1115.1 ± 3.5 KJ/100 g in stem and 1827.2 ± 35.2 KJ/100 g in fruit. The proximate analysis concluded that the fruit of *B. tinctoria* has achieved a higher amount of moisture, crude ash, crude lipid, crude protein, NFE, and energy. Moreover, the stem of *B. tinctoria* has high crude fibre, so the fruit has good energy sources compared to the leaf and stem. The *B. lyceum* fruit has the moisture and ash content of 83.2 ± 0.1% and 0.5 ± 0.1%. The nutritional characteristics of the feeds are judged by the moisture and ash content, as the result of their constant elemental composition. According to the Sood et al. (2010), the ash contents of the plant origin are poor nutritional indicators and also, they recorded the crude lipid (0.6 ± 0.2%) and crude protein (0.8 ± 0.1%) in the *B. lyceum* fruit. The gross energy of *B. lyceum* fruit is 52.1 ± 0.1 Kcal/100 g (Sood et al., 2010). It has been documented that there is a proximate relationship between the crude fibre and nutrition value of the feed, where higher the nutritional fibre, lower the nutritional value. Shah et al. (2003) have already reported the crude fibre of *B. lyceum* fruit being 0.8 ± 0.1%. Awan et al. (2014) reported the nutritional and phytochemical contents of three *Berberis* fruits. The moisture contents of *Berberis calliobotrys* Bien. ex Koehne., *Berberis orthobotrys* Bien. ex Aitch. and *Berberis psedumbellata* R.Parker. are respectively 80.47%, 74.96% and 80.13% accounting for high moisture compared to *B. tinctoria*. The ash content of *B. calliobotrys* showed 0.79% which is lower than *B. psedumbellata* (1.13%) and *B. orthobotrys* (1.05%) (Awan et al., 2014). The crude protein and crude fibre were 1.51% and 0.73% (*B. calliobotrys*), 0.96% and 0.78% (*B. psedumbellata*) and 1.10% and 0.94% (*B. orthobotrys*) respectively, among these *B. Calliobotrys* showed high nutritional contents (Awan et al., 2014). The results of the proximate analysis substantiates that the *B. tinctoria* fruit exhibited good results of moisture, crude ash, fiber, lipid, protein, NFE and energy which support that the fruit could be considered as a good nutrient-rich source.

3.2. Mineral contents of *B. tinctoria*

The mineral (macro and micro) nutrient profile of *B. tinctoria* leaf, stem and fruit were shown in Table 2. The ICP-MS analysis reported the presence of essential minerals such as potassium (K), aluminium (Al), sodium (Na) and magnesium (Mg), in which Na and K cations are located mainly inside and outside of the cell, which is engaged in the regulation of acid-base balance, plasma volume and muscle nerve contraction (Akpanyung, 2005). The fruit samples had more essential minerals compared to the leaf and stem, such as Na (20.296 ± 0.544 mg/100 g), Mg (75.477 ± 12.845 mg/100 g), Al (52.393 ± 15.986 mg/100 g) and K (177.898 ± 38.722 mg/100 g). The previous research reported the mineral contents of *B. lyceum* leaf with Zn, Mn, Fe, Cu, P, K, Na, Ca in which Zn, Cu, and Na were maximum (56.15 ± 0.01 µg/g, 95.67 ± 0.12 µg/g and 115.00 ± 0.03 µg/g) (Shah et al., 2003). Sood et al. (2010) also reported the potassium (161.42 ± 0.41 mg/100 g), sodium (14.5 ± 0.11 mg/100 g), and iron (2.61 ± 0.06 mg/100 g) contents of *B. lyceum* fruit extracts which have higher mineral content compared to the leaf. *B. tinctoria* has a significant amount of essential minerals and a low amount of heavy metals like Cd, Pb, Se, Cr and Cu compared to *B. aristata*, *Berberis*. *asiatica* Roxb. ex DC., *B. chitria* and *B. lyceum* (Markert, 1994; Srivastava et al., 2006). The results showed that *B. tinctoria* is a nutrient-rich plant and within the field given by Sood et al. (2010). The slight variations in results might be due to differences in the topography of soil, climate conditions coupled with a varietal difference.

3.3. Primary metabolites

The quantitative phytochemical screening of fresh leaf, stem and fruit of *B. tinctoria* are shown in Table 3. The results conceded that the primary metabolites such as carbohydrates, starch, reducing sugar, proteins, amino acids and vitamin C are present in an appreciable amount in plant parts. The total carbohydrates, starch, and reducing sugars were reported individually in which the total carbohydrates and starch of *B. tinctoria* were 305.7 ± 26.4 mg GLE/g and 177.3 ± 7.9 mg GLE/g in leaf; the stem had 265.2 ± 2.9 mg GLE/g and 84.7 ± 7.5 mg GLE/g, and the fruit contained 383.3 ± 14.3 mg GLE/g and 206.4 ± 12.1 mg GLE/g, respectively. The reducing sugar of 35.1 ± 1.2 mg GLE/g in leaf, 1.4 ± 0.1 mg GLE/g in stem and 29.4 ± 0.4 mg GLE/g in fruit was recorded. The total free amino acid content in leaf, stem and fruit are 0.3 ± 0.1 mg LE/g, 0.2 ± 0.1 mg LE/g and 1.3 ± 0.1 mg LE/g, respectively. Also, about 469.1 ± 12.4 BSA mg/g, 23.3 ± 0.3 BSA mg/g and 471.8 ± 15.8 BSA mg/g of protein and 12.3 ± 1.5

Table 2
Total content of macro and micronutrients of *Berberis tinctoria* leaf, stem and fruit on ICP-MS analysis.

Minerals	Total content of macro and micronutrients		
	Leaf (mg/100g)	Stem (mg/100g)	Fruit (mg/100g)
Be	0.001 ± 0.001	0.021 ± 0.014	0.932 ± 0.143
Na	8.153 ± 0.535	3.346 ± 0.519	20.296 ± 0.544
Mg	65.514 ± 4.357	32.171 ± 2.451	75.477 ± 12.845
Al	23.798 ± 1.660	25.214 ± 12.741	52.393 ± 15.986
K	95.678 ± 8.236	75.521 ± 21.36	177.898 ± 38.722
Cr	0.002 ± 0.001	0.034 ± 0.002	0.014 ± 0.001
Mn	0.073 ± 0.010	0.002 ± 0.001	0.005 ± 0.002
Fe	0.221 ± 0.032	0.127 ± 0.013	0.117 ± 0.047
Ni	0.001 ± 0.001	0.018 ± 0.00	0.008 ± 0.001
Cu	0.004 ± 0.001	0.002 ± 0.002	0.001 ± 0.005
Zn	0.007 ± 0.001	0.003 ± 0.00	0.006 ± 0.001
As	0.009 ± 0.001	0.022 ± 0.001	0.009 ± 0.001
Se	0	0.015 ± 0 .004	0
Mo	0.001 ± 0.001	0.009 ± 0.001	0.007 ± 0.001
Cd	0	0	0
Pb	0.001 ± 0.001	0.023 ± 0.001	0.001 ± 0.002

The macro and micronutrients analysis between the leaf, stem and fruit were analyzed the mean ± SE. **Be**: Beryllium; **Na**: Sodium; **Mg**: Magnesium; **Al**: Aluminium; **K**: Potassium; **Cr**: Chromium; **Mn**: Manganese; **Fe**: Iron; **Ni**: Nickel; **Cu**: Copper; **Zn**: Zinc; **As**: Arsenic; **Se**: Selenium; **Mo**: Molybdenum; **Cd**: Cadmium; **Pb**: Lead.

Table 3
Biochemical estimation of primary metabolites in different extracts of *Berberis tinctoria* leaf, stem and fruit.

Biochemical estimation	Biochemical estimation of primary metabolites			F=df (2,8)
	Leaf	Stem	Fruit	
Total Carbohydrates (GLE mg/g)	305.7 ± 26.4^{b}	265.2 ± 2.9^{a}	383.3 ± 14.3^{c}	35.4**
Starch (GLE mg/g)	177.3 ± 7.9^{a}	84.7 ± 7.5^{b}	206.4 ± 12.1^{c}	137.1**
Reducing sugar (GLE mg/g)	35.1 ± 1.2^{a}	1.4 ± 0.1^{b}	29.4 ± 0.4^{a}	1467.1**
Total Free Amino acid (LE mg/g)	0.3 ± 0.1^{a}	0.2 ± 0.1^{c}	1.3 ± 0.1^{b}	190.3*
Protein (BSA mg/g)	469.1 ± 12.4^{b}	23.3 ± 0.3^{c}	471.8 ± 15.8^{a}	559.5***
Vitamin- C (AA mg/g)	12.3 ± 1.5^{b}	4.4 ± 2.1^{c}	18.1 ± 1.9^{a}	124.9***

The Primary metabolites analysis between the leaf, stem and fruit were analyzed by DMRT. Mean ± SE followed by same letter (s) are not significantly ($p > 0.05$) different according to DMRT. *** Significant at 0.1% level, **Significant at 1% level, *Significant at 5% level. **GLE**: Glucose Equivalents; **LE**: Leucine Equivalents, **BSA**: Bovine Serum Albumin equivalents; **AA**: Ascorbic acid.

