T.C.
YEDITEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF PHARMACOGNOSY

EVALUATION OF EFFECTS OF *IN VITRO* HUMAN DIGESTION SIMULATION ON PHENOLIC PROFILE AND ANTIOXIDANT POTENTIAL OF ADOXACEAE FRUITS GROWING IN TURKEY

DOCTOR OF PHILOSOPHY THESIS

Timur Hakan Barak, Pharm.

ISTANBUL-2019

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APPROVAL

This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated 31.07.2019 and numbered 2019-13-41

Prof. Dr. Bayram YILMAZ

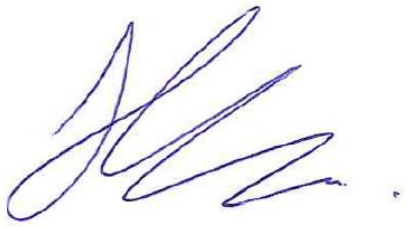
Director of Institute of Health Sciences

DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

14.06.2019

Timur Hakan Barak



Dedicated to my family,
Süheyla, Hasan and Ece Barak

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ABBREVIATIONS

AAE: Ascorbic acid equivalent

ABTS: 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)

ADC2: Automatic development chamber 2

ANOVA: Analysis of variance

BALF: Bronchoalveolar lavage fluid

BHA: Butylated hydroxyanisole

BHT: Butylated hydroxytoluene

CAT: Catalase

CUPRAC: Cupric Reducing Antioxidant Capacity

DMPD: (N,N-dimethyl-p-phenylendiamine)

DNA: Deoxyribonucleic acid

DPPH: 2,2-Diphenyl-1-picrylhydrazyl

EDTA: Ethylenediaminetetraacetic acid

FCR: Folin Ciocalteu Reagent

FRAP: Ferric Reducing Antioxidant Power

FW: Fresh weight

GAE: Gallic acid equivalent

GI: Gastro-intestinal

GPx: Glutathione peroxidase

GSH: Glutathione

HFD: High fat diet

HPTLC: High performance thin layer chromatography

IC_{50}: The half maximal inhibitory concentration

IL-1α: Interleukin 1 alpha

IL-1β: Interleukin 1 beta

LFD: Low fat diet

LOD: Limit of detection

LOQ: Limit of quantification

MDA: Malondialdehyde

MeOH: Methanol

MIC: Minimum inhibitory concentration

MRSA: Methicillin-resistant *Staphylococcus aureus*

n-BuOH: *n*-Butanol

ND: Not digested

ORAC: Oxygen Radical Absorbance Capacity

QE: Quercetin equivalent

ROS: Reactive Oxygen Species

RS: Reactive Species

SOD: Superoxide dismutase

SEM: *Sambucus ebulus* methanolic extract

SEA: *Sambucus ebulus* aqueous extract

SNM: *Sambucus nigra* methanolic extract

SNA: *Sambucus nigra* aqueous extract

TBARs: Thiobarbituric acid reactive substances

TE: Trolox equivalent

TEAC: Trolox equivalent antioxidant capacity

TNFα: Tumor necrosis factor alpha

TOAC: Total Antioxidant Capacity

TPC: Total phenolic content

TPAC: Total phenolic acid content

TPACC: Total proanthocyanidin content

TSH: Thyroid-stimulating hormone

VOM: *Viburnum opulus* methanolic extract

VOA: *Viburnum opulus* aqueous extract

ABSTRACT

Barak, TH. (2019). Evaluation of Effects of *in Vitro* Human Digestion Simulation on Phenolic Profile and Antioxidant Potential of Adoxaceae Fruits Growing in Turkey. Yeditepe University, Institute of Health Sciences, Department of Pharmacognosy, Ph.D. Thesis, İstanbul.

Although there is a wide array of antioxidant activity studies on plants and contribution of their phenolic contents in the determined activity, bioavailability phenomenon of the active ingredients is often neglected. Design of this study based on evaluation of difference in antioxidant potentials and phenolic profiles of Adoxaceae fruits growing in Turkey, before and after *in vitro* gastrointestinal human digestion simulation. For this purpose, two different solvent extracts, methanol and water, were prepared from the fruits of *Sambucus nigra* L., *Sambucus ebulus* L. and *Viburnum opulus* L. and their activity profiles were comparatively investigated. After the digestion simulation procedure, total phenolic, phenolic acid, flavonoid and total proanthocyanidin contents were determined for all phases of digestion. High performance thin layer chromatography (HPTLC) was used for the measurement of selected phenolic molecules. Bioavailability index was calculated for all phenolic content assays and for the selected bioactive compounds for accurate determination of alterations in phenolic profile via *in vitro* human digestion. For revelation of the precise antioxidant potential of fruit extracts, a couple of free radical scavenging (DPPH and DMPD) and metal reducing potential (CUPRAC and FRAP) assays and in addition a total antioxidant capacity assay were conducted on all phases. The results showed that human digestion might have significant effect on phenolic profile and antioxidant properties of herbal extracts.

Key words: *Sambucus nigra* L., *Sambucus ebulus* L., *Viburnum opulus* L., Antioxidant activity, Human digestion simulation, Chlorogenic acid

ÖZET

Barak, TH. (2019). *In Vitro* insan Sindirim Simülasyonu modelinin Türkiye'de yetişen Adoxaceae familyasına ait meyvelerin, fenolik profilleri ve antioksidan potansiyelleri üzerindeki etkilerinin incelenmesi. Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Farmakognozi ABD, Doktora Tezi, İstanbul.

Bitkilerde çok çeşitli antioksidan çalışmalar ve fenolik profil çalışmaları yapılmış olmasına rağmen, biyoyararlanım fenomeni sıklıkla ihmal edilir. Bu çalışmanın tasarımı, Türkiye'de yetişen Adoxaceae meyvelerinin antioksidan potansiyeli ve fenolik profilindeki farklılığın *in vitro* gastrointestinal insan sindiriminden önce ve sonra değerlendirilmesine dayanmaktadır. Bu amaçla, *Sambucus nigra* L., *Sambucus ebulus* L. ve *Viburnum opulus* L. meyveleri araştırıldı. Meyvelerinden iki ekstre (MeOH ve H_2O) hazırlandı. Sindirim simülasyon işleminden sonra, sindirimin bütün aşamaları için toplam fenolik, fenolik asit, flavonoit ve proantosiyanidin içerikleri karşılaştırmalı olarak belirlendi. Seçilen fenolik moleküllerin ölçümü için yüksek performanslı ince tabaka kromatografisi (YPİTK) kullanıldı. Biyoyararlanım indeksi, tüm fenolik içerik analizleri ve fenolik profildeki değişiklikler ve seçilen biyoaktif bileşikler, *in vitro* insan sindirimi yoluyla doğru bir şekilde belirlenmesi için hesaplandı. Meyve özütlerinin kesin antioksidan potansiyelinin açığa çıkarılması için, serbest radikal süpürücü (DPPH ve DMPD) ve metal indirgeme potansiyeli (CUPRAC ve FRAP) tahlilleri ve ayrıca tüm fazlarda toplam bir antioksidan kapasite tahlili gerçekleştirildi. Sonuçlar, insan sindiriminin bitki ekstrelerinin fenolik profili ve antioksidan özellikleri üzerinde önemli bir etkiye sahip olabileceğini göstermiştir.

Anahtar kelimeler: *Sambucus nigra* L., *Sambucus ebulus* L., *Viburnum opulus* L., Antioksidan aktivite, İnsan sindirim simülasyonu, Klorojenik asit

1. INTRODUCTION AND AIM

In vitro studies are generally the most preferable assays for research of various different bioactivities. The reasons for that are *in vitro* studies are cost-effective, reproducible, practical and deprived from ethical concerns (1). In spite of mentioned wide advantages, there are also crucial disadvantages leading to *in vivo* and clinical studies to be inevitable. First of all, generally the *in vitro* studies are incompetent for determining exact frame of mechanisms of bioactivity once applied to human body. The reason is that *in vitro* studies are incapable of evaluating and mimicking the precise properties of human body. *In vitro* bioactivity assays generally disregard specific conditions of human body therefore it is impossible to interpret whether bioactivity detected from *in vitro* assay is viable for actual clinic implementations. For all that reasons above, advanced enhanced *in vitro* assays for higher accuracy were required for utilization. *In vitro* human gastric simulation method is a well solution for these considerations. Through this method, it is possible to obtain higher precision when compared to direct application of the test sample to *in vitro* assay and furthermore metabolic transformations of the sample in the gastrointestinal system may be approximately estimated (2).

Numerous studies in scientific literature have proven that oxidative stress is an important precursor for various chronic diseases with high epidemiological rates around the world. Therefore, antioxidants are considered to be valuable tools for prophylaxis against such oxidative stress-induced illnesses. Particularly, among the vast types of phytochemials phenolic compounds exert significant antioxidant activity. There is a strong correlation between the phenolic content of a herbal extract and its antioxidant activity, hence determination of phenolic profile of a plant extract is expected to provide valuable hint for its possible antioxidant activity (3).

In the light of this information, it was aimed to comperatively investigate the antioxidant capacities and phenolics profiles of three fruits in Adoxaceae family, naturally growing in the flora of Turkey. *Sambucus nigra* L., *Sambucus ebulus* L. and *Viburnum opulus* L. are considered to be the most important species of this family (4). They all possess broad utilization in Turkish folk medicine and have economic importance due to their proven bioactivities (5). Although several *in vitro* studies have

previously reported the antioxidant potential of these fruits, changes in the activity due to possible transformations in the human gastro-intestinal system was disregarded (6). In this study, we enlightened the effects of human digestion on antioxidant capacity and phenolic profile of the entitled fruits with *in vitro* assays. Antioxidant capacity was determined through different mechanisms such as metal reducing, free radical scavenging and total antioxidant capacity. In addition, quantitative analysis was performed for chlorogenic acid and rutin through High Performance Thin Layer Chromatograpghy (HPTLC).

2. GENERAL DESCRIPTION

2.1. Botanical Information

2.1.1. Adoxaceae family

Adoxaceae family belongs to the order of Dipsacales comprising of five genera: *Sambucus, Viburnum, Adoxa, Sindoxa, Tetradoxa* with approximately 200 species. Leaves are characteristically opposite and serrated. Small flowers in cymose inflorescences have five (infrequently four) petalled. Fruits are drupe (7,8).

2.1.1.1. Genus *Sambucus*

Even though in the Flora of Turkey and the East Aegean Islands genus *Sambucus* was classified in Caprifoliaceae, recent morphological and genetic studies lead to the transfer of *Sambucus* genus to Adoxaceae (7,8).

Plants in genus *Sambucus* are shrubs with a large pith or herbaceous with imparipinnate and opposite leaves. There may be stipules but sometimes absent. Flowers are compound paniculate or umbellate and usually 5-merous. They contain regular and rotate corolla. Ovary contains three to five cells and three to five loci which one seed in each of them. Fruit is drupe. There are twenty five species in the world and two species in the flora of Turkey of *Sambucus* genus: *Sambucus nigra* L. and *Sambucus ebulus* L.

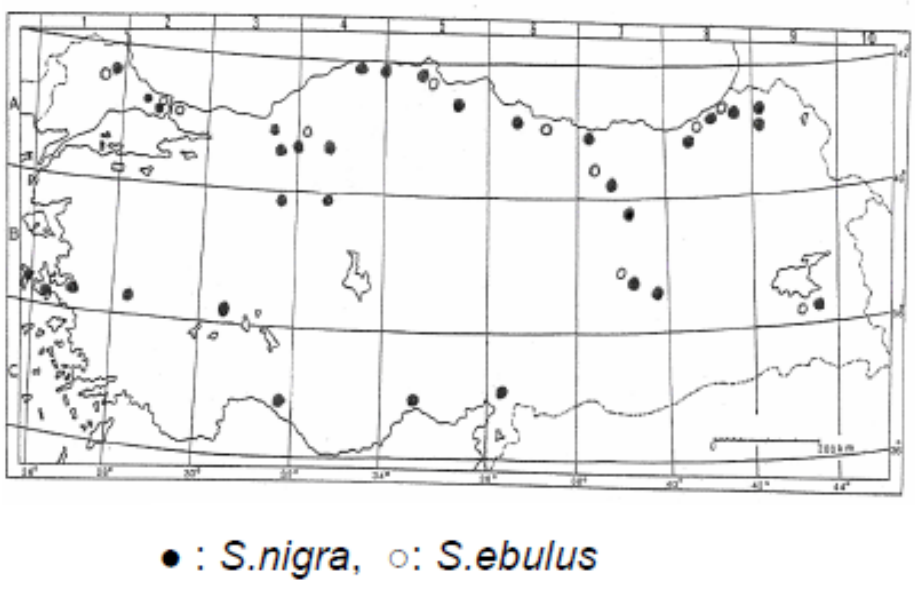

Figure 1. Distribution of *Sambucus* species in Turkey (9)

2.1.1.1.1. *Sambucus nigra* L.

Small trees of shrubs, length between four to ten meters. Unpleasant smell. Ovate-lanceolate or ovate elliptic leaflets, serrate, veins on the underside and lightly hairy. Subulate or absent stipules. Inflorescence generally with five primary rays, flat topped with ten to twenty centimeter diameter. Flowers are in cream color. Fruits is drupe globose with six to eigth millimeter diameter, blackish purple color (7).

Flowering: From April to July depending on geographic conditions.

Altitude: Ranges from sea level to 1700m altitude.

Turkish names: Kara mürver, Melesir, Mındar, Mindiraç, Patlak, Patlangaç, Patlangıç, Patlangoz, Patlankuç, Patlavuç, Patlayak, Şişni, Yalangoz, Yalankoz (10).

Names in other languages: Sauko-Italian (11), black elderberry -English (12), astunpa, intsusa, sabuko, saúco-Basque (13), angure koli-Azarbaijani (14) Holunderbluten, Aalhornblüten, Fliedertee, Schwitztee (Almanya), Fleurs de sureau (Fransa) (15).

Figure 2. *Sambucus nigra* L. fruits (16)

2.1.1.1.2. *Sambucus ebulus* L.