AA mg/g, 4.4 ± 2.1 AA mg/g and 18.1 ± 1.9 AA mg/g of vitamin C were estimated, respectively. Among the different parts used, the fruit showed a high nutritional value of carbohydrates, amino acids, vitamins, and protein in the order, i.e. fruit > leaf > stem indicates the antioxidative, anticarcinogenic, antihypertension, anti-inflammatory, antimicrobial, immunostimulating, and cholesterol-lowering properties (Tabeshpour et al., 2017). The ascorbic acid in *B. lycium* fruit was reported as 83 mg/g so that the raw berries were prevalently consumed by the rural population because of their taste, and being rich source of vitamin C and anthocyanin (Bober et al., 2018). Most of the *Berberis* species have been used in traditional folk medicine, especially in Asian regions. Phytochemicals such as isoquinoline alkaloid berberine (useful in treating a wide array of diseases including cancer) have been isolated from *Berberis* plants (Bhat and Paliyath, 2016). The reducing sugar content of fruit of *B. calliobotrys* (9.00%), *B. orthobotrys* (7.23%) and *B. psedumbellata* (7.68%) was already reported by Awan et al. (2014). The nutritional and antinutritional qualities of the pulp and seed portions

of edible fruits (*Berberis jaeschkeana* C.K.Schneid.*; B. asiatica; B. aristata; B. lycium; B. pseudumbellata*) from the Southern Western Ghats and Western Himalayas plants showed higher carbohydrates, fibre, protein, fat, tannins, and vitamin C (Bhat and Paliyath, 2016). Moreover, *B. tinctoria* raw fruits are consumed by the rural population of the Nilgiri hills, which has valuable primary metabolites and major active constituents. The obtained results were leads to further phytochemical analysis.

3.4. Extracts yield percentage

The yield percentage of leaf, stem and fruit extracts of *B. tinctoria* in different solvents extraction was calculated. The maximum yield was obtained in methanol and the extract recovery percentage was found to be higher in leaf (20.30 g per 50 g sample) compared to fruit (12.32 g per 50 g sample) and stem (4.15 g per 50 g sample). The yield percentage in petroleum ether, chloroform and ethyl acetate of leaf, stem and fruit was found to be low compared to methanol and water (Supplementary Table 1). The water extraction of leaf, stem and fruit showed extract recovery percentage of 4.93 g, 3.60 g and 2.54 g, respectively. The Soxhlet extraction yield of *B. tinctoria* showed a higher yield percentage in the alcohol and water extracts compared to the other solvents (Srivastava and Rawat, 2007). Therefore, it indicates that the extraction efficiency favours the high polar solvents.

3.5. Secondary metabolites

The quantitative phytochemical screening of leaf, stem and fruit extracts of *B. tinctoria* has been represented in Table 4. As seen, the total phenolics were found to be higher in ethyl acetate leaf extract (689.5 ± 2.3 GAE mg/g), methanol extract of stem (128.1 ± 1.8 GAE mg/g) and fruit (498.6 ± 6.9 GAE mg/g), compared to other solvent extracts. From the results, the leaf and fruit extracts were observed to have higher phenolics compared to the stem. A previous study has reported the phenolic content of *B. tinctoria* fresh fruit as 410.1 ± 0.1 GAE mg/g (Sasikumar et al. 2012). The phenolic content of *B. calliobotrys* (689.82 mg/100 g), *B. orthobotrys* (675.68 mg/100 g) and *B. psedumbellata* (702.94 mg/100 g) fruits have been reported by Awan et al. (2014). *B. vulgaris* fruit methanolic extract showed higher phenolic contents of 28000 mg/g (Motalleb et al., 2005). Plenty of inflating evidence turned out that consumption of polyphenolic-rich foods may lower the risk of health disorders because of their antioxidant activity (Shahidi and Ambigaipalan, 2015). Therefore, the higher amounts of phenolics in *B. tinctoria* are subjected for their antioxidant capacity. Flavonoids are organic compounds that are indirectly involved in metabolic activities present abundantly in fruits and vegetables which include flavonols, flavones, flavanones, and anthocyanins (Alén-Ruiz et al., 2008). The flavonoid and tannin contents were found to be more in methanol extract of leaf: 411.6 ± 4.7 QE mg/g and 251.4 ± 9.6 GAE mg/g, fruit: 389.7 ± 5.7 QE mg/g and 236.6 ± 8.1 GAE mg/g as well as stem: 332.1 ± 6.5 QE mg/g and 223.3 ± 6.7 GAE mg/g, respectively. The total flavonoid content was already reported for the same species as 320.1 ± 0.1 QE mg/g (Sasikumar et al., 2012). Awan et al. (2014) reported flavonoids of three *Berberis spp*. fruit such as *B. calliobotrys* (385.52 mg/100 g), *B. orthobotrys* (376.93 mg/100 g) and *B. psedumbellata* (395.09 mg/100 g). Also, Belwal et al. (2016) reported higher tannin content of 77.8 ± 2.5 CGE mg/g in *B. asiatica* fruit methanolic extract. Hanachi and Golkho (2009) reported that *B. vulgaris* fruit has higher tannin content of 20.5 ± 0.5 GAE mg/g. Among the reports of different *Berberis spp*., total tannins and flavanoids revealed that the proportion of free phenolics was higher in methanol extracts of leaf and fruit compared to the stem. The *in vitro* studies related to health and disease were advised to intake diet with vast quantities of flavonoids; which would increase defence mechanism related to oxidative stress (Nithiyanantham et al., 2012). The greater amounts of tannins in the extracts of fruit of *B. tinctoria* are due to the higher polymerization of existing polyphenolic compounds. It has been reported that high molecular weight phenolics such as tannins have more ability to quench/scavenge free radicals (Hagerman et al. 1998). Tannins are constituents of several drugs because of their astringent property, used in the treatment of haemorrhoids, diarrhoea, dysentery, leucorrhoea, and throat infections (Allport, 1970). Therefore, the quantification of flavonoid and tannin content is important, since it can be correlated with the anticancer and radical scavenging activity of the plant. Since *B. tinctoria* possesses good flavonoid and tannin content in fruit and leaf, it has high free radical scavenging activity which involves the transfer of electron or hydrogen atom from flavonoids to free radicals. Alkaloids are the largest group known for a variety of biological activities having specific mechanism of action. They have long been used as psychoactive substances but don't have strong psychoactive effects. However, they have been shown as precursors for semi-synthetic psychoactive drugs. In fact, alkaloids act directly on the central nervous system (CNS) to increase feelings of pleasure and warm relaxation and reduce pain. So, they definitely treat as a powerful painkiller medication (Raffauf, 1996) and antihypertensive agent (Zee-Cheng, 1997). *Berberis spp*. are rich in alkaloids (Hinz and Zenk, 1981) and the results of *B. tinctoria* methanolic extract showed the highest amount of alkaloids in leaf (392.5 ± 10.2 COLE mg/g), fruit (363.9 ± 23.1 COLE mg/g) and stem (163.6 ± 4.8 COLE mg/g) compared to the other solvent extracts. Previously, Lamichhane et al. (2014) reported a higher amount of alkaloids in the *B. aristata* fruit methanolic extract. Hence, the present study showed that the *B. tinctoria* has rich alkaloids in the ascending order of leaf > fruit > stem. Saponins are a group of plant compounds, with the number of the stereochemistry of aglycone moieties and sugars, producing a distinct array of compounds with potent biological effects (Francis et al., 2002) such as precipitating and coagulating of red

blood cells (Sodipo et al., 2000). The highest amount of saponins was observed in the methanolic extract of leaf (11.7 ± 0.8 RUTE mg/g), stem (8.1 ± 0.5 RUTE mg/g), and fruit (10.3 ± 0.7 RUTE mg/g). The earlier reports on *B. tinctoria* revealed that higher amount of secondary metabolic activity of phenolics (10.2 ± 0.12%), flavonoids (1.86 ± 0.31%), tannins (0.40 ± 0.11%), alkaloids (0.28 ± 0.12%) and saponins (0.34 ± 0.20%) in leaf methanolic extract (Doss et al., 2009). The leaf, stem and fruit of methanol and ethyl acetate extracts have excellent secondary metabolic activities especially polyphenolics and alkaloids followed the trend, fruit >leaf > stem. According to Lamichhane et al. (2014), methanolic solvent used for the extraction was effective in extracting secondary metabolites especially polyphenols in which lower polyphenols are extracted efficiently in methanol. Also, the antioxidant properties of plant extracts have been attributed to their phenolic contents. The phenolics and flavonoids have much attention in day-to-day life due to their anti-mutagenic, antitumor and antioxidant activities (Lamichhane et al., 2014). Based on the secondary metabolites, *B. tinctoria* has a major alkaloid, group of benzylisoquinoline isoform of oxyberberine, jatrorrhizine, isotettrandine, berberine, berbamine and palmatine. Amidst of them, berberine is the most responsible compound for the antioxidant and antidiabetes (Andola, 2012). Therefore, the present study clearly revealed that *B. tinctoria* has rich secondary metabolites and further confirmation studies would be carried out for validation of the phytocompounds in this plant.

Table 4

Biochemical estimation of secondary metabolites in different extracts of *Berberis tinctoria* leaf stem and fruit.

Secondary metabolites	Biochemical estimation of secondary metabolites						
	Plant extracts	Petroleum ether	Chloroform	Ethyl acetate	Methanol	Hot water	F=df (14,44)
Total phenolic (GAE mg/g)	Leaf	19.5 ± 4.1^{f}	73.1 ± 2.7^{e}	689.5 ± 2.3^{a}	512.2 ± 12.2^{b}	137.2 ± 5.1^{d}	376.5**
	Stem	1.6 ± 2.1^{f}	120.1 ± 1.2^{d}	111.1 ± 7.2^{d}	128.1 ± 1.8^{d}	13.9 ± 4.3^{f}	
	Fruit	15.7 ± 5.8e^{f}	132.7 ± 1.9^{d}	386.8 ± 5.8^{b}	498.6 ± 6.9^{c}	74.9 ± 5.9^{e}	
Tannin (GAE mg/g)	Leaf	139.5 ± 2.5^{f}	169.1 ± 5.1^{e}	224.3 ± 3.1b^{c}	251.4 ± 9.6^{a}	197.5 ± 4.8^{d}	140.1**
	Stem	30.4 ± 5.7^{g}	197.7 ± 87.2^{d}	168.3 ± 8.1^{e}	223.3 ± 6.7^{bc}	140.6 ± 10.6^{f}	
	Fruit	128.6 ± 6.6^{f}	141.9 ± 5.9^{f}	210.5 ± 4.1^{ed}	236.6 ± 8.1^{b}	214.6 ± 11.8^{c}	
Total flavonoid (QE mg/g)	Leaf	88.3 ± 12.3f^{g}	176.3 ± 15.5^{d}	388.6 ± 4.7^{a}	411.6 ± 4.7^{a}	88.6 ± 6.1f^{g}	172.8**
	Stem	117.6 ± 9.8e^{f}	163.1 ± 16.5^{d}	258.3 ± 15.5^{c}	332.1 ± 6.5^{b}	35.6 ± 7.6^{h}	
	Fruit	64.3 ± 5.6^{gh}	132.6 ± 12.6^{e}	328.6 ± 11.8^{b}	389.7 ± 5.7^{a}	65.6 ± 4.8^{gh}	
Alkaloid (COLE mg/g)	Leaf	62.3 ± 1.2^{h}	102.4 ± 1.1^{g}	323.7 ± 3.5^{c}	392.5 ± 10.2^{a}	129.8 ± 2.5^{f}	838.3**
	Stem	18.1 ± 0.8^{j}	33.4 ± 1.9^{i}	117.2 ± 1.1^{f}	163.6 ± 4.8^{e}	33.9 ± 1.1^{i}	
	Fruit	24.2 ± 2.1^{ij}	27.8 ± 0.9^{ij}	211.8 ± 12.1^{d}	363.9 ± 23.1^{b}	93.4 ± 5.1^{g}	
Saponin (RUTE mg/g)	Leaf	0.7 ± 0.1^{f}	0.6 ± 0.4^{f}	7.9 ± 0.1^{b}	11.7 ± 0.8^{a}	7.4 ± 0.3^{bc}	24.7***
	Stem	0.1 ± 0.1^{f}	1.1 ± 0.1^{f}	5.9 ± 0.1^{bcd}	8.1 ± 0.5^{b}	3.7 ± 0.2^{de}	
	Fruit	0.7 ± 2.6^{ef}	0.6 ± 0.5^{f}	4.7 ± 0.3^{d}	10.3 ± 0.7^{a}	5.4 ± 0.3^{cd}	

The secondary metabolites analysis between the leaf, stem and fruit were analyzed by DMRT. Mean ± SE followed by same letter (s) are not significantly (p >0.05) different according to DMRT. *** Significant at 0.1% level, **Significant at 1% level. **GAE**- Gallic acid equivalents; **QE**- Quercetin equivalent; **COLE**- Colchicine; **RUTE**- Rutin equivalents.

3.6. Antioxidant activities

The inhibition of free radicals by different antioxidants can be measured using several stable free radicals such as DPPH, $ABTS^{\bullet+}$, phosphomolybdenum, FRAP and metal chelating activity. The DPPH radical has been extensively handled to analyze the capacity of compounds to scavenge the free radicals and to figure out the antioxidant activity of the sample (Soare et al., 1997). The effect of solvent concentration on the DPPH radical scavenging activities of *B. tinctoria* leaf, stem and fruit extracts were evaluated and exhibited in Fig. 2a. The DPPH radical scavenging activity was expressed in IC_{50} value in micrograms per mL of fresh weight. The IC_{50} values have been widely used to measure the antioxidant activity of the test samples. It is calculated as an amount of sample needed to inhibit 50% of free radicals in the site of action. Thus, lower the IC_{50} value, higher the antioxidant activity (Rivero-Cruz et al., 2020). Among the different solvent extracts, ethyl acetate extract of leaf: 34.1 ± 9.8 µg/mL and fruit: 38.2 ± 11.9 µg/mL showed higher DPPH radical scavenging activity. Ascorbic acid and BHT were used as standard and their IC_{50} values were found to be (11.2 ± 6.3 µg/mL) and (10.2 ± 7.4 µg/mL), respectively. A previous study reported the DPPH scavenging activity of *B. tinctoria* fruit with an inhibition of 96.6 ± 0.2% (Sasikumar et al., 2012). Farmani et al. (2018) also implied the DPPH scavenging activities of *B. asistita* having an IC_{50} value of 33.3 µg/mL, while the column fraction *Berberis integerrima* Bunge. extracts showed an IC_{50} value of 495.5 ± 1.8 µg/mL. Based on these results, the crude extracts of

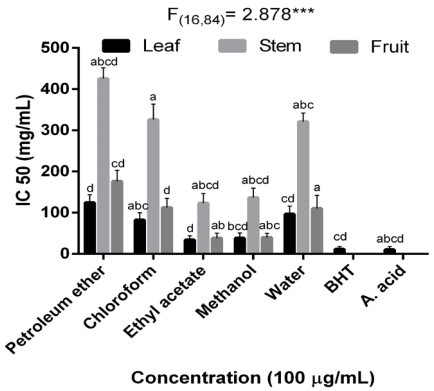

Fig. 2a. The antioxidant and anti- inflammatory analysis of the leaf, stem and fruit extracts of *B. tinctoria*. **a**-The DPPH* radical assay was to test the ability of free radical scavengers to evaluate the IC_{50} values on leaf, stem and fruit extracts of *B. tinctoria* was compared with BHT and Ascorbic acid.

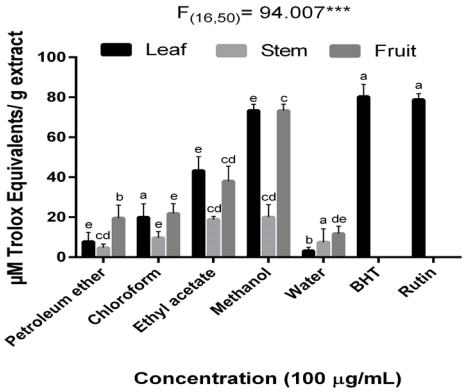

Fig. 2b. The antioxidant and anti- inflammatory analysis of the leaf, stem and fruit extracts of *B. tinctoria*. **b**- The ABTS^{+} scavenging activities on different extracts of leaf, stem and fruits was compared with BHT and rutin standard.

B. tinctoria plant showed better activity which could quench more free radicals relative to other species of *Berberis*. The enhanced activity of ethyl acetate extract may be due to the mid-polar nature and extracting ability of phenolic compounds (Brand-Williams et al., 1995). On the other hand, the DPPH radical scavenging efficiency of extracts from *B. tinctoria* might have also been partly attributed to Millard reaction products than phenolic constituents because of radical scavengers (Brand-Williams et al., 1995). Thus, antioxidants that react quickly with peroxyl radicals may react slow or may be inert to the DPPH radical. Steric accessibility is a major determinant of the reaction mechanism; hence small molecules have higher apparent antioxidant capacity due to their better access to the DPPH radical site (Prior et al., 2005). Hence, apropos to these factors, ethyl acetate and methanol have higher DPPH scavenging activity. Therefore, further antioxidant studies were carried out to estimate the antiradical ability of the plant extracts. The ABTS$^{\bullet+}$ was activated by a reaction of strong oxidizing agent e.g., potassium permanganate or potassium persulfate with the ABTS$^{\bullet+}$ salt (Miller and Rice-Evans, 1997). The ABTS$^{\bullet+}$ cation radical scavenging activities of methanol leaf extract showed higher activity (73.3 ± 3.1 µM TE/g) followed by fruit: 73.4 ± 3.2 µM TE/g and stem: 20.1 ± 6.2 µM TE/g, compared to the standards BHT and rutin (89.3 ± 4.9 µM TE/g, and 85.2 ± 5.3 µM TE/g, respectively) (Fig. 2b). Sasikumar et al. (2012) already reported that *B. tinctoria* fruit extract has scavenging activity ranged from 1.0063 to 2.364 mM TE/g. This study indicated that the extracts of *B. tinctoria* have strong hydrogen donating ability that could serve as free radical scavengers by acting as primary antioxidants. The phosphomolybdenum assay was used to determine the ability of extracts to reduce Mo(VI) to Mo(V) and successive development of green phosphate/Mo(V) complex at an acidic pH (Prieto et al, 1999). Among the different extraction used, the methanol extract of fruit showed higher activity (20.1 ± 0.9 mg AAE/g) followed by ethyl acetate extract of leaf (14.1 ± 1.6 mg AAE/g) and stem (16.7 ± 0.5 mg AAE/g).

Also, the standard BHT (24.7 ± 3.7 mg AAE/g) and ascorbic acid (29.9 ± 2.4 mg AAE/g) showed relative activity and the results were represented in Fig. 2c.

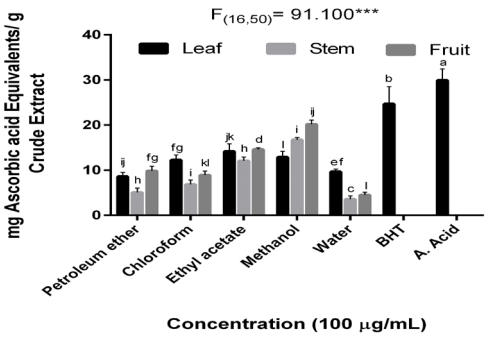

Fig. 2c. The antioxidant and anti- inflammatory analysis of the leaf, stem and fruit extracts of *B. tinctoria*. **c**- The total antioxidants activity on phosphomolybdenum method. *B. tinctoria* extracts compared with BHT and Ascorbic acid.