Sambucus ebulus is a perennial plant which is glabrous and grows up to two meters. It generally has unbrenched stems and creepy rhizome. The leaves are pinnate and opposite, can reach 30 cm long. Leaflets are three to six pairs and serrate. Inflorescence is with three rays, flat topped and 10 cm diameter. Flowers are hermaphrodite, usually in cream color but sometimes pink and anthers are purple. Fruits are globose drupe and have 6 mm diameter and dark purple or black color (7).

Flowering: Generally from June to September.

Altitude: 500-2000 m

Turkish names: Sultanotu, cüce mürver, pıyran, haptovina, ademotu, piran, mulver, sahmelik, buzka, ancura, sahmehlemi, yigdan, yılgın, azıotu, Ayı otu, Hekimana, Kımçırık, Livor, Mürver otu, Patpatik, Pellempüs, Purtlak, Şahmelik, Telligelin, Yivdim, Yivdin (10, 17).

Other languages: Bazak -Bulgarian, daneworth, dwarf elder-English, Eurepean Dwarf Elder, Danewort, Walewort, Blood elder, Blood hilder (18, 19, 20, 21).

Figure 3. *Sambucus ebulus* L. fruits (21)

2.1.1.2. Genus *Viburnum*

All of the species in genus *Viburnum* are small deciduous trees or shrubs. Leaves are simple and opposite. Flowers are compound umbellate cymes, actinomorphic, 5-merous; corolla rotate to campanulate; ovary is 1-celled; stigmas 3. Fruits are drupe (7).

2.1.1.2.1. *Viburnum opulus* L.

Shrub, 2-4 m. Twigs greyish, glabrous; buds scaly. Leaves palmately 3(-5) lobed, smooth, greenish becoming red in autumn, glabrous or sparsely simplepubescent beneath, irregularly dentate. Inflorescence 5-10 cm diam., outer sterile flowers 15-20 mm, inner fertile flowers c. 6 mm. Fruit globose, c. 8 mm, bright red (7).

Flowering: From March to April

Altitude: From sea level to 1400 m

Turkish names: Gilaburu (10)

Other names: Water elder, Cramp bark, Snowball tree and European cranberrybush (22, 23, 24, 25)

Figure 4. *Viburnum opulus* L. fruits (26)

2.2. Ethnobotanical Information

2.2.1. Ethnobotanical records of *Sambucus nigra*

2.2.1.1. Ethnobotanical records of *Sambucus nigra* from Turkey

Ethnobotanical data demonstrated that different parts of *S. nigra* are widely used against hemorrhoids in all across Anatolia. Mature fruits are swallowed in Rize province, south part of Izmit Gulf and Kadışehir, Yozgat (27-29), while crashed fruits are used externally in Bozüyük, Bilecik for the same purpose (30). Infusion from flowers is used in Pertek, Tunceli (31), while decoction of flowers are prepared in Kırklareli for hemorrhoids (32). Besides, seeds are also used against hemorrhoids. Decoction prepared from seeds is used in Alaşehir and Sarıgöl, Manisa (33, 34), while infusion from seeds is used in Kırklareli, also for hemorrhoids (32).

Another widespread application of *S. nigra* in Anatolia is against rheumatism. In Solhan, Bingöl (35) leaves, while in Northwest Anatolia and Adana province aerial parts with leaves and branches are boiled in water and used as bath externally (36,37).

In Edremit, infusion from flowers is used against stomachache, dizziness, nausea and flu symptoms (38). In İzmir province, infusion from leaves and flowers is used as diuretic, expectorant, sedative, laxative and sudorific (39). In Bayramiç, Çanakkale, plant is used as antitussive (40).

2.2.1.2. Ethnobotanical records of *Sambucus nigra* from other countries

S. nigra has broad utilization in folk medicine of different cultures from all around the world. In Bosnia and Herzegovina infusion made from flowers are used against fever and cold (41). In Italy, decoction prepared from leaves of *S. nigra* is used against abscesses (42). Also in Eastern Europe, several preparations form different parts of *S. nigra* are used against various dermatological purposes (43). In Spain, leaves are used for various ophthalmological disorders such as conjunctivitis (44).

2.2.2. Ethnobotanical records of *Sambucus ebulus*

2.2.2.1. Ethnobotanical records of *Sambucus ebulus* from Turkey

S. ebulus exerts a widepread distribution all across Turkey. Therefore, various local names and utilizations have been described in ethnobotanical surveys. Ethnobotanical data pointed out that two noticeable medical utilizations are distinguished: against rheumatism and hemorrhoids.

It was recorded that leaves and fruits of *S. ebulus* are used against rheumatism in Bafra, Rize and Adana (45,27,46). In Bayramiç, Uzunköprü, Kütahya and Northwest Anatolia, only leaves were used against rheumatism externally (40,47,48,36). Herbs and roots are also used as a folk remedy in Yalova and Andırın, respectively (49,50).

Fruits are the most common parts that are used against hemorrhoids in Turkey. In Trabzon, Yalova, İzmit, Çatalca fruits are used internally (51,48,27,52). In Karaisalı and Rize leaves are used together with fruits as infusions (53,27). Decoction of aerial parts is used in Uzunköprü (47), while seeds are the parts are used in Andırın (50).

In Aydın, decoction of leaves and in Çatalca (İstanbul) infusion of flowers is used as diuretic agent (54,52).

S. ebulus is also reported to be used for respiratory system disorders in Turkey. In Çatalca decoction of flowers is used as antitussive, while infusion of flowers is used against asthma. In Karaman, leaves are used against symptoms of common cold (52,46).

Fruits are used externally for wound healing in Kastamonu (55), while leaves are also used for bruises and injuries in Bafra (45). In Kırklareli whole aerial parts and in Karaman leaves are used for snake bites, and in Yalova, roots are used against bee and scorpion bites (32,46,49). In Şile, roots are used for eczema (56).

In Kastamonu and İzmit, fruits are used against stomachache by swallowing the hole fruit (55,28).

2.2.2.2. Ethnobotanical records of *Sambucus ebulus* from other countries

In Palestine, leaves are used as infusions for enuresis and against prostatic enlargement (57). In Razgrad district of Bulgaria marmalade of fruits is used against gastric ulcer, while decoction from fruits is used against hemorrhoids and syrup made from mature fruits is used for cardiac diseases (58). It is also known that flowers, fruits and rhizomes of *S. ebulus* used as purgative, tonic and diuretic in traditional Bulgarian medicine (59). Besides, aerial parts of the plant is externally used against Malta fever in Spain (60). It is recorded that *S. ebulus* is used as diuretic in Romania (61) and also is against rheumatism and common cold. In Iranian folk medicine, *S. ebulus* is recommended for arthiritis, as purgative and diuretic agent, against sore throat and bee bites (62).

2.2.3. Ethnobotanical records of *Viburnum opulus*

2.2.3.1. Ethnobotanical records of *Viburnum opulus* from Turkey

Ethnobotanical data represent that *V. opulus* is used to pass kidney stones, to ease cough and against nephralgia. In eastern Anatolia, decoctions from fruits of *V. opulus* are used internally as antitussive and against nephralgia (63). In Tokat province, fruits are used to pass kidney stone, as hypoglycemic and to ease cough (64).

2.2.3.2. Ethnobotanical records of *Viburnum opulus* from other countries

Barks and fruits of *V. opulus* are used as antirheumatic, laxative and for increment of heart muscle tune in Azerbaijan (65). In Russia, decoction made from barks of *V. opulus* is used as hemostatic agent (66) while *Viburnum* species, especially *V. opulus* is used as diuretic, antispasmodic and sedative in Britain (67).

2.3. Bioactivity studies

2.3.1. Bioactivity studies of *Sambucus nigra*

2.3.1.1. Antioxidant activities

Duymuş et al. investigated *in vitro* antioxidant activity of *S nigra* fruits. DPPH radical scavenging activity assay was conducted for the determination of *in vitro* antiradical activity. IC_{50} value of 70% acetone extract was 117 μg/mL and 123 μg/mL for aqueous extracts while for reference substance ascorbic acid had significantly lower IC_{50} value; 8 μg/mL. ABTS radical scavenging activity (TEAC) was also measured as the highest in acetone extract, very close to ascorbic acid (1.96 and 1.97 mM Trolox equivalent/L respectively). In inhibition of linoleic acid oxidation assay, 70% ethanolic extract exhibited the highest activity but the activity was significantly lower than BHT (68).

Various *in vitro* digested elderberry extracts were investigated for their protective role against oxidative stress on *in vitro* cultured human colonic cells. Non-digested elderberry extract in 1 mg/mL (freeze dried powder) concentration. Extracts reduced the ROS production in intracellular liquid by 22% and DNA damage caused by oxidation in colonic cells by 46%. *In vitro* digestion procedure caused no significant alteration on bioactivity of extracts (69). In another study T_{EC50} value (time for reaching EC_{50} value) in DPPH radical scavenging activity was 23-75 seconds for flowers, however this value was significantly higher in fruits (91-133 s) (70). Similar results were obtained by Dawidowicz et al. DPPH radical scavenging activity was found to be

higher in flowers (91.95–94.15%) when compared to leaves (16.76–48.52%) and berries (50.25–67.69%) (71). In addition flowers of *S. nigra* showed remarkable antioxidative properties in another study. Flowers of *S. nigra* exhibited higher anti-radical activity than reference substances rutin, BHT and BHA. Flower extract showed 97.70% inhibition in concentration of 10 μg/mL. Rutin displayed 77.47% inhibition in 40 μg/mL, while BHT inhibited 82.40% in 20 μg/mL and BHA 89.98% in 20 μg/mL (72).

2.3.1.2. Antimicrobial activities

Hearst et al. (2010) investigated antimicrobial activities of aqueous and ethanolic extracts from flowers, fruits and leaves of *S. nigra* against 13 nosocomial pathogens. Fruits and flowers inhibited the growth of both gram-positive (*Staphylococcus* and *Bacillus*) and gram-negative (*Salmonella* and *Pseudomonas*) bacteria, even in 100-fold dilution. Flowers exerted significantly higher inhibition with respect to other parts. Flower extracts were especially effective against methicillin-resistant *Staphylococcus aureus* (MRSA), which is an important pathogen for various infections. Aqueous extract of the leaves showed only moderate inhibition against *Bacillus cereus* and *Serratia marcescens* in 10-fold dilution, while they were found to be ineffective against nosocomial pathogens (73). Krawitz et al. (2011) investigated a standardized liquid extract of *S. nigra* fruits (Rubini®) for its antimicrobial activity against some pathogenic bacteria for humans. At 10% concentration, extract decreased the growth of both gram-positive and gram-negative bacterial cultures, more than 70%. When the concentration was increased to 20%, bacterial development was inhibited 99% (74).

2.3.1.3. Antiviral activities

Krawitz et al. (2011) investigated antiviral activities of Rubini®, a standardized liquid extract of *S. nigra* fruits. Pathogenic virus strains –influenza A (KAN-1, H5N1) and influenza B (B/Mass)- were incubated in Madin-Darby kidney cell culture. Extract significantly reduced the replication of influenza B virus. Influenza A virus strain was inflamed, however numbers were lesser when compared to control (74).

In another study, fruit extract of *S. nigra* was investigated against H1N1 virus. IC_{50} value was achieved at 252 μg/mL concentration, when virus was applied and infected the kidney cells. When the concentration was increased to 1mg/mL inhibition of virus infection ascended to 100%. In addition two phenolic compounds were identified as active constituents for binding hemagglutinin at the surface of H1N1,

thereby blocking its ability to infect human cells. These compounds were 5,7,3,4-tetra-O-methylquercetin and 5,7-dihydroxy-4-oxo-2-(3,4,5-trihydroxyphenyl) chroman-3-yl-3,4,5-trihydroxycyclohexanecarboxylate. These compounds were separately synthesized and investigated against H1N1 virus. Compounds reached IC_{50} values of 0.13 μg/mL and 2.8 μg/mL, respectively. These results indicated that these compounds which were isolated from *S. nigra* fruits had comparable bioactivity to Oseltamivir and Amantadine, known anti-influenza medicines (75).

In another study concentrated fruits juice was investigated against early stage of H1N1 infection. An IC_{50} value of 720 μg/mL was achieved when samples were applied during the infection. IC_{50} value was increased to 3600 μg/mL, when samples were applied immediately after the viral infection. In addition *in vivo* experiments were conducted to fractions of fruit juice and high-molecular-weight fraction which showed suppressor effect on viral yield in the Bronchoalveolar lavage fluid (BALF) and lungs, was determined as the most effective fraction (76).

In other *in vitro* study, barks of *S. nigra* were investigated against Feline immunodeficiency virus (FIV), which is a common virus that affects cats. A dose of 500 μg/ml extract of *S. nigra* barks inhibited 100% of syncytia formation of virus (77).

In a double-blind, placebo-controlled clinical trial a standardized elderberry extract (Sambucol® syrup) was investigated against influenza A and B virus infections. 60 patients –age ranged from 18 to 54- were randomly divided into two groups. One group received 15 mL of standardized elderberry extract, while the other group was treated with placebo. Results indicated that elderberry syrup made the symptoms diminish four days earlier in average when compared to placebo (78). In a similar clinical trial, 64 patients presenting minimum three flu symptoms -such as coughing, fever etc. were divided into two groups randomly. One group received lozenges containing 175 mg of elderberry extracts, while the other group received placebo. After 24 hours of treatment, placebo group exhibited no improvement or even increment in symptoms, while 28% of the symptoms in patients treated with lozenges were totally ebbed and 60% of patients exhibited significant relief of symptoms (79).

2.3.1.4. Activities against metabolic disorders

In an *in vitro* study, flower extract of *S. nigra* promoted insulin secretion by inducing clonal pancreatic cells at 1 g/L dose. Results indicated that glucose uptake,

glycogenesis and glucose oxidation were improved in abdominal muscles of mice without adding insulin, at *in vitro* conditions (80).