The reduction of Mo(VI) to Mo(V) by the fruit and leaf extracts of *B. tinctoria* specifically contains phenolics and flavonoids that are responsible for the electron or hydrogen ion transfer (Fernández-Poyatos et al., 2019). Fernández-Poyatos et al. (2019) reported 5.7 ± 0.3 mg/TE g for phosphomolybdenum activity of the *Berberis thunbergii* DC. leaf methanol extract. FRAP antioxidant potential of the prepared extracts was estimated from their ability to reduce TPTZ-Fe(III) complex to TPTZ-Fe(II) and the results of leaf, stem and fruit extracts of *B. tinctoria* were given in Fig. 2d. The methanol extract of leaf: 56.3 ± 4.9 mM Fe(II)/g extract and fruit: 55.1 ± 2.1 mM Fe(II)/g extract accounted for higher ferric reducing power than stem (39.6 ± 6.2 mM Fe(II)/g extract). The standard antioxidants BHT: 67.1 ± 4.1 mM Fe(II)/g and rutin: 77.6 ± 4.1 mM Fe(II)/g showed more antioxidant activity than the plant extracts. Farmani et al. (2018) reported that the IC_{50} value of the FRAP activity of *B. integerrima* fruit was 65.98 ± 0.66 µg/mL and 11 ± 0.16 µg/mL, before and after anthocyanin

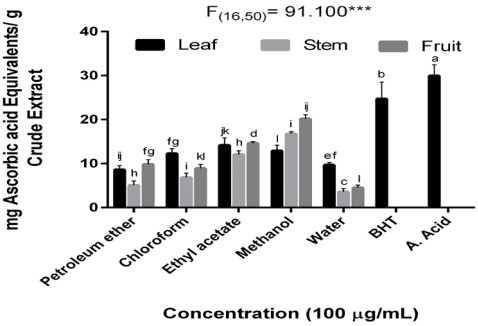

Fig. 2d. The antioxidant and anti- inflammatory analysis of the leaf, stem and fruit extracts of *B. tinctoria*. **d**- The FRAP activity of different extracts of *B. tinctoria* in terms of Trolex equivalence to compared with BHT and rutin standard.

separation on column fraction. Also, the *B. thunbergii* leaf methanol extract has an FRAP activity of 620 ± 10 mg TE/g (Fernández-Poyatos et al., 2019). Thus, the ferric reducing power of different extracts of *B. tinctoria* revealed that there are compounds in the methanolic extract which have a high affinity to the ferrous ions and thereby quench/scavenge them through redox reactions. In lipid peroxidation, the catalyzing transition forms α-bonds with metal and the redox potential forms metal ions by stabilization (Duh et al., 1999). The higher metal chelating activity was shown by the ethyl acetate extract of leaf (125.3 ± 4.6 mg EDTA E/g), fruit (115.5 ± 5.2 mg EDTA E/g) and stem (80.3 ± 3.2 mg EDTA E/g of *B. tinctoria*) also standard EDTA showed 135.5 ± 4.7 mg/g. The results were presented in Fig. 2e.

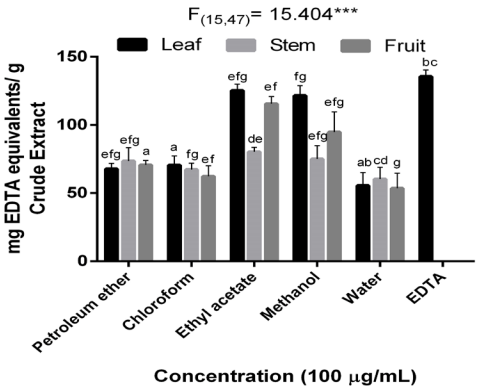

Fig. 2e. The antioxidant and anti-inflammatory analysis of the leaf, stem and fruit extracts of *B. tinctoria*. . **e**- The metal chelating activity of leaf, stem and fruit extracts of *B. tinctoria* was compared with EDTA equivalence. **BHT**- Butylated hydroxyl toluene: **A. acid**- Ascorbic acid; **EDTA**- Ethylene diamine tetra acetic acid.

Sasikumar et al. (2012) also reported that the *B. tinctoria* fruit has a metal chelating effect of 96.1 ± 1.6 mg EDTA E/mL, which confirmed that our study also has an equal chelating effect. Fernández-Poyatos et al. (2019) reported that the metal chelating activity of *B. thunbergii* was found to be 4.54 ± 0.01 mg EDTA E/g in leaf methanol extract. These results indicated the development of insoluble metal complexes with ferrous ions and the prevention of metal-lipid interaction by antioxidants. Hence, the metal chelating assay of *B. tinctoria* revealed that water extract of all the parts has shown effective activity, by antioxidant related to its sequestering of Fe^{2+} ions that may otherwise catalyze fenton type reactions or participate in metal-catalyzed hydroperoxide decomposition reactions. *B. tinctoria* leaf, stem and fruit showed higher antioxidant activities in methanol and ethyl acetate extracts. From the results, we observed that the *B. tinctoria* plant has excellent antioxidant activities which would be used against chronic diseases.

3.7. Correlation analysis between antioxidants activities and secondary metabolites

The antioxidant activity and secondary metabolites correlation is a challenging task because plant extracts have complex mixtures of various metabolic actions with antioxidant and pro-oxidant properties that show synergistic effects. A strong correlation between antioxidant activity and phenolic content has been documented in several studies, indicating the significance of polyphenols as a potent antioxidant component that can be noted in numerous plant species (Rana et al., 2019). The evaluation of antioxidant activities of plant extracts is of greater importance to provide novel and safe approaches for developing functional products with more nutritional and antioxidant ability (Rana et al., 2019). In this study, the secondary metabolites of the plant extract were found to possess higher antioxidant activity and the significant positive correlation analyses were demonstrated between secondary metabolites and antioxidant activities as being shown in Table 5. Pearson's correlation studies also revealed a positive correlation between antioxidant activity and phenolic content (Wang and Lin, 2000; Podsedek et al., 2014). It is pointed out that the secondary metabolic compounds are the chief source of plant antioxidants which can prevent reactive free radicals and have progression against cancers, cardiovascular diseases, neurodegeneration, inflammatory mediated diseases, and chronic diseases, including diabetes (Podsedek et al., 2014).

3.8. Principal component analysis of secondary metabolites and antioxidant activities

Principal component analysis (PCA) was performed for the given dataset including secondary metabolites and antioxidant activities. A correlation study was conducted between secondary metabolites and antioxidant activities among different parts viz., leaf, stem and fruit extracts of *B. tinctoria* using 30 variable components of phenolics, tannins, flavonoids, alkaloids, saponins,DPPH assay, $ABTS^{\bullet+}$ assay, phosphomolybdenum assay, FRAP assay and metal chelating activity. PCA technique is an efficient technique used for the

Table 5
Pearson's correlation coefficient of secondary metabolites content with their antioxidant activities of leaf, stem and fruit extracts of *Berberis tinctoria*.

Variables	Ple	Pst	Pfr	Tle	Tst	Tfr	Fle	Fst	Ffr	Ale	Ast	Afr	Sle	Sst	Sfr	DPPHle	DPPHst	DPPHfr
Pst	0.621	1																
Pfr	0.978	0.752	1															
Tle	0.844	0.616	0.837	1														
Tst	0.528	0.866	0.630	0.753	1													
Tfr	0.716	0.338	0.655	0.916	0.594	1												
Fle	0.927	0.812	0.967	0.840	0.666	0.624	1											
Fst	0.766	0.804	0.836	0.678	0.580	0.410	0.934	1										
Ffr	0.904	0.781	0.943	0.857	0.655	0.651	0.989	0.938	1									
Ale	0.927	0.681	0.930	0.939	0.655	0.803	0.957	0.856	0.968	1								
Ast	0.887	0.699	0.908	0.914	0.652	0.754	0.957	0.892	0.973	0.991	1							
Afr	0.821	0.578	0.818	0.931	0.609	0.825	0.879	0.809	0.917	0.969	0.975	1						
Sle	0.747	0.323	0.680	0.925	0.516	0.962	0.685	0.511	0.723	0.859	0.823	0.904	1					
Sst	0.749	0.490	0.730	0.842	0.578	0.853	0.742	0.596	0.765	0.833	0.815	0.831	0.822	1				
Sfr	0.628	0.358	0.587	0.878	0.543	0.897	0.648	0.514	0.695	0.817	0.810	0.895	0.943	0.795	1			
DPPHle	-0.929	-0.838	-0.967	-0.898	-0.797	-0.723	-0.955	-0.822	-0.935	-0.934	-0.911	-0.836	-0.714	-0.772	-0.653	1		
DPPHst	-0.960	-0.755	-0.972	-0.931	-0.733	-0.782	-0.955	-0.807	-0.943	-0.965	-0.935	-0.882	-0.794	-0.801	-0.715	0.988	1	
DPPHfr	-0.936	-0.752	-0.949	-0.942	-0.778	-0.810	-0.921	-0.754	-0.907	-0.942	-0.907	-0.860	-0.803	-0.788	-0.716	0.982	0.992	1
ABTSle	0.779	0.772	0.832	0.816	0.671	0.606	0.933	0.949	0.946	0.925	0.957	0.919	0.691	0.721	0.707	-0.853	-0.854	-0.823
ABTSst	0.809	0.685	0.830	0.813	0.623	0.566	0.854	0.778	0.833	0.836	0.824	0.780	0.643	0.570	0.583	-0.839	-0.852	-0.829
ABTSfr	0.681	0.657	0.737	0.763	0.572	0.562	0.857	0.908	0.902	0.874	0.921	0.917	0.685	0.681	0.717	-0.744	-0.760	-0.718
PHOSle	0.803	0.896	0.877	0.703	0.771	0.469	0.854	0.743	0.818	0.751	0.732	0.604	0.435	0.549	0.400	-0.915	-0.862	-0.859
PHOSst	0.814	0.766	0.869	0.811	0.628	0.581	0.954	0.950	0.970	0.934	0.963	0.919	0.686	0.690	0.705	-0.864	-0.869	-0.827
PHOSfr	0.732	0.674	0.780	0.672	0.453	0.440	0.898	0.963	0.922	0.858	0.901	0.855	0.580	0.634	0.611	-0.743	-0.755	-0.690
FRAPle	0.724	0.688	0.776	0.754	0.566	0.567	0.888	0.934	0.920	0.895	0.941	0.915	0.669	0.690	0.722	-0.777	-0.787	-0.741
FRAPst	0.758	0.634	0.797	0.832	0.589	0.651	0.877	0.874	0.901	0.914	0.956	0.935	0.737	0.743	0.773	-0.798	-0.816	-0.778
FRAPfr	0.727	0.863	0.822	0.645	0.624	0.347	0.922	0.980	0.914	0.817	0.861	0.760	0.437	0.556	0.477	-0.818	-0.782	-0.732
METAle	0.895	0.676	0.911	0.739	0.488	0.515	0.929	0.884	0.911	0.885	0.882	0.804	0.627	0.518	0.554	-0.849	-0.870	-0.835
METAst	0.557	0.366	0.559	0.232	0.029	0.059	0.603	0.627	0.582	0.475	0.487	0.381	0.190	0.341	0.158	-0.432	-0.444	-0.371
METAfr	0.841	0.509	0.837	0.556	0.268	0.375	0.795	0.747	0.781	0.742	0.730	0.633	0.463	0.372	0.358	-0.721	-0.750	-0.708