In another study, fruit extract of *S. nigra* was investigated against diabetic osteoporosis. Results showed that extract restored mineral density and decreased the body fat of diabetic rats. Malondialdehyde, an indicator for lipid peroxidation, was significantly decreased in serum, thus the extract apparently caused improvement on the condition on osteoporosis (81).

In another study, Salvador et al. studied the effects of polar and lipophilic extracts against diabetes in rats. Streptozotocin induced rats were fed with a high-fat diet. They were separated into two groups, one group received 190 mg/kg lipophilic, while the other group 350 mg/kg polar elderberry extract. Both extracts assisted reducing insulin resistance and no significant variation in homeostasis was observed (82). In another study, effect of anthocyanin-rich (13%) elderberry extracts on metabolic disorders was investigated in obese mice. 8 weeks-old male mice were divided into four groups: low-fat diet (LFD), high-fat lard-based diet (HFD), HFD with in dose of 0.25% of elderberry extract and HFD with elderberry extract in dose of 1.25%. Triglyceride concentration was significantly lowered in elderberry treated group of mice. Besides, markers of inflammation and insulin resistance were lowered in the same groups (83).

2.3.1.5. Antidepressant activity

Mahmoudi et al. investigated antidepressant activity potential of *S. nigra* fruits in mice. Tail suspension test (TST) and forced swimming test (FST) were conducted for evaluation. Results indicated that the fruit extract increased the physical activity of mice, while the immobility was shortened when compared with the control group. Additionally, 1200 mg/kg fruit extract promoted significantly higher activity on mice in the FST than 10 mg/kg imipramine, a known effective antidepressant medicine (84).

2.3.2. Bioactivity studies of *Sambucus ebulus*

2.3.2.1. Antioxidant activity

Various scientists investigated *S. ebulus* fruits for their antioxidant activity and active principles (85). Results demonstrated that chlorogenic acid showed the highest $ORAC_{FL}$ activity among other phenolic compounds by 9648.9 ± 180.2 μL TE/g. DPPH radical scavenging activity was measured numerous times. Balkan et al measured DPPH

radical scavenging activity of *S. ebulus* 161.97 mgGA/g (86), Hosseinimehr et al. studied DPPH scavenging activity of the whole plant. They measured rate of inhibition (91.3% ± 0.29) in 0.8 mg/mL concentration, while 0.4 mg/mL concentration exhibited 93.6% ± 0.47 inhibition, when compared to BHT (87). In another study, DPPH and CUPRAC assays were performed (88). DPPH scavenging activity of ripe fruits was measured as 3.07 ± 0.08 mg TE/g FW and CUPRAC was measured 3.53 ± 0.07 mg GAE/g FW. Another research group reported that extract of leaves showed higher DPPH radical scavenging activity than fruits, while fruits showed higher activity than roots (89). Anton et al. showed that fruit extracts of *S. ebulus* exhibited higher DPPH scavenging activity compared to BHT (83.17% ± 1.21 and 81.39% ± 0.85, respectively) (90).

2.3.2.2. Anti-inflammatory activity

Yeşilada et al. performed a bioactivity guided fractionation study on the aerial parts of *S. ebulus,* in order to investigate its anti-inflammatory activity. MeOH extract of aerial parts was used and n-hexane, chloroform and n-butanol fractions were investigated. *In vitro* PLA_2-inhibitory activity, *in vivo* carrageenan and serotonin-induced hind paw edema in mice, adjuvant–induced chronic arthritis in rats models were conducted for determination. Chlorogenic acid was determined as the active principle for anti-inflammatory activity in butanol fraction (91). Schwaiger et al. also investigated the active principle responsible for anti-inflammatory activity. Researchers used inhibition of induced expression of vascular cell adhesion molecule 1 (VCAM-1) by TNF-α method on human imbilical vein endothelial cells (HUVECs). Ursolic acid was determined as the active compound for anti-inflammatory activity and it was found to be present in diethyl ether fraction of EtOH extracts of the leaves (92). Yeşilada et al. investigated *in vitro* IL-1α, IL-1*β and* TNFα biosynthesis inhibitory effect of leaves and flowers of *S. ebulus.* MeOH extract of leaves showed significant inhibitory effect for IL-1α and IL-1*β* but no inhibitory effect for TNF-α. Aqueous extract of leaves only exhibited IL-1*β* inhibitory effect. Hexane subfraction still showed significant IL-1α and IL-1*β* inhibitory effect but unlike other fractions, it showed high TNF-α inhibitory effect. MeOH extract of flowers showed significant inhibitory effect for IL-1α, IL-1β and TNF-α. Hexane fraction showed inhibitory effect for IL-1α and IL-1β and chloroform fraction showed only notable TNF-α inhibitory activity (93). In a previous study, nitric oxide scavenging activity (a known indicator of inflammation) of *S. ebulus*

flowers was measured among other plant extracts. It was shown that *S. ebulus* flowers had noteworthy nitric oxide scavenging activity (94). Methanolic extracts of fruits and leaves showed significant inhibition of carrageenan-induced edema, when compared to Diclofenac. Fruits exhibited higher inhibition than leaves inhibition was close to diclofenac (86%, 71% respectively) (95).

2.3.2.3. Anti-microbial activity

Two previous studies investigated *S. ebulus* against *Helicobacter pylori.* First study investigated the fruits of *S. ebulus* for their inhibition of *Helicobacter pylori* growth and susceptibility enhancement for clarythromisin. Results showed that fruit extracts were effective in both manners that were investigated (96). Yeşilada et al. studied alcoholic extract and sub-extracts of aerial parts of the plant (97). Results exhibited that chloroform sub-extract had a MIC of 31.2 mg/mL while other fractions were inactive.

2.3.2.4. Anti-ulcer activity

Yesilada et al. investigated anti-ulcerogenic activity and the active principles of *S. ebulus* leaves. Male Wistar rats were used for experiments. Results indicated that *S. ebulus* leaves had significant anti-ulcer activity as ethnobotanic records displayed. Study also revealed that active principles for anti-ulcer activity of the extracts were quercetin-3-*O*-monoglycoside and isorhamnetin-3-*O*-monoglycoside (98).

2.3.2.5. Wound healing activity

In a comperative study, wound healing activity of aerial parts of *S. ebulus* and *Urtica dioica* were investigated. 2% and 5% ointment and combinations in same percentage were used in full thickness wound model in rats. Phenytoin and eucerin were used as positive control. 2% *S. ebulus* ointment showed the highest activity in all studied samples (99). Another study also showed remarkable wound healing activity of MeOH extract of leaves of *S. ebulus* at 1% concentration ointment. Linear incision and circular excision wound models were employed and Madecassol® was used as positive control. *S. ebulus* ointment showed 84.3% healing while Madecassol® showed 100%. Furthermore, bioactivity guided fractionation was used for determination of the compound responsible for bioactivity. It was revealed that quercetin-3-*O*-glucoside was the main compound for wound healing activity (100).

2.3.2.6. Antidepressant activity

Fruit extracts of *S. ebulus* were investigated for its antidepressant activity by forced swimming test and tail suspension test on albino male swiss mice. Results indicated that 1200 mg/kg showed higher activity compared to imipramine in 10 mg/kg dose. *S. ebulus* fruit extracts shortened the immobility time effectively. No mortality was observed in mice after two days with a dose of 3g/kg. (84).

2.3.2.7. Activity against knee osteoarthritis

Aqueous extract of *S. ebulus* leaves was investigated against knee osteoarthritis in a randomized double-blind clinical study. 79 patients were divided in two groups. One group was treated with 10% *S. ebulus* gel and other group was treated with 1% diclofenac gel. Both treatments were applied three times a day for four weeks. Visual analogue pain scale and Western Ontario and McMaster University osteoarthritis index questionnaire were used for determination of effectiveness. Results indicated that *S. ebulus* gel had higher activity than diclofenac gel (101).

2.3.2.8. Activity against metabolic disorders

Fruit infusion of *S. ebulus* was studied for its metabolic effects on lipid profile of 21 healthy volunteers. 200 ml of fruit infusion was consumed for a month. Results exhibited that LDL levels dropped 24.67% and HDL levels raised 42.77%. Both triglyceride and total cholesterol levels reduced significantly (14.92% and 15.04% respectively). (102)

2.3.3. Bioactivity studies of *Viburnum opulus*

2.3.3.1. Antioxidant Activity

Karaçelik et al. investigated total phenolic content and antioxidative effects of fruit juice and methanol, aqueous, acetonitrile, extracts of seed and skin of *Viburnum opulus* fruits. Results showed that fruit juice had higher phenolic content and higher activity in ABTS radical scavenging and ferric reducing power. Methanol extract of seeds had second higher activity and phenolic content (103).

Kraujalyte et al. studied total phenolic content assay and various *in vitro* antioxidant assays on six different genotypes of *V. opulus* fruits. Total phenolic content of fruit genotypes varied from 5.47 ± 0.24 to 10.61 ± 0.42 mg Gallic acid equivalent/g.

ABTS radical scavenging activity results varied from 31.95 ± 0.94 to 109.81 ± 1.09 μmol Trolox eq/g, ferric reducing power of genotypes were determined between 55.47 ± 1.77 and 109.76 ± 1.37 Fe^{+2} μmol/g and ORAC assay resulted between 127.37 ± 5.44 and 260.38 ± 7.38 μmol Trolox/g (104).

Altun et al. conducted DPPH radical scavenging assay on different branches, fruits and leaves of both *V. opulus* and *V. lanata* aqueous macerates. In 10 mg/ml concentration branches showed 94% of inhibition values while in same concentration fruits and leaves exhibited 47% and 81% inhibition values respectively. Results also indicated that *V. opulus* showed higher DPPH radical scavenging activity than *V. lanata.* (105)

In another study, methanolic macerate of *V. opulus* fruits were investigated against ischemia and reperfusion induced oxidative stress in lung transplanted rats. Fruits were macerated for three days in methanol. 30 female rats were divided into three groups. One group was ischemia-reperfusion group, second group was ischemia-reperfusion group treated with *V. opulus* and third group was control. Results indicated that malondialdehyde (MDA) and protein carbonyl levels were significantly lower in the treated group. Also superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) levels showed significant induction. These results exhibited that *V. opulus* fruits may be beneficial against lung toxicity (106).

2.3.3.2. Antimicrobial properties

Antimicrobial effects of methanolic *V. opulus* fruit extracts were evaluated against 10 different bacteria strains. Results showed that methanolic extract of *V. opulus* fruits were highly effective on *Aeromonas hydrophila* and *Staphylococcus aureus* among others (107). In another study, antimicrobial effects of fruit juice of *V. opulus* was evaluated against six different strains. Results exhibited that fruit juice is more effective on *Lysteria monocytogenes* and *Enterococcus aureus* than on other strains (108).

2.3.3.3. Antiurolithiatic activity

Ethnobotanical records suggested that fruits of *V. opulus* had high prominence against kidney stones in traditional medicine in Turkey. In an *in vivo* study sodium-oxalate induced male wistar rats were used to investigate the antiurolithiatic activity of *V. opulus* fruit juice. 70 mg/kg sodium oxalate was injected to induce urolithiasis.

Biochemical (urine and serum parameters), histopathological and antioxidant (TBARs, TSH and GSH levels in serum) parameters were evaluated on 48 male rats for determination. Results indicated that liyophilized fruits juice of *V. opulus* fruits had beneficial effect on all parameters, confirming the ethnobotanical data. (109)

2.4. Phytochemical Information

2.4.1. Phytochemical Studies on *Sambucus nigra*

2.4.1.1. Terpenic compounds from *Sambucus nigra*

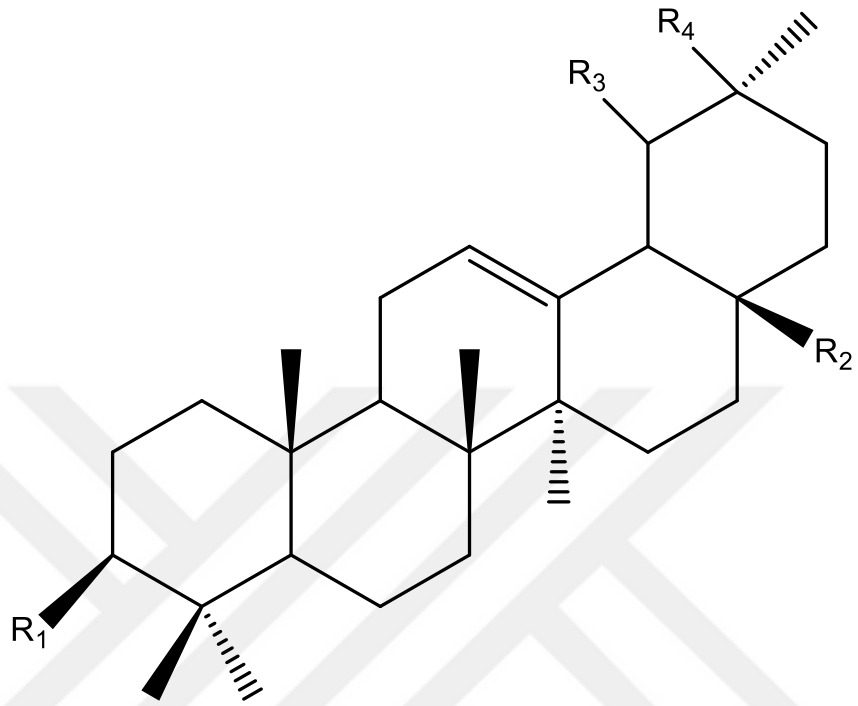

Table 1. Terpenic compounds from *S. nigra*

Compound	R_1	R_2	R_3	R_4	Plant Part	Reference
α-Amyrin	H	CH_3	CH_3	H	Barks	(110)
Oleanolic Acid	OH	COOH	H	CH_3	Barks	(110)

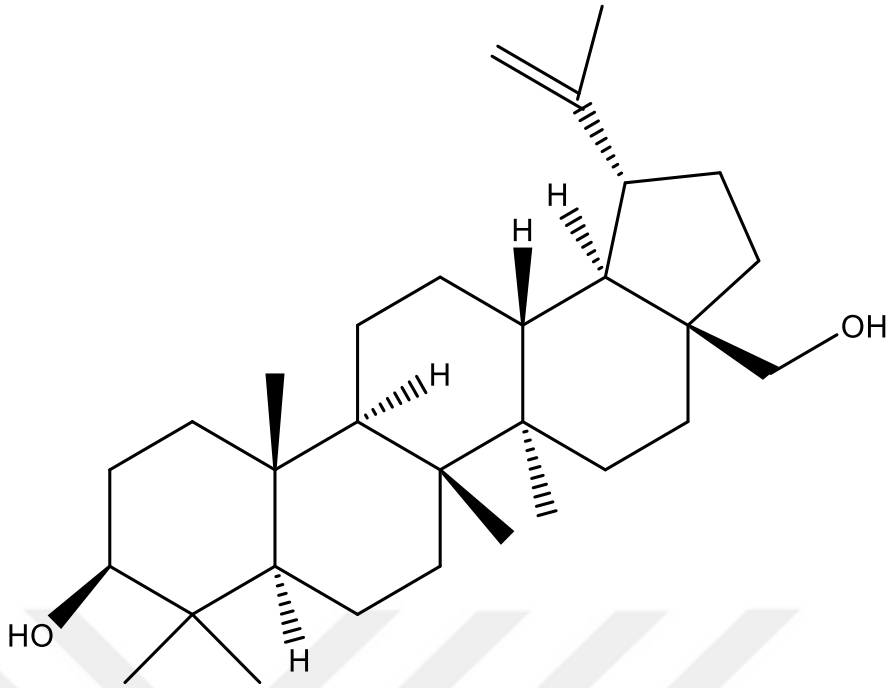

Table 2. Terpenic compounds from *S. nigra* (cont.)

Compound	Plant Part	Reference
Betulin	Barks	(110)

2.4.1.2. Phenolic Acids from *Sambucus nigra*

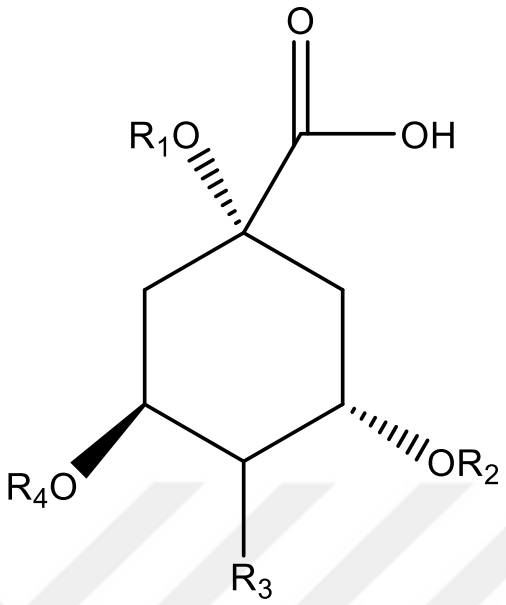

Table 3. Phenolic Acids from *S. nigra*

Compound	R_1	R_2	R_3	R_4	Plant Part	Reference
3-*O*-caffeoylquinic acid	H	H	OH	Caffeoyl	Flowers	(111)
5-*O*-caffeoylquinic acid	H	H	OH	Caffeoyl	Flowers	(111)
1,5-Dicaffeoylquinic acid	Caffeoyl	H	OH	Caffeoyl	Flowers	(111)
3,4-Dicaffeoylquinic acid	H	Caffeoyl	Caffeoyl	H	Flowers	(111)
4,5-Dicaffeoylquinic acid	H	H	Caffeoyl	Caffeoyl	Flowers	(111)
3,5-dicaffeoylquinic acid	H	Caffeoyl	OH	Caffeoyl	Flowers	(111)

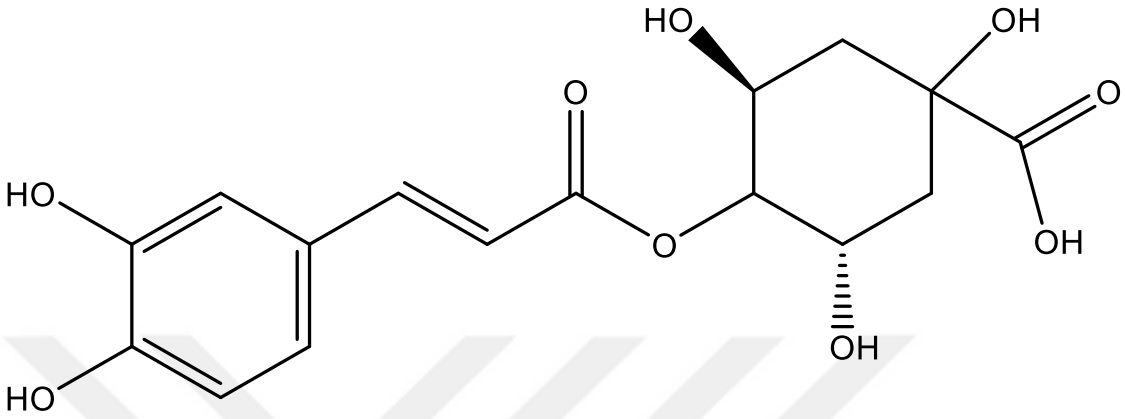

Table 4. Phenolic Acids from *S. nigra* (cont.)

Compound	Plant Part	Reference
4-*O*-Caffeoylquinic acid	Flowers	(111)

2.4.1.3. Flavonoids from *S. nigra*

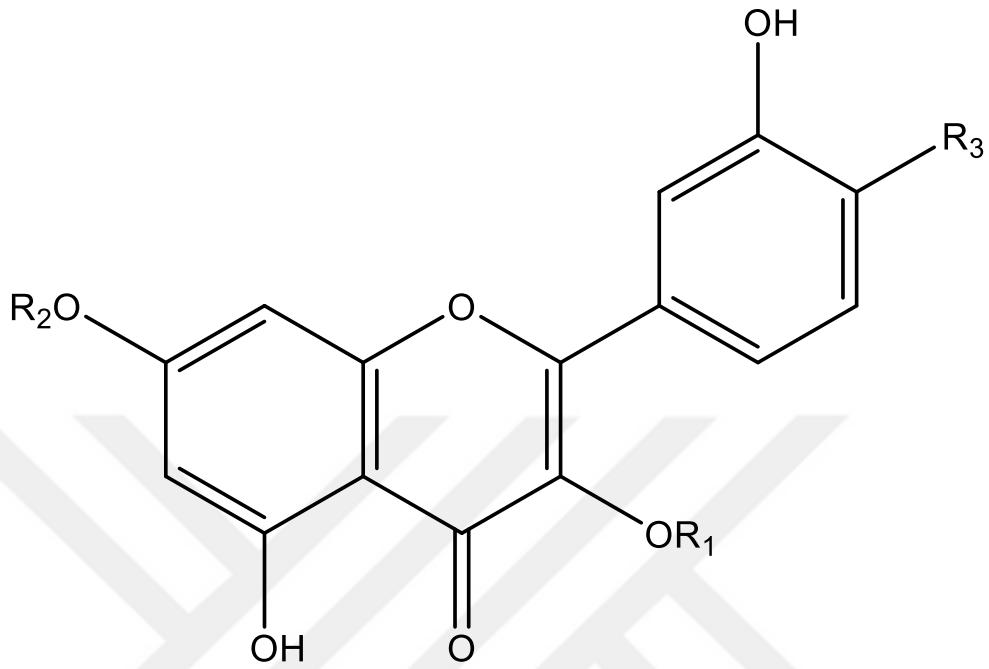

Table 5. Flavonoids from *S. nigra*

Compound	R_1	R_2	R_3	Plant Part	Reference
Quercetin	H	H	OH	Flowers	(111)
Quercetin-3-*O*-glucoside	Glucose	H	OH	Berries	(112)
Quercetin-3-*O*-galactoside	Galactose	H	OH	Flowers	(113)
Quercetin-3-*O*-rutinoside	Rutinose	H	OH	Flowers	(113)
Quercetin-3-*O*-neohesperoside	Neohesperose	H	OH	Flowers	(112)
Quercetin-3-*O*-(6’’-acetylglucoside)	6’’-acetylglucose	H	OH	Flowers	(111)

Compound	**R_1**	**R_2**	**R_3**	**Plant Part**	**Reference**
Rhamnetin-3-*O*-glucoside	Glucose	CH_3	OH	Flowers	(113)
Isorhamnetin-3-*O*-glucoside	Glucose	H	CH_3	Flowers	(111)
Isorhamnetin-3-*O*-rutinoside	Rutinose	H	CH_3	Flowers	(111)
Kamepferol-3-*O*-glucoside	Glucose	H	H	Flowers	(111)
Kaempferol-3-*O*-rutinoside	Rutinose	H	H	Flowers	(111)
Kaempferol-3-*O*-neohesperoside	Neoherperose	H	H	Flowers	(111)

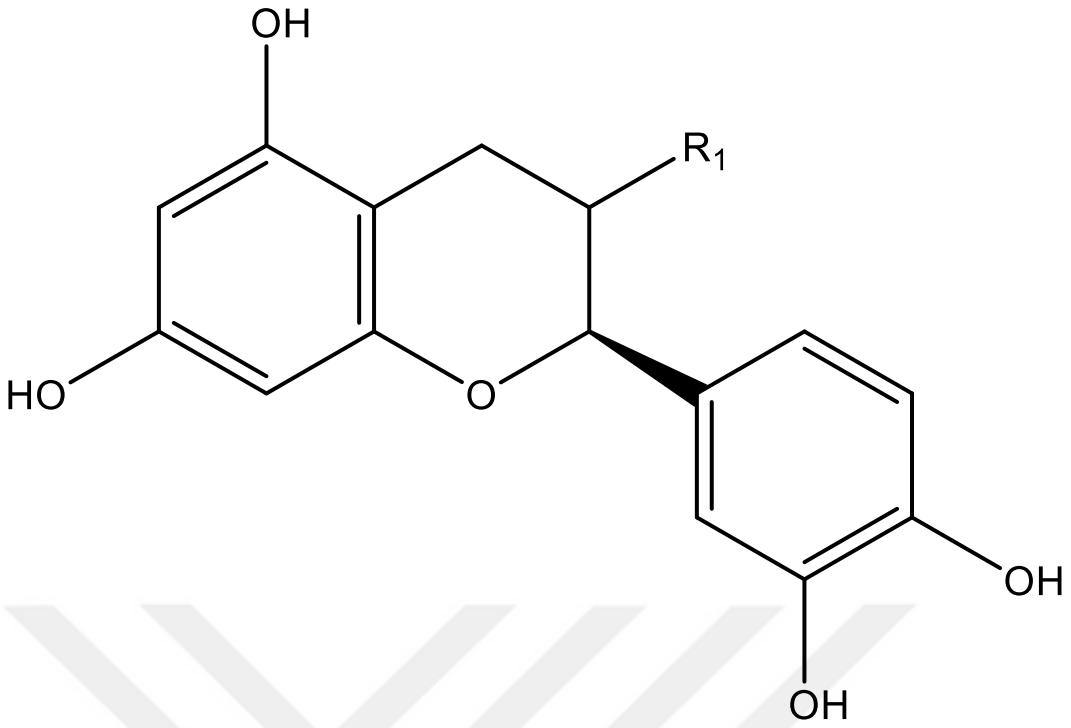

Table 6. Flavonoids from *S. nigra* (Cont.)

Compound	R_1	Plant Part	Reference
(+)-catechin		Flowers	(111)
(-)-epicatechin		Flowers	(111)

2.4.1.4. Anthocyanins from *S. nigra*

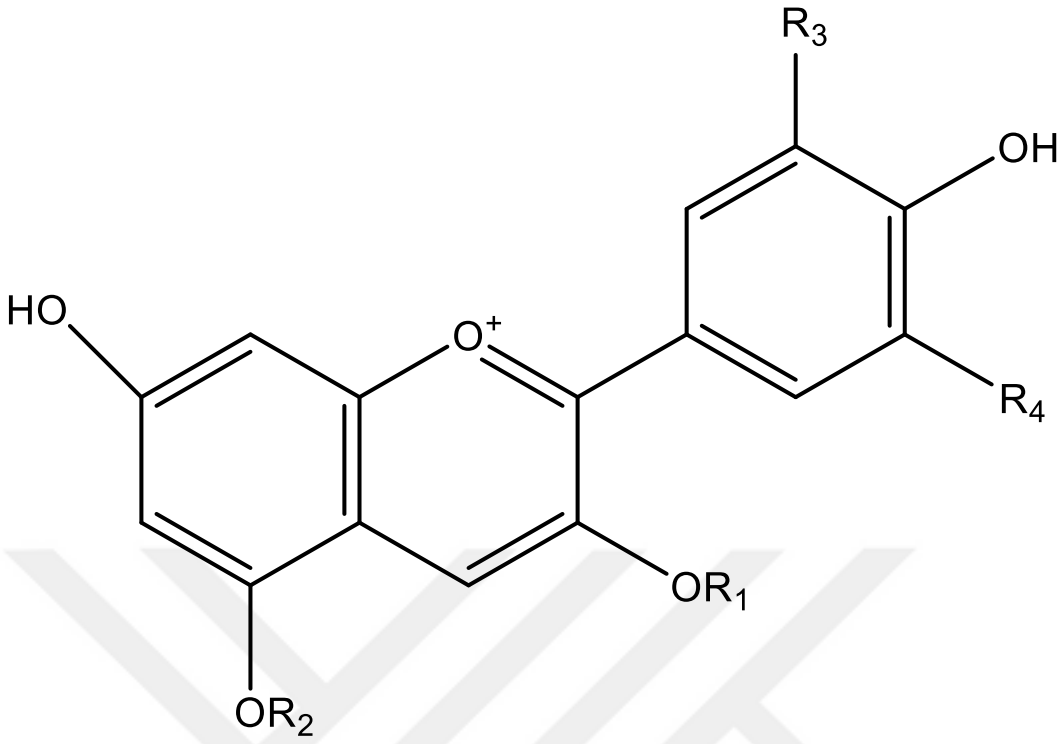

Table 7. Anthocyanins from *S. nigra*

Compound	R_1	R_2	R_3	R_4	Plant Part	Reference
Cyanidin-3-glucoside	Glucose	H	OH	H	Fruits	(112)
Cyanidin-3-sambubioside	Sambubiose	H	OH	H	Fruits	(112)
Cyanidin-3-rutinoside	Rutinose	H	OH	H	Fruits	(112)
Cyanidin-3,5-diglucoside	Glucose	Glucose	OH	H	Fruits	(112)
Cyanidin-3-sambubioside-5-glucoside	Sambibiose	Glucose	OH	H	Fruits	(112)
Delphinidin-3-rutinoside	Rutin	H	OH	OH	Fruits	(112)
Pelargonidin-3-glucoside	Glucose	H	OCH_3	H	Fruits	(112)