Table 5 (continued)

Variables	ABTSle	ABTSst	ABTSfr	PHOSle	PHOSst	PHOSfr	FRAPle	FRAPst	FRAPfr	METALle	METALst
Pst											
Pfr											
Tle											
Tst											
Tfr											
Fle											
Fst											
Ffr											
Ale											
Ast											
Afr											
Sle											
Sst											
Sfr											
DPPHle											
DPPHst											
DPPHfr											
ABTSle	1										
ABTSst	0.790	1									
ABTSfr	0.950	0.729	1								
PHOSle	0.713	0.717	0.558	1							
PHOSst	0.970	0.834	0.957	0.728	1						
PHOSfr	0.946	0.733	0.945	0.621	0.958	1					
FRAPle	0.968	0.704	0.964	0.619	0.962	0.959	1				
FRAPst	0.936	0.772	0.945	0.599	0.942	0.899	0.950	1			
FRAPfr	0.929	0.769	0.882	0.780	0.944	0.937	0.907	0.857	1		
METAle	0.858	0.833	0.785	0.760	0.898	0.854	0.823	0.803	0.853	1	
METAst	0.492	0.331	0.488	0.429	0.536	0.652	0.489	0.450	0.612	0.609	1
METAfr	0.674	0.626	0.594	0.699	0.723	0.707	0.683	0.629	0.701	0.904	0.587

Ple- Phenolic leaf, **Tle**- Tannin leaf, **Fle**- Flavonoid leaf, **Ale**- Alkaloid leaf, **Sle**- Saponin leaf, **DPPHle**- DPPH leaf, **ABTSle**- ABTS leaf, **PHOSle**- Phosphomolybdenum leaf, **FRAPle**- FRAP leaf and **METAle**- Metal chelating leaf. **Pst**- Phenolic stem, **Tst**- Tannin stem, **Fst**- Flavonoid stem, **Ast**- Alkaloid stem, **Sst**- Saponin stem, **DPPHst**- DPPH stem, **ABTSst**- ABTS stem, **PHOSst**- Phosphomolybdenum stem, **FRAPst**- FRAP stem and **METAst**- Metal chelating stem. **Pfr**- Phenolic fruit, **Tfr**- Tannin fruit, **Ffr**- Flavonoid fruit, **Afr**- Alkaloid fruit, **Sfr**- Saponin fruit, **DPPHfr**- DPPH fruit, **ABTSfr**- ABTS fruit, **PHOSfr**- Phosphomolybdenum fruit, **FRAPfr**- FRAP fruit and **METAfr**- Metal chelating fruit. *** Significant at p< 0.001, **Significant at 1% level, respectively.

reduction of dimensionality of the dataset and to locate the most influencing variables in the study. This technique also identified uncorrelated variables that maximize the variance in the dataset. The auto-scaled data eigenvectors in PCA (van den Berg et al., 2006) are proportional to the correlation coefficients between the PC scores and the variables shown in Fig. 3 and Fig. 4. In the multivariate analysis, this fact is well-known but does not seem to be understood in metabolomics (Afifi et al., 2011). In the present study, the correlation coefficients between the PC scores and the variables are known as 'factor loading'. This concept can be used to conduct statistical hypothesis testing and to objectively use statistical criteria to pick relevant metabolites (Weston and Gore, 2006). In supervised learning approaches, significant metabolites are selected according to the significance of factor loading or other variable selection methods, such as support vector machine, random forest and then biological inferences are made by biologists for these metabolites. With respect to a biological functional unit, such as a metabolic pathway, biologists also draw these inferences (Yamamoto et al., 2014). However, there was no efficient tool for a single framework that can perform

our workflow, including the statistical hypothesis testing of factor loading in PCA (Yamamoto et al., 2014). In the present study, we performed statistical hypothesis testing of the factor loading in PCA for two metabolomics datasets from secondary metabolites and antioxidants activity. The PCA resulted in finding a high positive correlation between all variables by reduction of dimensionality in dataset with minimum variance that showed ethyl acetate and methanolic extracts as pivotal extracts of *B. tinctoria*. All the experiments exhibited that, the antioxidant effect of *B. tinctoria* extracts followed the same arrangement that higher the metabolites, higher the free radical quenching which was also confirmed by correlation analysis and PCA.

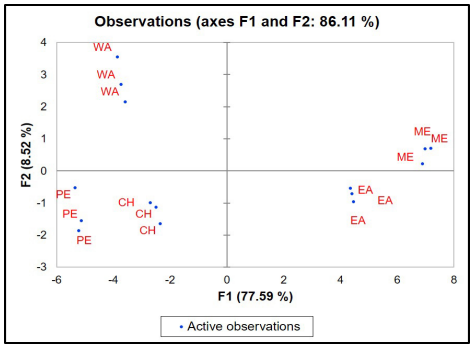

Fig. 3. PCA active observations of secondary metabolites content with their antioxidant activities of leaf, stem and fruit extracts of *B. tinctoria*.

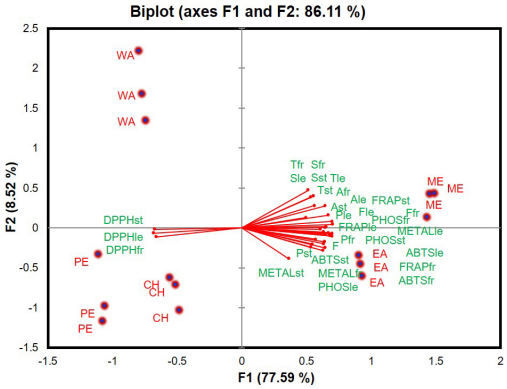

Fig. 4. PCA of secondary metabolites content with their antioxidant activities of leaf, stem and fruit extracts of *B. tinctoria*.

3.9. Anti-inflammatory activity

In the protein denaturation process, secondary and tertiary structures of the protein are drifted by an external stress which leads to loss of biological function such that catalytic ability, solubility and structure of the enzyme are due to the aggregation and exposure of hydrophobic groups. Denaturation of proteins is a fundamental element of inflammation (Matarredona et al., 2020). Hence, the anti-inflammatory activity of *B. tinctoria* leaf, stem and fruit extracts were effective by inhibiting heat-induced bovine serum albumin denaturation where the ethyl acetate fruit extract showed maximum inhibition of 95.2% at 500 µg/mL. It is also noteworthy that the methanol fruit extract exhibited 93.9%, respectively. Aspirin, a standard anti-inflammation drug showed the maximum inhibition of 95.6% at the concentration of 100 µg/mL responded as control (Fig. 2f). Alamgeer et al. (2015) reported that the *B. calliobotrys* crude methanol extract (800 µg/mL) inhibited 89.8% against bovine serum albumin (BSA) denaturation which showed maximum anti-inflammatory activity against the standard drug. In the current investigation, ethyl acetate and methanol fruit extract of *B. tinctoria* showed maximum inhibition of protein denaturation mainly due to the presence of high quantities of phenolics, alkaloids, amino acids, and flavonoids. Thus, both ethyl acetate and methanol fruit extracts have nearly equal inhibition to the standard aspirin for the effective anti-inflammatory activity than the synthesized drugs.