2.4.1.5. Phenolic glycosides from *S. nigra*

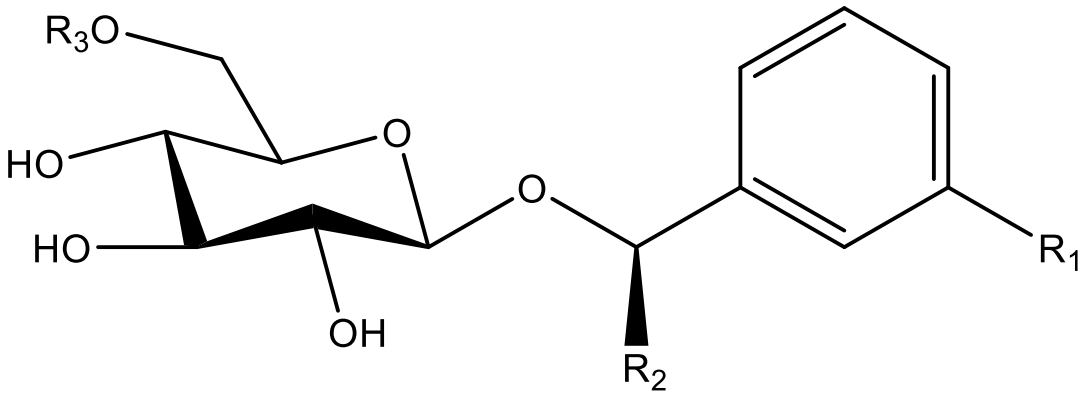

Table 8. Phenolic glycosides from *S. nigra*

Compound	R_1	R_2	R_3	Plant Part	Reference
(2*S*)-2-*O*-β-D-glucopyranosyl-2-hydroxy-phenyl-acetic acid	H	COOH	OH	Leaves	(113)
3-Hydroxybenzyl-1-*O*-β-D-glucopyranoside	H	H	H	Leaves	(113)
1-*O*-β-D-glucopyranosyl-2-(3-hydroxyphenyl)-ethanol	OH	H	H	Leaves	(113)
Benzylalcohol-β-D-apiofuranosyl-(1-6)-β-D-glucopyranoside	OH	H	Apiofuranose	Leaves	(113)

2.4.1.6. Lignans from *S. nigra*

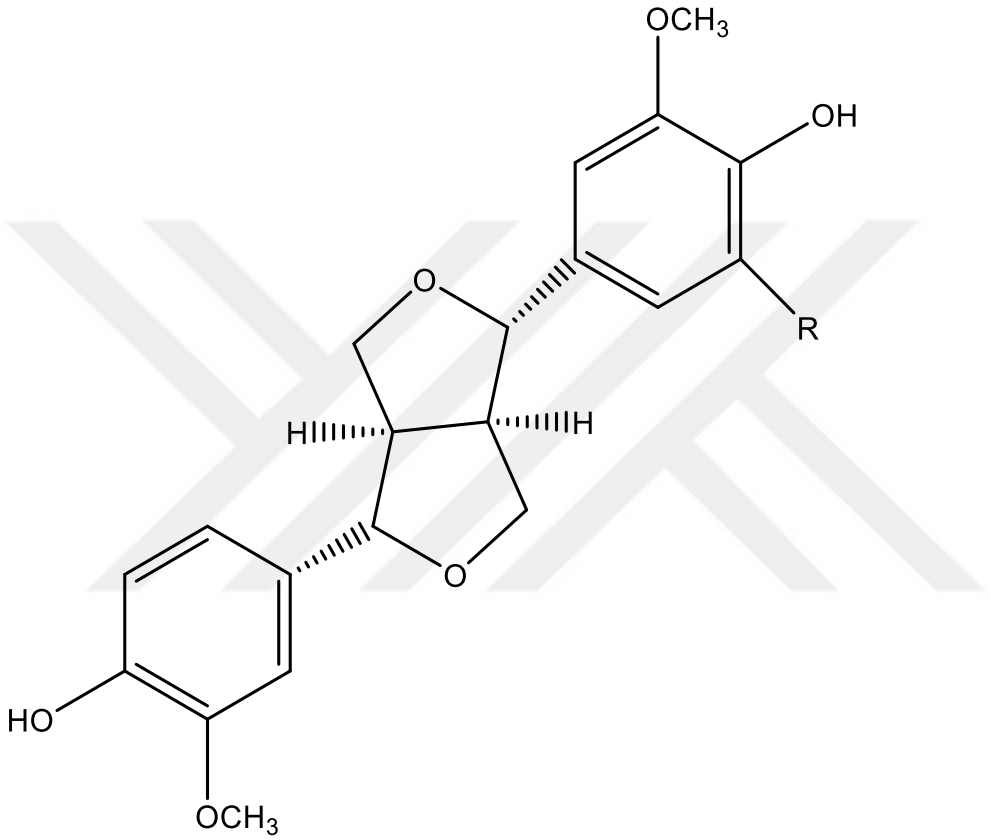

Table 9. Lignans from *S. nigra*

Compound	R	Plant Part	Reference
(+) Pinoresinol	H	Leaves	(113)
(+) Medioresinol	OCH_3	Leaves	(113)

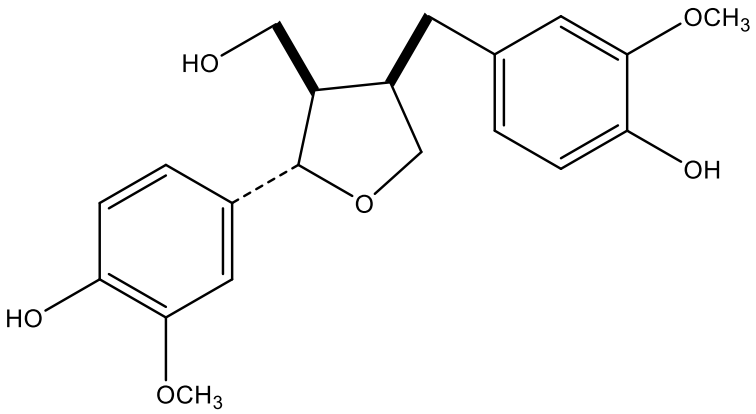

Table 10. Lignans from *S. nigra* (Cont.)

Compound	Plant Part	Reference
(+) lariciresinol	Leaves	(113)

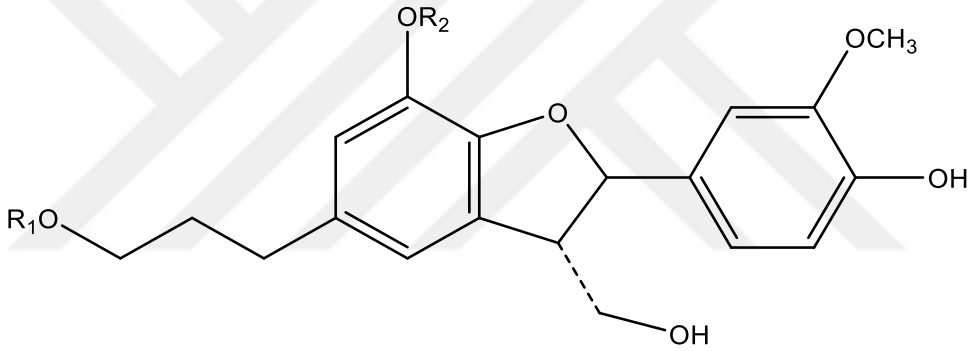

Table 11 Lignans from *S. nigra* (Cont.)

Compound	R_1	R_2	Plant Part	Reference
(2*R*-trans) 2,3-dihydro-2-(4- hydroxy-3-methoxyphenyl)-3-(hydroxymethyl)-7-methoxy-5-benzofuranpropanol acetate	Ac	CH_3	Leaves	(113)
(2*R*-trans)-2,3-dihydro-2-(4- hydroxy-3-methoxyphenyl)-3-(hydroxymethyl) -7-hydroxy-5-benzofuranpropanol	H	CH_3	Leaves	(113)
(2*R*- trans)-2,3-dihydro-2-(4-hydroxy-3-methoxyphenyl)-3-(hydroxymethyl)-7-methoxy-5-benzofuranpropanol	H	H	Leaves	(113)

2.4.1.7. Cyanogenetic glycosides from *Sambucus nigra*

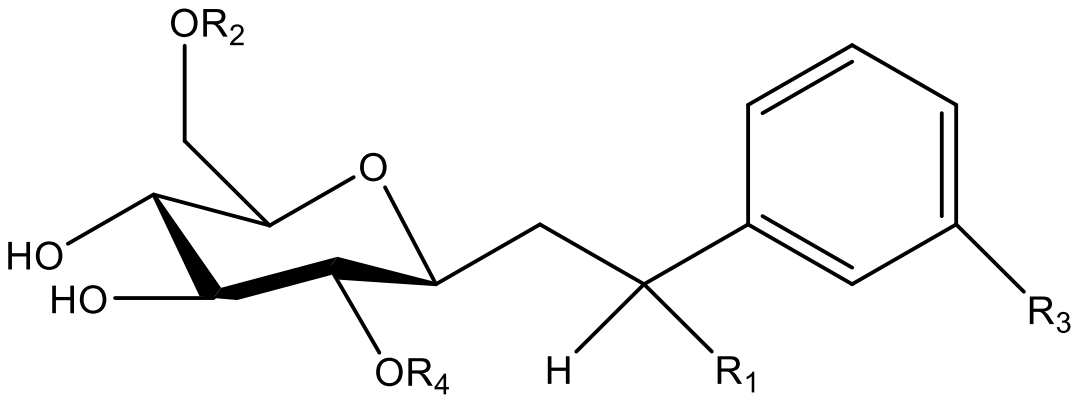

Table 12. Cyanogenins and cyanohydrines from *S. nigra*

Compound	R_1	R_2	R_3	R_4	Plant Part	Reference
Sambunigrin		H	H	H	Leaves	(113)
Holocalin		H	OH	H	Leaves	(113)
6-Acetyl Holocalin		H	OH	Acetyl	Leaves	(113)
Prunasin		H	H	H	Leaves	(113)
(*2S*)-*[*β-D-apiofuranosyl-(*1-2*)*]*-β-D-glucopyranosyl-Mandelonitrile		H	H	Apiose	Leaves	(113)

2.4.2. Phytochemical Studies on *Sambucus ebulus*

2.4.2.1. Terpenic Compounds of *S. ebulus*

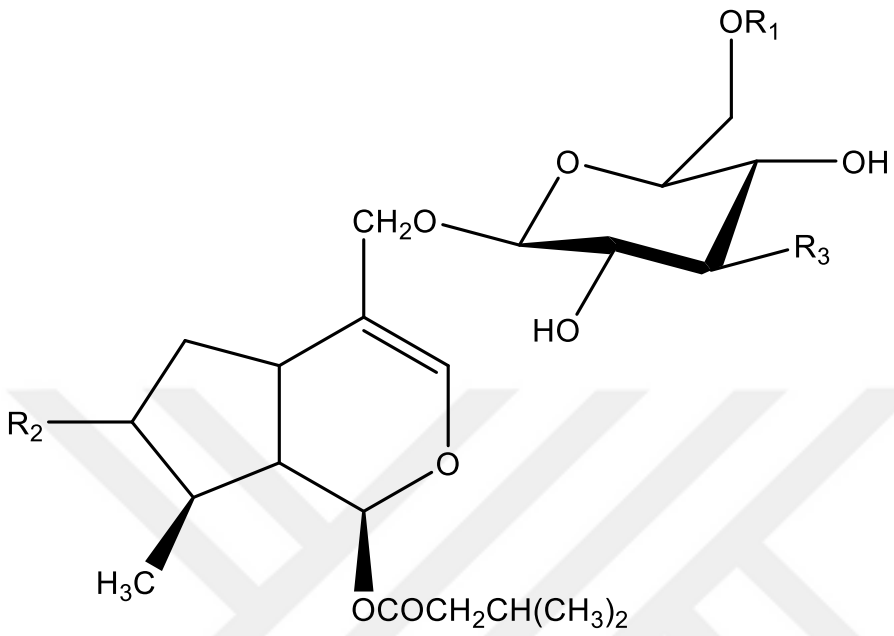

Table 13. Iridoids from *S. ebulus*

Compound	R_1	R_2	R_3	Plant Part	Reference
Ebuloside	H	═══	OH	Leaves	(114)
6'-*O*-apiosylebuloside	Apiose	═══	OH	Roots	(115)
7,7-*O*-dihydroebuloside	CH_2OH	◄OH	H	Roots	(116)

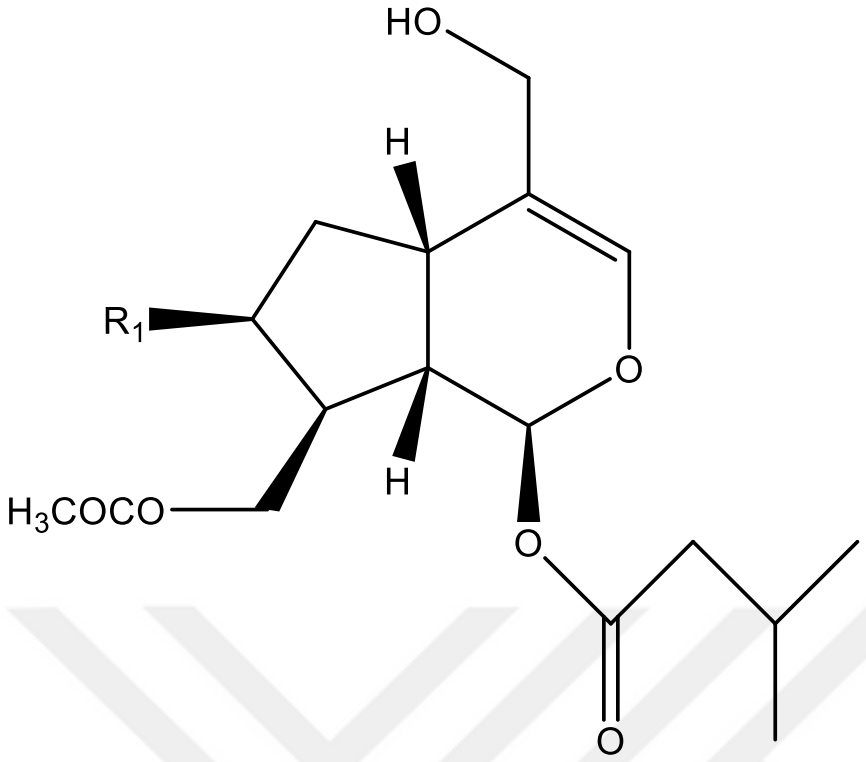

Table 14. Iridoids from *S. ebulus* (Cont.)

Compound	R_1	Plant Part	Reference
Sambulin A	OH	Leaves	(117)
Sambulin B	$OCOCH_3$	Leaves	(117)

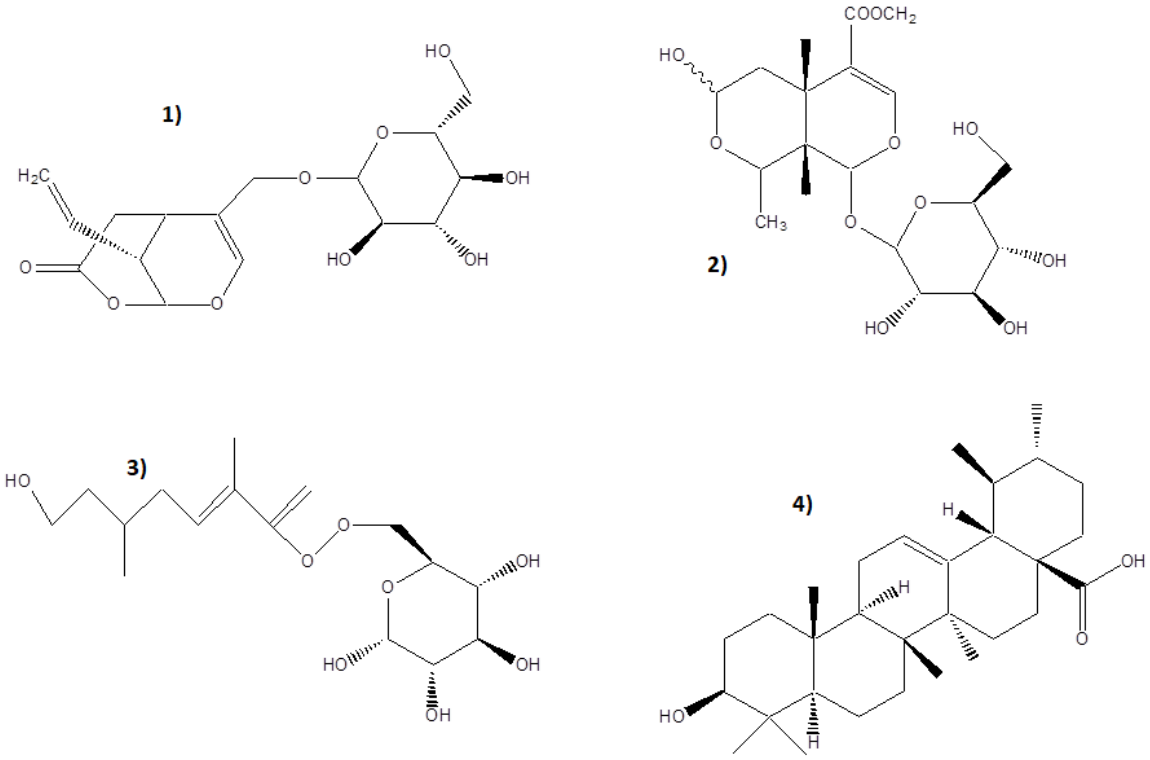

Table 15. Triterpens from *S. ebulus* (cont.)

Compound	Plant Part	Reference
1) Isosweroside	Leaves	(114)
2) Morronoside	Root bark	(118)
3) (β-D-glucopyranosyl)-8-hydroxy-2,6-dimetyloct-2-enoat	Root	(115)
4) Ursolic acid	Leaves	(92)

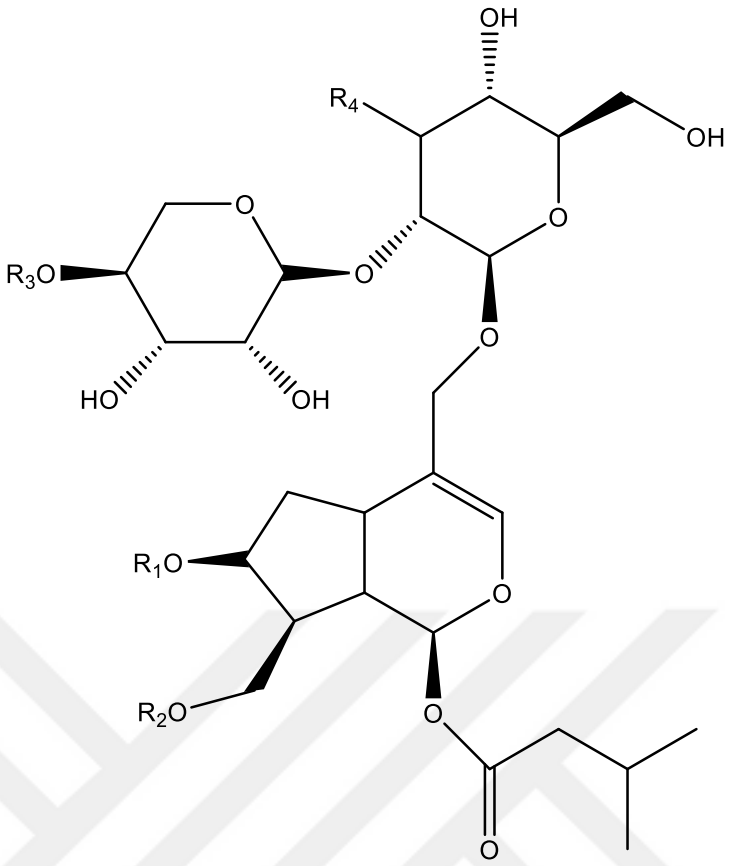

Table 16. Iridoid glycosides from *S. ebulus*

Compound	R_1	R_2	R_3	R_4	Plant Part	Reference
10-*O*-acetylpatrinoside-aglycone-11-*O*-[4''-*O*-acetyl-α-L-rhamnopyranosyl-(1-2)-β-D-ribohexo-3-ulopyranoside]	H	H	Acetyl	=O	Leaves	(119)
7-*O*-acetylpatrinoside-aglycone-11-*O*-[4''-*O*-acetyl-α–L-rhamnopyranosyl-(1-2)-β-D-ribohexo-3-ulopyranoside]	Acetyl	H	Acetyl	=O	Leaves	(119)
10-*O*-acetylpatrinoside-aglycone-11-*O*-[α-L-rhamnopyranosyl-(1-2)-β-D-ribohexo-3-ulopyranoside]	H	Acetyl	H	=O	Leaves	(119)

Compound	R_1	R_2	R_3	R_4	Plant Part	Reference
Patrinoside-aglycone-11-*O*-[4’’-*O*-acetyl-α-L-rhamnopyranosyl-(1-2)-β-D-ribohexo-3-ulopyranoside]	H	H	Acetyl	=O	Leaves	(119)
10-*O*-acetylpatrinoside-aglycone-11-*O*-[4’’-O-acetyl-α–L-rhamnopyranosyl-(1-2)-β-D-glucopyranoside]	H	Acetyl	Acetyl	OH	Leaves	(119)

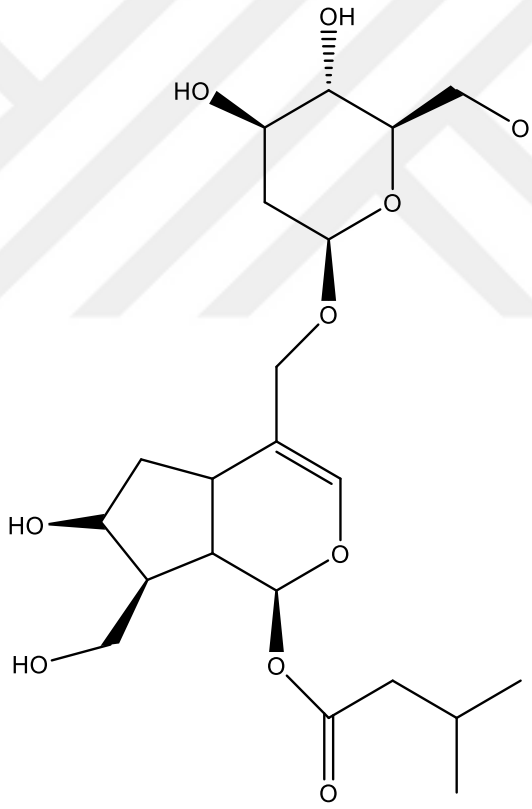

Table 17. Iridoid glycosides from *S. ebulus* (Cont.)

Compound	Plant Part	Reference
Patrinoside-aglycone-11-*O*-2’-deoxy-β-D-glucopyranoside	Leaves	(119)

2.4.2.2. Phenolic acids of *S. ebulus*

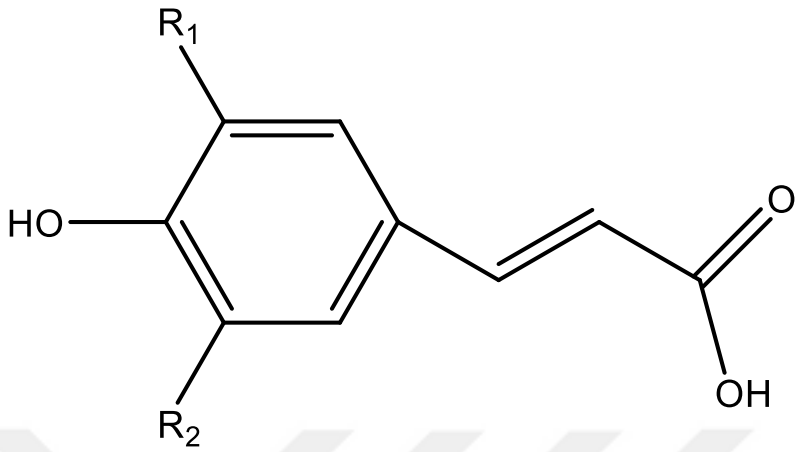

Table 18. Phenolic acids from *S. ebulus*

Compound	**R_1**	**R_2**	**Plant Part**	**Reference**
Caffeic acid	OH	H	Leaves	(119)
p- Coumaric acid	H	H	Leaves	(120)
(E)-Ferulic acid	OCH_3	H	Leaves	(121)
Sinapic Acid	OCH_3	OCH_3	Leaves	(89)

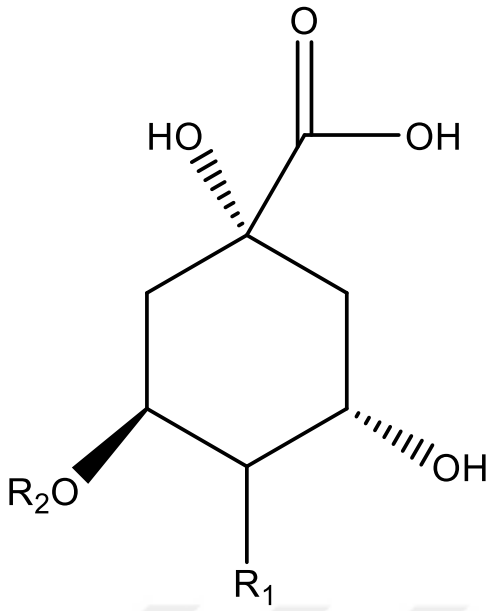

Table 19. Phenolic acids from *S. ebulus* (Cont.)

Compound	R_1	R_2	Plant Part	Reference
3-*O*-caffeoylquinic acid		Caffeoyl	Flowers	(122)
5-*O*-caffeoylquinic acid		Caffeoyl	Flowers	(123)

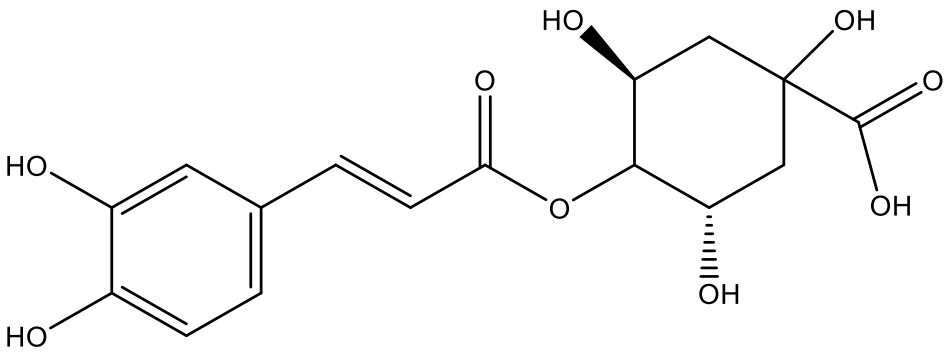

Table 20. Phenolic acids from *S. ebulus* (Cont.)