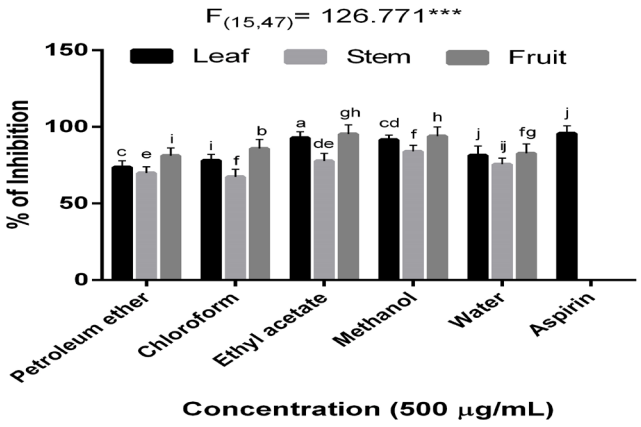

Fig. 2f. The antioxidant and anti- inflammatory analysis of the leaf, stem and fruit extracts of *B. tinctoria*. **f**- The Anti-inflammatory activity by **BSA** protein denaturation method of leaf, stem and fruit extracts of *B. tinctoria* was compared with Aspirin standard. **BSA**- Bovine Serum Albumin. Data are presented as Mean ± SE followed by same letter (s) are not significantly ($p > 0.05$) different according to DMRT. *** Significant at 0.1% level.

3.10. *In vitro* antidiabetic activity

The leaf and fruit of *B. tinctoria* plant have been widely used by the tribes in and around the Nilgiri hill region for diabetes mellitus (Latha et al., 2015). The previous studies revealed the free radical scavenging activity of plant extracts with remarkable antidiabetic potentiality (Gudise et al., 2019). In this relation, an attempt was made to find the *in vitro* antidiabetic activity of different extracts of *B. tinctoria* leaf, stem and fruit using α-amylase and α-glucosidase enzyme inhibition methods. The enzyme activities are responsible for postprandial hyperglycemia (break down of dietary carbohydrates to glucose), and the selected plant extracts were tested against these enzymes which may reduce the postprandial hyperglycemic condition considered to be a major risk factor for cardiovascular disorders (CVD) (Cavarape et al., 2001). The hydrolysis of α-1,4-glycosidic linkage of starch, glycogen, and different oligosaccharide was catalyzed by α-amylase which separates the disaccharides into straight simple sugars, promptly accessible for intestinal assimilation (Hara and Honda, 1990). The inhibition of α-amylase and α-glucosidase in the digestive tract would be an active tool to control diabetes (Hara and Honda, 1990) and the inhibition assay was expressed in IC_{50} value micrograms per mL of fresh weight. The IC_{50} values have been widely used to measure the antidiabetic activity of test samples. It is calculated as an amount of sample needed to inhibit the α-amylase and α-glucosidase enzyme concentration by 50% (Kazeem et al., 2013), thus, lower the IC_{50} value, higher the antidiabetic activity. The *B. tinctoria* methanolic extract exhibited significant antidiabetic activity on both α-amylase and α-glucosidase enzyme inhibitors (Table 6). Whereas, higher antidiabetic activity was observed in

Table 6
In vitro antidiabetic activities of different extracts of *B. tinctoria* leaf, stem and fruit.

***In vitro* Antidiobetic inhibitors assay**	***In vitro* antidiabetic activities of different extracts of *Berberis tinctoria***							
	Plant Extracts	**Petroleum Ether**	**Chloroform**	**Ethyl acetate**	**Methanol**	**Hot water**	**Acarbose**	**F=df (14,44)**
α - Amylase inhibition assay	Leaf IC_{50} (µg/mL)	184.2 ± 11.8^{abc}	176.3 ± 17.2^{abcd}	120.1 ± 7.7^{efg}	86.2 ± 9.5^{g}	157.5 ± 15.8^{abc}	38.6 ± 3.7^{b}	8.4***
	Stem IC_{50} (µg/mL)	278.5 ± 27.8^{ab}	274.7 ± 23.9^{ab}	124.1 ± 15.8^{abc}	101.2 ± 10.7^{abcd}	277.5 ± 25.9^{a}		
	Fruit IC_{50} (µg/mL)	109.6 ± 11.8^{bcd}	197.5 ± 13.8^{cde}	92.5 ± 11.9^{def}	75.5 ± 6.8^{fg}	206.6 ± 12.8^{abc}		
α -Glucosidase inhibitors assay	Leaf IC_{50} (µg/mL)	125.2 ± 12.3^{abc}	125.4 ± 11.2^{a}	73.2 ± 11.9^{abcd}	58.8 ± 13.2^{cd}	146.5 ± 14.9^{a}	32.8 ± 0.3^{b}	8.3***
	Stem IC_{50} (µg/mL)	134.7 ± 7.1^{a}	188.3 ± 14.4^{a}	159.3 ± 12.4^{cd}	128.6 ± 9.9^{bcd}	150.9 ± 16.7^{a}		
	Fruit IC_{50} (µg/mL)	198.3 ± 11.2^{ab}	144.5 ± 5.3^{ab}	76.5 ± 9.4^{d}	45.4 ± 11.1^{e}	182.3 ± 7.8^{a}		

The *in vitro* Antidiabetic activities analysis between the leaf, stem and fruit were analyzed by DMRT. Mean ± SE followed by the same letter (s) are not significantly ($p > 0.05$) different according to DMRT. *** Significant at 0.1% level. **IC_{50}**- The half-maximal inhibitory concentration.

the hierarchy of methanolic fruit extract (75.5 ± 6.8 µg/mL and 45.4 ± 11.1 µg/mL), leaf (86.2 ± 9.5 µg/mL and 58.8 ± 13.2 µg/mL) and stem (101.2 ± 10.7 µg/mL and 128.6 ± 9.9 µg/mL). Acarbose a monetarily known antidiabetic inhibitor showed strong inhibitory activity of 38.6 ± 3.7 µg/mL (α-amylase) and 32.8 ± 0.3 µg/mL (α-glucosidase), where the plant extracts showed the comparatively equal potential to that of positive control. The antidiabetic activity of *B. tinctoria* methanol leaf extract of 150 mg/kg and 300 mg/kg body weight in a streptozotocin-induced diabetes rat model showed a serious reduction of blood glucose levels (Murugesh et al., 2006). The α-glucosidase inhibitory activity of *Berberis amurensis* Rupr. methanol extract of leaf and stem showed lower activity (IC_{50}s of 90.5 ± 0.6 and 147.1 ± 10.1 µg/mL) (Hyun et al., 2015). Upwar et al. (2011) reported that *B. tinctoria* shows a powerful antidiabetic activity. Preceded findings on *B. vulgaris* extracts reported excellent inhibition of α-amylase and α-glucosidase activity and also confirmed that the compound berberine plays a vital role in the antidiabetic activity (Boudjelthia et al., 2017; Dulić et al., 2019). The compound berberine present in *B. tinctoria* could block K^+ channels on colonic cell lines and

also inhibit brain Na^+, K^+, ATPase and various cations dependent phosphohydrolase (Amritpal et al., 2010). The compound triggers the adenosine monophosphate which activates protein kinase for the development of insulin and the mechanism of berberine may also be associated with promoting renewal and functional rescue of β cells (Amritpal et al., 2010). Therefore, the methanol extract showed the greatest activity responsible for the inhibition of α-amylase and α-glucosidase synergistically relative to the positive control acarbose. Hence, the results substantiate that the traditional medicinal plant *B. tinctoria* could be used as an antidiabetic remedy and further investigations are still required to authenticate the antidiabetic activity of the plant through compound isolation and acute toxicity studies in *in vivo* models.

4. Concluding remarks

The results obtained from *B. tinctoria* exhibited that it possesses immense phytochemicals with high nutritional, antioxidant, anti-inflammatory and antidiabetic activities. The primary and secondary metabolites particularly polyphenols, alkaloids and flavanoids are liable as potential nutritional and antioxidant agents. The natural antioxidants provide supplements and are performed as health hoarders which resist many chronic diseases. As a result, we should seek out the natural antioxidants in plants like *B. tinctoria* to form a healthy diet. The exploration of phytochemical screening for compounds with valuable medicinal properties will provide a reliable and profitable source of medicine to the humanity. To the best of our knowledge, the present research is the first scientific report regarding the comparison of secondary metabolites and antioxidant activities of *B. tinctoria*. Therefore, further study in phytocompounds isolated from *B. tinctoria* and other research on isolated compounds are still necessary to reveal more biological effects of this herbal species for the treatment of various chronic disorders.

Acknowledgments

The present study was supported by Tamil Nadu State Council Science and Technology, Chennai, Tamil Nadu. India (TNSCST/&T Project/AS/RJ/2013-2014) and I would like to thanks the Dr. V. S. Ramachandran (Rtd), Professor, Department of Botany, Dr. C. Samydurai, Dr. P. Rajasekar, and Mr. I. Kanivalan, Department of Botany, Bharathiar University, Coimbatore, Tamil Nadu, India for the plant collection and identification.

Conflict of Interest

The authors declare that there is no conflict of interest.

Reference

Afifi, T.O., Mather, A.,Boman, J., Fleisher, W., Enns, M.W., MacMillan, H., Sareen, J., 2011. Childhood adversity and personality disorders: results from a nationally representative population-based study. J. Psychiatr. Res. 45(6), 814-822.

Akpanyung, E., 2005. Proximate and mineral element composition of bouillon cubes produced in Nigeria. Pak. J. Nutr. 4(5), 327-329.

Alamgeer, Hasan, U.H., Uttra, A.M., Rasool, S., 2015. Evaluation of *in vitro* and *in vivo* anti-arthritic potential of *Berberis calliobotrys*. Bangladesh J. Pharmacol. 10(4), 807-819.

Alén-Ruiz, F., Pérez-Gregorio, M. R., Martínez-Carballo, E., García-Falcón, M. S., Simal-Gándara, J., 2008. Influence of polyphenols on color and antioxidant value in plant foods. Electron. J. Environ. Agric. Food Chem. 7, 3171-3176.

Ali, A., Deokule, S., 2009. Studies on nutritional values of some wild edible plants from Iran and India. Pak. J. Nutr. 8(1), 26-31.