Compound	Plant Part	Reference
4-*O*-Caffeoylquinic acid	Fruits	(123)

2.4.2.3. Flavonoids of *S. ebulus*

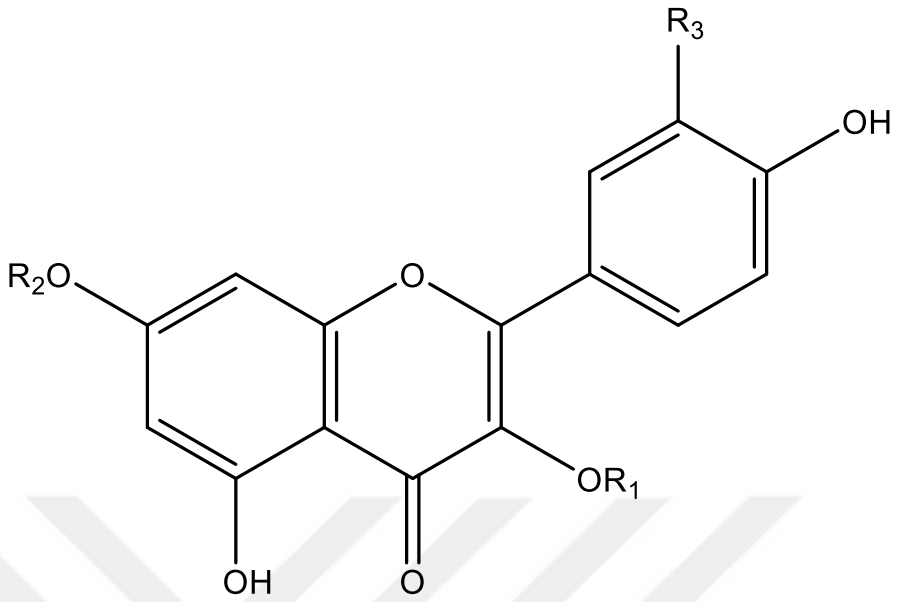

Table 21. Flavonoids from *S. ebulus*

Compound	R_1	R_2	R_3	Plant Part	Reference
Quercetin 3-*O*-rutinoside	Rutinose	H	OH	Leaves	(85)
Quercetin 3-*O*-glucoside	Glucose	H	OH	Leaves	(85)
Quercetin	H	H	OH	Leaves	(85)
Kaempferol-3-O-rutinoside	Rutinose	H	H	Leaves	(85)
Isorhamnetin-3-O-rutinoside	Rutinose	H	OCH_3	Leaves	(85)

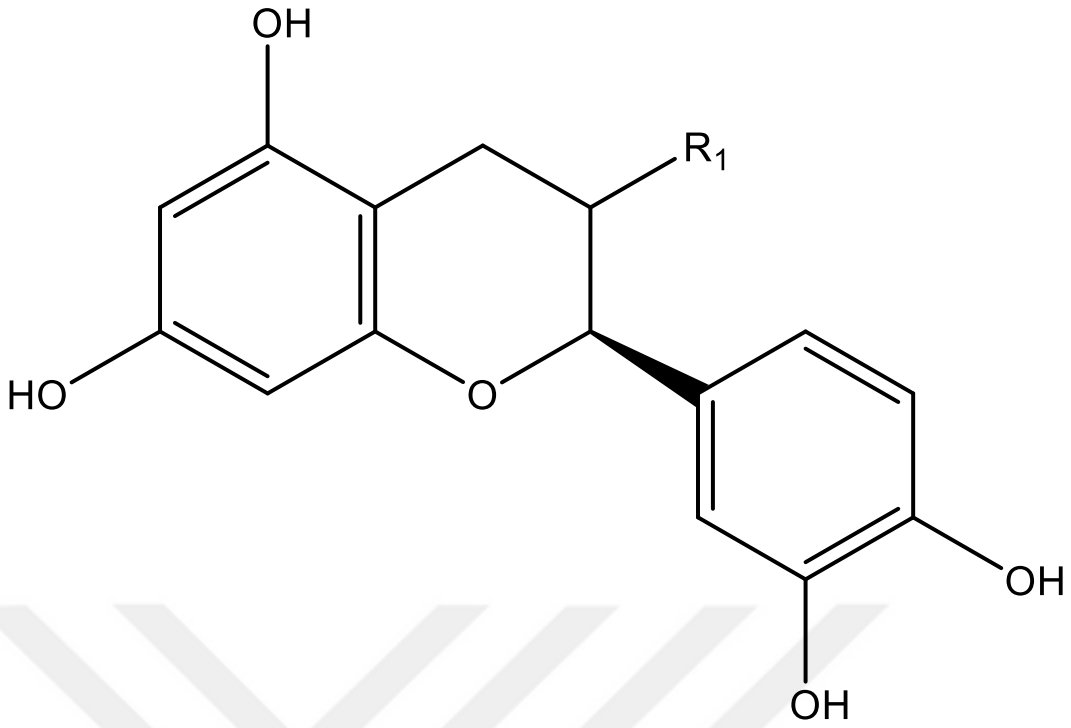

Table 22. Flavonoids from *S. ebulus* (cont.)

Compound	R_1	Plant Part	Reference
(+)-catechin		Flowers	(124)
(-)-epicatechin		Flowers	(124)

2.4.2.4. Anthocyanins of *S. ebulus*

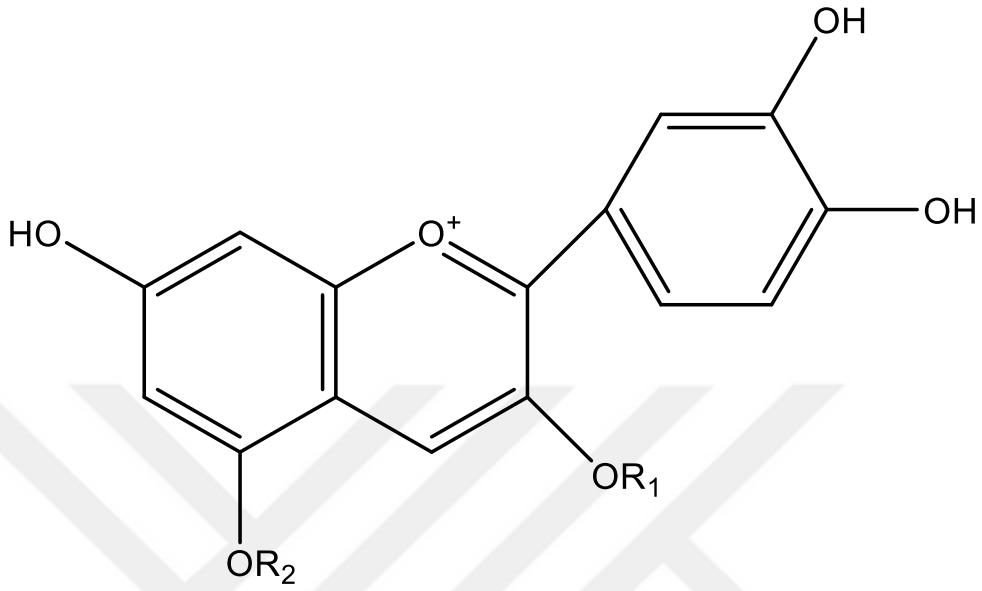

Table 23. Anthocyanins from *S. ebulus*

Compound	R_1	R_2	Plant Part	Reference
Cyanidin-3-glucoside	Glucose	H	Fruits	(90)
Cyanidin-3,5-diglucoside	Glucose	Glucose	Fruits	(90)
Cyanidin-3-sambubioside	Sambubiose	H	Fruits	(90)
Cyanidin-3-sambubioside-5-glucoside	Sambubiose	Glucose	Fruits	(90)

2.4.3. Phytochemical Studies on *Viburnum opulus*

2.4.3.1. Terpenic Compounds of *V. opulus*

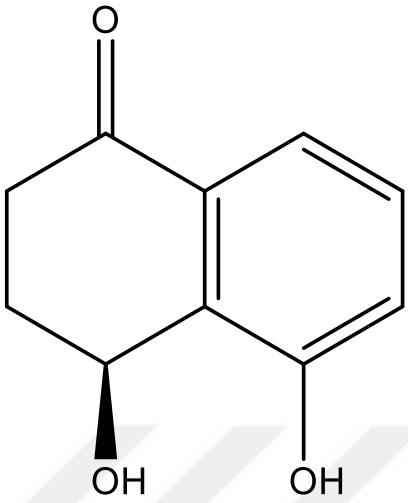

Table 24. Naphtaquinon Derivates from *V.opulus*

Compound	Plant Part	Reference
Sclerone	Fruits	(121)

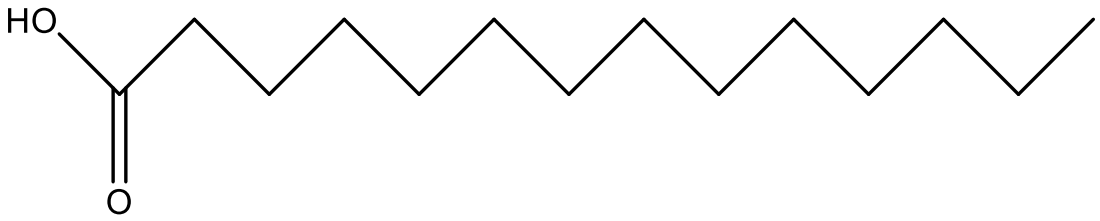

Table 25. Organic acids from *V.opulus* (Cont.)

Compound	Plant Part	Reference
Myristic Acid	Fruits	(121)

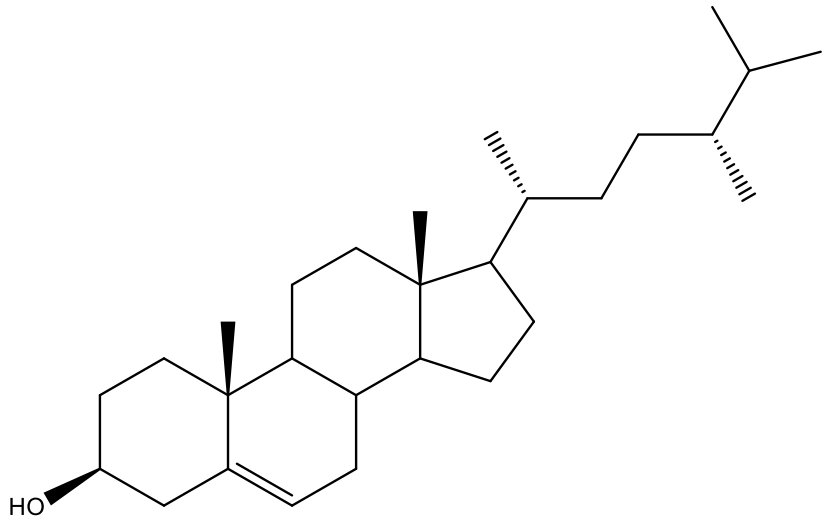

Table 26. Phytosterols from *V.opulus* (Cont.)

Compound	Plant Part	Reference
Campesterol	Fruits	(121)

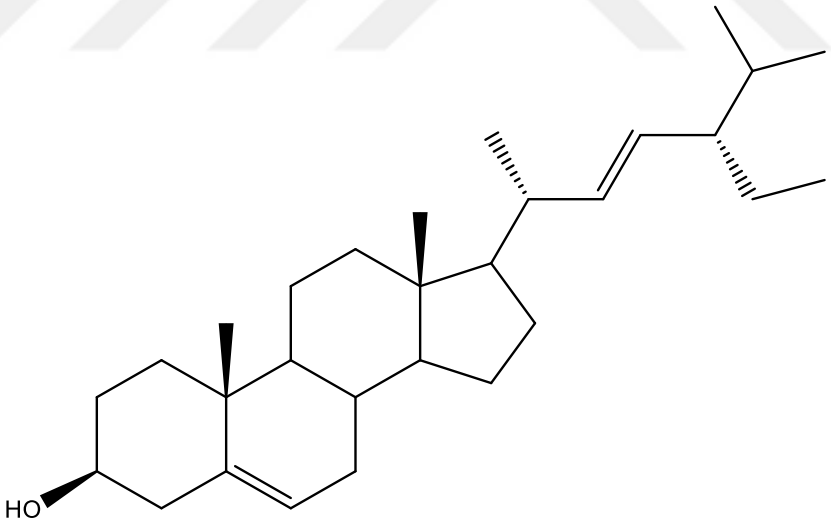

Table 27. Terpenic Compounds from *V.opulus* (Cont.)

Compound	Plant Part	Reference
Stıgmasterol	Fruits	(121)

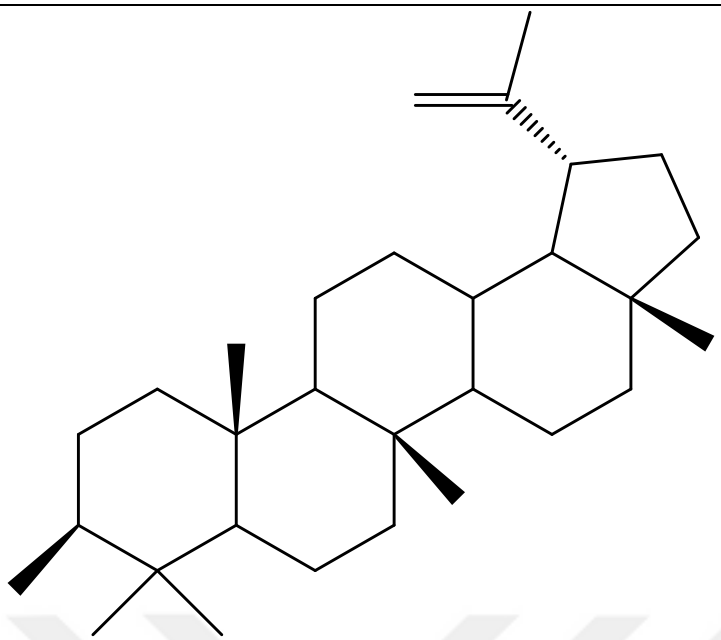

Table 28. Triterpenic compounds from *V. opulus* (Cont.)