Ali, H., Houghton, P., Soumyanath, A., 2006. α-Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to *Phyllanthus amarus*. J. Ethnopharmacol. 107(3), 449-455.

Amritpal, S., Sanjiv, D., Navpreet, K., Jaswinder, S., 2010. Berberine: alkaloid with wide spectrum of pharmacological activities. Indian J. Nat. Prod. Resour. 3, 64-75.

Andola, H.C., 2012. Genus *Berberis* and berberine HPLC: an overview. JBAPN. 2(5), 256-264.

AOAC., 2000. Official Methods of Analysis, 17th Ed. The Association of Official Analytical Chemists, Gaithersburg, MD, USA. Methods 925.10, 65.17, 974.24, 992.16.

Arango Duque, G., Descoteaux, A., 2014. Macrophage cytokines: involvement in immunity and infectious diseases. Front. Immunol. 5(491), 1-12.

Awan, M.S., Ali, S., Ali, A., Hussain, A., Ali, M., 2014. A comparative study of barberry fruits in terms of its nutritive and medicinal contents from CKNP region, Gilgit-Baltistan, Pakistan. J. Bio. Env. Sci. 5(2), 9-17.

Belwal, T., Dhyani, P., Bhatt, I.D., Rawal, R.S., Pande, V., 2016. Optimization extraction conditions for improving phenolic content and antioxidant activity in *Berberis asiatica* fruits using response surface methodology (RSM). Food Chem. 207, 115-124.

Benderitter, M., Maupoil, V., Vergely, C., Dalloz, F., Briot, F., Rochette, L., 1998. Studies by electron paramagnetic resonance of the importance of iron in the hydroxyl scavenging properties of ascorbic acid in plasma: effects of iron chelators. Fundam. Clin. Pharmacol. 12(5), 510-516.

Bhat, R., Paliyath, G., 2016. Fruits of tropical climates: Biodiversity and Dietary Importance, in: B, Caballero., P, Finglas., F, Tol-drá. (Eds.), Encyclopedia of Food and Health, Oxford: Academic Press (Elsevier), UK., pp. 138-144.

Bober, Z., Stępień, A., Aebisher, D., Ożóg, Ł., Bartusik-Aebisher, D., 2018. Fundamentals of the use of *Berberis* as a medicinal plant. Eur. J. Clin. Exp. Med. 1, 41-46.

Boudjelthia, K., Hammadi, K., Kouidri, M., Djebli, N., 2017. Evaluation of antidiabetic activity of two plants *Berberis vulgaris* and *Zygophyllum geslini*. J. Phys. Chem. Biophys. 7(1), 1-7.

Braca, A., De Tommasi, N., Di Bari, L., Pizza, C., Politi, M., Morelli, I., 2001. Antioxidant principles from B*auhinia tarapotensis*. J. Nat. Prod. 64(7), 892-895.
Brand-Williams, W., Cuvelier, M.E., Berset, C.L.W.T., 1995. Use of a free radical method to evaluate antioxidant activity. LWT- Food Sci. Technol. 28(1), 25-30.
Cavarape, A., Feletto, F., Mercuri, F., Quagliaro, L., Damante, G., Ceriello, A., 2001. High-fructose diet decreases catalase mRNA levels in rat tissues. J. Endocrinol. Invest. 24(11), 838-845.
Cencic, A., Chingwaru, W., 2010. The role of functional foods, nutraceuticals, and food supplements in intestinal health. Nutrients 2(6), 611-625.
Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., Li, Y., Wang, X., Zhao, L., 2018. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget. 9(6), 7204-7218.
Dinis, T.C., Madeira, V.M., Almeida, L.M., 1994. Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys. 315(1), 161-169.
Doss, A., 2009. Preliminary phytochemical screening of some Indian medicinal plants. Anc. Sci. Life. 29(2), 12-16.
Duh, P.D., Tu, Y.Y., Yen, G.C., 1999. Antioxidant activity of water extract of Harng Jyur (*Chrysanthemum morifolium Ramat*). LWT - Food Sci. Technol. 32(5), 269-277.
Dulić, M., Ciganović, P., Vujić, L., Zovko Končić, M., 2019. Antidiabetic and cosmeceutical potential of common Barbery (*Berberis vulgaris* L.) root bark extracts obtained by optimization of 'Green' ultrasound-assisted extraction. Molecules 24(19),1-17.
Farmani, F., Moein, M., Khoshnood, M.J., Sabahi, Z., 2018. Anthocyanin isolation from *Berberis integerrima* Bunge. fruits and determination of their antioxidant activity. Free Radic. Antioxid. 8(1), 1-5.
Fernández-Poyatos, M.D.P., Ruiz-Medina, A., Zengin, G., Llorent-Martínez, E.J., 2019. Phenolic characterization, antioxidant activity, and enzyme inhibitory properties of *Berberis thunbergii* DC. leaves: A valuable source of phenolic acids. Molecules 24(22), 4171.
Francis, G., Kerem, Z., Makkar, H.P., Becker, K., 2002. The biological action of saponins in animal systems: a review. Br. J. Nutr. 88(6), 587-605.
Fyson, P.F., 1974. The Flora of the Nilgiris and Pulney Hill Tops, in: Bishen Singh and Mahendra Pal Singh, vol. 1, 1st Ed, Dehradun, India.
Gudise, V., Chowdhury, B., Manjappa, A.S., 2019. *In vitro* free radical scavenging and antidiabetic activity of aqueous and ethanolic leaf extracts: a comparative evaluation of *Argyreia pierreana* and *Matelea denticulata*. Futur J. Pharm. Sci. 5(13), 1-11.
Gurib-Fakim, A., 2006. Medicinal plants: traditions of yesterday and drugs of tomorrow. Mol. Asp. Med. 27(1), 1-93.
Hagerman, A.E., Riedl, K.M., Jones, G.A., Sovik, K.N., Ritchard, N. T., Hartzfeld, P. W., Riechel, T. L., 1998. High molecular weight plant polyphenolics (tannins) as biological antioxidants. J. Agric. Food Chem. 46(5), 1887-1892.
Hanachi, P., Golkho, S., 2009. Using HPLC to determination the composition and antioxidant activity of *Berberis vulgaris*. Eur. J. Res. 29(1), 47-54.
Hara, Y., Honda, M., 1990. The inhibition of α-amylase by tea polyphenols. Agric. Biol. Chem. 54(8), 1939-1945.
Hinz, H., Zenk, M., 1981. Production of protoberberine alkaloids by cell suspension cultures of *Berberis* species. Naturwissenschaften 68(12), 620-621.
Hodge, J., 1962. Determination of Reducing Sugars And Carbohydrates. Methods in Carbohydrate Chemistry. Vol. 1, 380-394.
Hyun, T.K., Kim, H.C., Kim, J.S., 2015. *In vitro* screening for antioxidant, antimicrobial, and antidiabetic properties of some Korean native plants on Mt. Halla, Jeju Island. Indian J. Pharm. Sci. 77(6), 668-674.
Jayasri, M., Lazar, M., Radha, A., 2009. A report on the antioxidant activity of leaves and rhizomes of *Costus pictus* D. Don. Int. J. Integr. Biol. 5(1), 20-26.
Kaur, S., Syed Ali, M., Anuradha, V., Suganya, V., Ashashalini, A., Bhuvana, P., 2018. *In vitro* anti-inflammatory activity of mangrove plant *Rhizophora mucronata* Lam. (Malpighiales: Rhizophoraceae). Brazilian J. Biol. Sci. 5(10), 417-426.
Kazeem, M.I., Adamson, J.O., Ogunwande, I.A., 2013. Modes of inhibition of α-amylase and α-glucosidase by aqueous extract of *Morinda lucida* Benth. leaf. BioMed Res. Int. 2013, 1-6.
Kim, G.N., Shin, J.G., Jang, H.D., 2009. Antioxidant and antidiabetic activity of Dangyuja (*Citrus grandis Osbeck*) extract treated with *Aspergillus saitoi*. Food Chem. 117(1), 35-41.
Kirtikar, K., Basu, B., 1995. Indian Medicinal Plants. International Book Distributors Vol. 1, Dehradun, India. pp. 371-372.
Lamichhane, B., Adhikari, S., Shrestha, P., Shrestha, B.G., 2014. Study of phytochemical, antioxidant, antimicrobial and anticancer activity of *Berberis aristata*. J. Trop. Life Sci. 4(1), 01-07.
Latha, R., Sagaya Jansi, R., Agastian, P., 2015. Physicochemical and phytochemical investigation on medicinal plants used by ethnic tribes of Nilgiri Mountains, South India. World J. Pharm. Sci. 3(3), 632-644.
Li, Z., Geng, Y.N., Jiang, J.D., Kong, W.J., 2014. Antioxidant and anti-inflammatory activities of berberine in the treatment of diabetes mellitus. J. Evid. Based Complementary Altern. Med. 2014, 1-12.
Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem.193, 265-275.
Makkar, H., Francis, G., Becker, K., 2007. Bioactivity of phytochemicals in some lesser-known plants and their effects and potential applications in livestock and aquaculture production systems. Animal 1(9), 1371-1391.
Makkar, H.P., 2003. Quantification of Tannins in Tree and Shrub Foliage: A Laboratory Manual. Springer, Dordrecht, Netherlands.
Marchetti, P., Del Prato, S., Lupi, R., Del Guerra, S., 2006. The pancreatic beta-cell in human Type 2 diabetes. Nutr. Metab. Cardiovasc. Dis. 16, S3-S6.
Markert, B., 1994. Plants as Biomonitors- Potential

Advantages and Problems, in: D.C. Adriano, Z.S. Chen and S.S. Yang (Ed), Biogeochemistry of Trace Elements. Science and Technology Letters. Northwood, New York, pp. 601-613.

Matarredona, L., Camacho, M., Zafrilla, B., Bonete, M. J., Esclapez, J., 2020. The role of stress proteins in haloarchaea and their adaptive response to environmental shifts. Biomolecules 10(10), 1390.

Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31(3), 426-428.

Miller, N.J., Rice-Evans, C.A., 1997. Factors influencing the antioxidant activity determined by the $ABTS^{\bullet+}$ radical cation assay. Free Radic. Res. 26(3), 195-199.

Moore, S., Stein, W.H., 1948. Photometric nin-hydrin method for use in the ehromatography of amino acids. J. Biol. Chem. 176, 367-388.

Motalleb, G., Hanachi, P., Kua, S., Fauziah, O., Asmah, R., 2005. Evaluation of phenolic content and total antioxidant activity in *Berberis vulgaris* fruit extract. J. Biol. Sci. 5(5), 648-653.

Murugesh, K., Yeligar, V.C., Dash, D.K., Maiti, B., Maity, T.K., 2006. Effect of B*erberis tinctoria* leaf (Berberidaceae) extract on antidiabetic, antihyperlipidemic and antioxidant status in streptozotocin induced diabetes in rats. Orient. Pharm. Exp. Med. 6(4), 336-343.

Nithiyanantham, S., Selvakumar, S., Siddhuraju, P., 2012. Total phenolic content and antioxidant activity of two different solvent extracts from raw and processed legumes, *Cicer arietinum* L. and *Pisum sativum* L. J. Food Compos. Anal. 27(1), 52-60.

Ogurtsova, K., da Rocha Fernandes, J., Huang, Y., Linnenkamp, U., Guariguata, L., Cho, N.H., Cavan, D., Shaw, J., Makaroff, L., 2017. IDF diabetes atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. Diabetes Res. Clin. Pract. 128, 40-50.

Paredes-López, O., Cervantes-Ceja, M.L., Vigna-Pérez, M., Hernández-Pérez, T., 2010. Berries: improving human health and healthy aging, and promoting quality life- a review. Plant Foods Hum. Nutr. 65(3), 299-308.

Pham-Huy, L.A., He, H., Pham-Huy, C., 2008. Free radicals, antioxidants in disease and health. Int. J. Biomed. Sci. 4(2), 89-96.

Podsedek, A., Majewska, I., Redzynia, M., Sosnowska, D., Koziołkiewicz, M., 2014. *In vitro* inhibitory effect on digestive enzymes and antioxidant potential of commonly consumed fruits. J. Agric. Food Chem. 62(20), 4610-4617.

Prieto, P., Pineda, M., Aguilar, M., 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal. Biochem. 269(2), 337-341.

Prior, R.L., Wu, X., Schaich, K., 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J. Agric. Food Chem. 53(8), 3101-3113.

Pulido, R., Bravo, L., Saura-Calixto, F., 2000. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. J. Agric. Food Chem. 48(8), 3396-3402.

Raffauf, R.F., 1996. Plant Alkaloids: A Guide to Their Discovery and Distribution. Food Products Press, Northeastern University, Boston, Massachusetts, USA.

Rana, Z.H., Alam, M.K., Akhtaruzzaman, M., 2019. Nutritional composition, total phenolic content, antioxidant and α-amylase inhibitory activities of different fractions of selected wild edible plants. Antioxidants 8(7), 203.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26(9-10), 1231-1237.

Ricciotti, E., FitzGerald, G.A., 2011. Prostaglandins and inflammation. Arterioscler. Thromb. Vasc. Biol. 31(5), 986-1000.

Rivero-Cruz, J.F., Granados-Pineda, J., Pedraza-Chaverri, J., Pérez-Rojas, J.M., Kumar-Passari, A., Diaz-Ruiz, G., Rivero-Cruz, B.E., 2020. Phytochemical constituents, antioxidant, cytotoxic, and antimicrobial activities of the ethanolic extract of Mexican brown propolis. Antioxidants 9(1), 70.

Sadasivam, S., Manickam, A., 2008. Biochemical Methods, 3rd Ed, New Age International Publishers, New Delhi, India.

Sampson, S., Bucris, E., Horovitz-Fried, M., Parnas, A., Kahana, S., Abitbol, G., Chetboun, M., Rosenzweig, T., Brodie, C., Frankel, S., 2010. Insulin increases H_2O_2-induced pancreatic beta cell death. Apoptosis 15(10), 1165-1176.

Sasikumar, J., Maheshu, V., Smilin, A., Gincy, M., Joji, C., 2012. Antioxidant and antihemolytic activities of common Nilgiri barberry (*Berberis tinctoria* Lesch.) from south India. Int. Food Res. 19(4), 1601-1607.

Shah, H., Shad, A.A., Perveen, S., Khattak, S., Khanzadi Fatima Khattak, 2003. PPhysiochemical composition of wild medicinal plant Berberislycium.J.Appl.Sci.3,370-375.

Shahidi, F., Ambigaipalan, P., 2015. Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects-A review. J. Funct. Foods 18(B), 820-897.

Shenoy, R.S., Prashanth, K., Manonmani, H., 2018. *In vitro* antidiabetic effects of isolated triterpene glycoside fraction from *Gymnema sylvestre*. Evid.-Based Complementary Altern. Med. 2018, 1-12.

Shigwan, H., Saklani, A., Hamrapurkar, P.D., Mane, T., Bhatt, P., 2013. HPLC method development and validation for quantification of berberine from *Berberis aristata* and *Berberis tinctoria*. Int. J. Appl. Sci. Eng. 2, 203-211.

Siddhuraju, P., Vijayakumari, K., Janardhanan, K., 1996. Chemical composition and protein quality of the little-known legume, velvet bean (*Mucuna pruriens* (L.) DC.). J. Agric. Food Chem. 44(9), 2636-2641.

Singh, D.K., Srivastava, B., Sahu, A., 2004. Spectrophotometric determination of rauwolfia alkaloids: estimation of reserpine in pharmaceuticals. Anal Sci. 20(3), 571-573.

Soare, J.R., Dinis, T.C., Cunha, A.P., Almeida, L., 1997. Antioxidant activities of some extracts of *Thymus zygis*. Free Radic. Res. 26(5), 469-478.

Sodipo, O., Akinniyi, J.A., Ogunbameru, J., 2000. Studies

on certain characteristics of the extracts of bark of *Pausinystalia johimbe* and *Pausinystalia macroceras* (K Schum) Pierre ex Beille. Global J. Pure Appl. Sci. 6(1), 83-88.

Sood, P., Modgil, R., Sood, M., 2010. Physico-chemical and nutritional evaluation of indigenous wild fruit Kasmal, *Berberis lyceum* Royle. Indian J. Nat. Prod. Resour. 1(3), 362-366.

Srivastava, S. K., Rai, V., Srivastava, M., Rawat, A. K. S., Mehrotra, S., 2006. Estimation of heavy metals in different *Berberis* species and its market samples. Environ. Monit. Assess. 116(1), 315-320.

Srivastava, S., Srivastava, M., Misra, A., Pandey, G., Rawat, A., 2015. A review on biological and chemical diversity in *Berberis* (Berberidaceae). EXCLI. J. 14, 247-267.

Srivastava, S.K., Rawat, A.K.S., 2007. Pharmacognostic evaluation of the roots of *Berberis tinctoria* Lesch. Nat. Prod. Sci. 13(1), 27-32.

Tabeshpour, J., Imenshahidi, M., Hosseinzadeh, H., 2017. A review of the effects of *Berberis vulgaris* and its major component, berberine, in metabolic syndrome. Iran J. Basic Med. Sci. 20(5), 557-568.

Tacon, A., Ferns, P., 1979. Activated sewage sludge, a potential animal foodstuff I. Proximate and mineral content: seasonal variation. J. Agri. Envi. 4(4), 257-269.

Tan, B.L., Norhaizan, M.E., Liew, W.P.P., Sulaiman Rahman, H., 2018. Antioxidant and oxidative stress: a mutual interplay in age-related diseases. Front. pharmacol. 9(1162), 1-28.

Upwar, N., Patel, R., Waseem, N., Mahobia, N.K., 2011. Hypoglycemic effect of methanolic extract of *Berberis aristata* DC stem on normal and streptozotocin induced diabetic rats. Int. J. Pharm. Pharm. Sci. 3(1), 222-224.

Van den Berg, R.A., Hoefsloot, H.C., Westerhuis, J.A., Smilde, A.K., van der Werf, M.J., 2006. Centering, scaling, and transformations: improving the biological information content of metabolomics data. BMC Genom. 7(1), 142.

Vasarri, M., Leri, M., Barletta, E., Ramazzotti, M., Marzocchini, R., Degl'Innocenti, D., 2020. Anti-inflammatory properties of the marine plant *Posidonia oceanica* (L.) Delile. J. Ethnopharmacol. 247, 112252.

Wang, S.Y., Lin, H.S., 2000. Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. J. Agric. Food Chem. 48(2), 140-146.

Weston, R., Gore Jr, P.A., 2006. A brief guide to structural equation modeling. Couns. Psychol. 34(5), 719-751.

Yamamoto, H., Fujimori, T., Sato, H., Ishikawa, G., Kami, K., Ohashi, Y., 2014. Statistical hypothesis testing of factor loading in principal component analysis and its application to metabolite set enrichment analysis. BMC bioinformatics 15(1), 51.

Zee-Cheng, R.Y., 1997. Anticancer research on Loranthaceae plants. Drugs Future 22(5), 519-530.

Zhuang, X., Lu, Y., Yang, G., 1992. Extraction and determination of flavonoid in ginkgo. Chin. Herb. Med. 23, 122-124.