Compound	Plant Part	Reference
Lupeol	Fruits	(121)

2.4.3.2. Phenolic acids of *V.opulus*

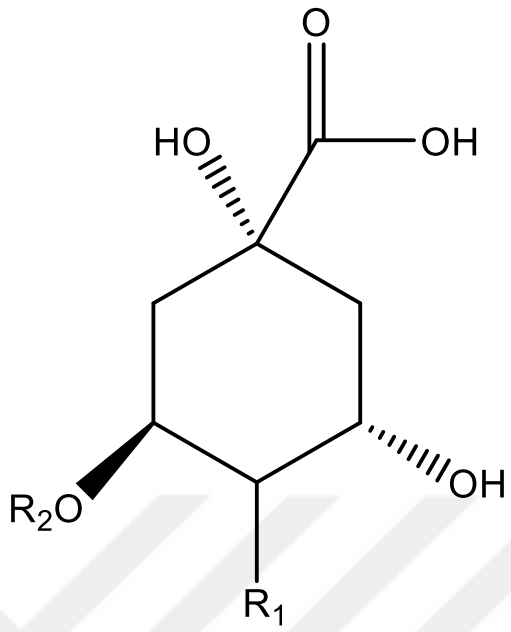

Table 29. Phenolic acids from *V. opulus*

Compound	R_1	R_2	Plant Part	Reference
Quinic Acid	OH	H	Fruits	(104)
3-*O*-caffeoylquinic acid		Caffeoyl	Flowers	(104)
5-*O*-caffeoylquinic acid		Caffeoyl	Flowers	(104)

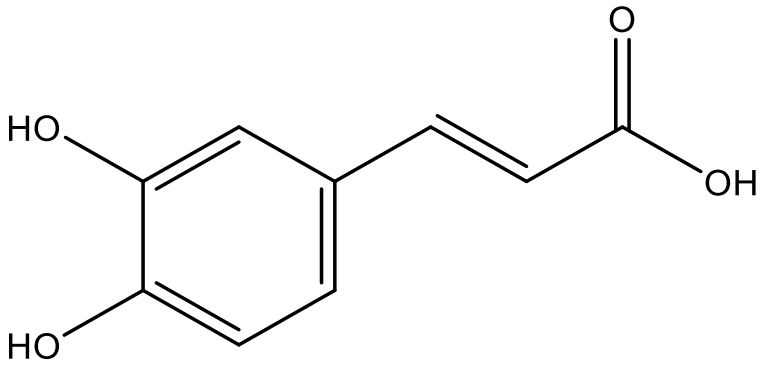

Table 30. Phenolic acids from *V. opulus* (Cont.)

Compound	Plant Part	Reference
Caffeic acid	Fruits	(104)

2.4.3.3. Anthocyanins of *V.opulus*

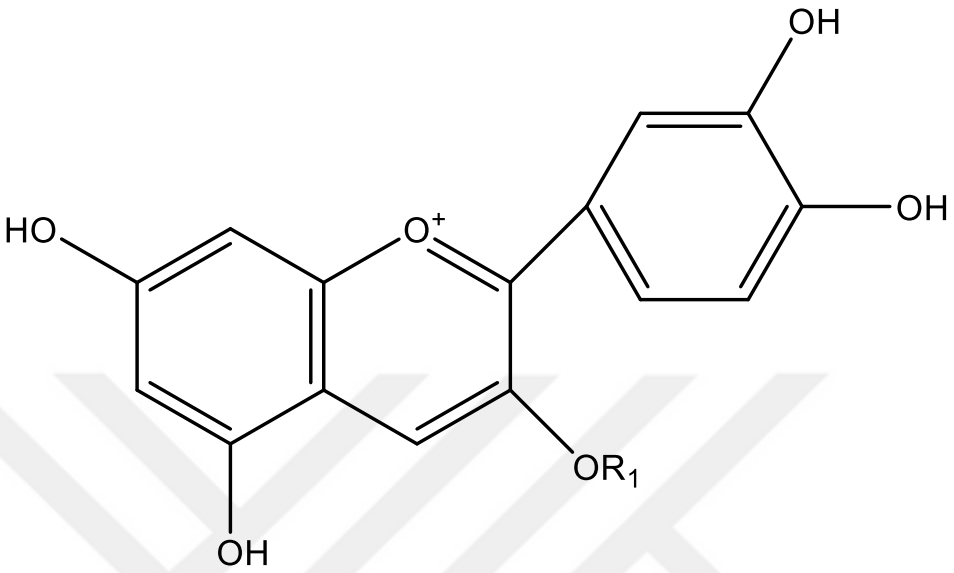

Table 31. Anthocyanins from *V. opulus*

Compound	R_1	Plant Part	Reference
Cyanidin-3-*O*-rutinoside	Rutinose	Fruits	(125)
Cyanidin-3-*O*-vicianoside	Vicianose	Fruits	(125)
Cyanidin-3- *O*-sambubioside	Sambubiose	Fruits	(125)
Cyanidin-3- *O*-glucoside	Glucose	Fruits	(125)

2.4.3.4. Flavonoids of *V. opulus*

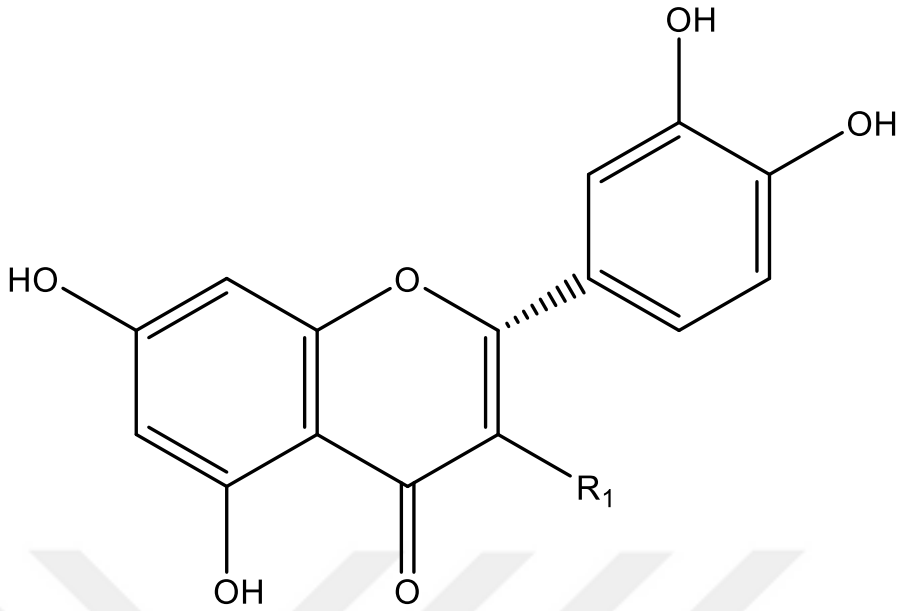

Table 32. Flavonoids from *V. opulus*

Compound	R_1	Plant Part	Reference
(+)-catechin		Flowers	(125)
(-)-epicatechin		Flowers	(125)

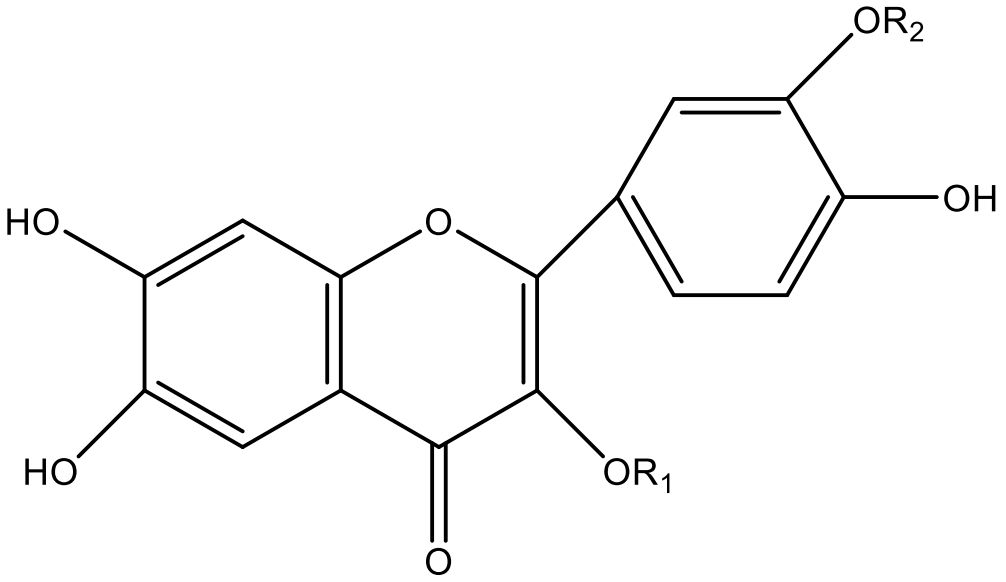

Table 33. Flavonoids from *V. opulus* (Cont.)

Compound	**R_1**	**R_2**	**Plant Part**	**Reference**
Quercetin-3-*O*-rhamnoside	Rhamnose	H	Fruits	(125)
Quercetin-3-*O*-rutinoside	Rutinose	H	Fruits	(103)
Quercetin-3-*O*-sambubioside	Sambubiose	H	Fruits	(103)
Isorhamnetin-3-*O*-rutinoside	Rutinose	CH_3	Fruits	(103)
Isorhamnetin-3-*O*-sambubioside	Sambubiose	CH_3	Fruits	(103)

2.5. High Performance Thin Layer Chromatography (HPTLC)

Chemical composition of the plant materials, including their chemical structure and pharmacological properties which are responsible for their therapeutic actions, is the issue of modern pharmacognosy. Plant materials, galenic preparations, and isolated compounds are supposed to meet various standards which are in pharmacopoeias, monographs etc. Standardization of the plant materials and of herbal preparations mean to guarantee their therapeutic effect and as a result of examination of biologically active components, for the establishment of a consistent biological activity.

Phytochemical studies involve a variety of goals, such as determination of the substance groups, quantitative analysis of active compounds, isolation of substances from the plant materials for their further identification, or physicochemical characterization, and finally, structural analysis of the isolated unknown compounds. There are a wide number of methods for the quality control of herbal components such as chemical methods, spectroscopy, liquid chromatography, gas chromatography, thin layer chromatography, electrophoresis and recently high performance thin layer chromatography (HPTLC). However, there is a widespread need for simple and rapid analytical method for plant based medicines. Among these methods, chromatographic analysis plays a major/critical role, and it has been introduced to all the modern pharmacopoeias (126).

Like all chromatographic techniques, TLC is based on a multistage distribution process, including an adsorbent as a stationary phase and the mobile phase. Chromatographic separation is rely on different retention times of the individual sample components on adsorbents. HPTLC is the advanced and automated form of instrumental TLC. The modern HPTLC technique involves automated sample application and densitometric scanning, is sensitive and completely reliable, suitable for use in qualitative and quantitative analysis. HPTLC is an important and valuable tool for reliable identification, since it provides chromatographic fingerprints that can be visualized and stored as electronic images. There are a broad range of advantages of HPTLC with respect to other methods such as HPLC, spectroscopy, etc. In HPTLC separation process is observable, especially with coloured compounds, several samples can be analyzed on the same plate resulting high throughput screening and a rapid low-

cost analysis, and chromatographic development and detection of the separated spots on a plate are generally separate processes in time, therefore following separation process, the plates can be stored for a long time, and detection performed at a later stage to obtain the analytical information. Important parameters in an HPTLC analyses are selection of appropriate stationary and mobile phase, use of appropriate derivatizing reagent, proper wavelength and interpretation of results.

HPTLC finds applications in diverse fields such as clinical laboratories (drug monitoring, metabolism studies, doping control), environmental analysis (residual, soil, water analyses), and pharmacognosy. Apart from being mainly used to quantitate active ingredients in galenicals or herbal materials, it is also used for testing their stability (127). Hence, the practise of HPTLC has become increasingly practicing at industrial level for routine analysis of many herbal medicines.

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Plant Material

Fruits of *Sambucus ebulus* and *Sambucus nigra* were collected from Yedigöller province just outside of Yedigöller national park. In the first week of September, 2016. Fruits of *Viburnum opulus* were purchased from a local farmer from Kayseri and fruits were harvested in September, 2016. The plant materials were authenticated by Prof. Dr. Erdem Yesilada before any process. Voucher specimens for *S. nigra* L. (YEF 16 0011) and *S. ebulus* L. (YEF 16 0012) have been deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Yeditepe University, İstanbul, Turkey.

3.1.2. Chemicals and Solvents

1,1,3,3-Tetramethoxypropane	Sigma Aldrich; MKBB0326
2,2-diphenyl picryl hydrazil	Sigma Aldrich; 056K1147
2-Thiobarbituric acid	Sigma Aldrich; STBB0632
Acetic acid	Riedel de Haen; 30990
Aluminum chloride	Merck; 8.01081.1000
Ammonium acetate	Carlo Erba; 313507
Ammonium molybdate	Riedel de Haen; 30590
Ascorbic acid	Sigma Aldrich; 065K0003
Bile acids	Sigma Aldrich; CBA-1KT
Butylated hydroxytoluene	Sigma Aldrich; MKBD8339
Chloroform	Lab-Scan; 0344/6
Copper sulphate	Carlo Erba; 364757
Dialysis tubing cellulose membrane	Sigma Aldrich; D9277
Dichloromethane	Merck; 1.06044.2500
Epigallocatechin gallate	Teavigo; WB00044001
Ethyl acetate	Sigma Aldrich; SZBA113S
Ethylenediaminetetraacetic acid disodium salt dihydrate(EDTA)	Sigma Aldrich; BCBC1356
Ferric chloride hexahydrate	Riedel de Haen; 41250
Ferrous chloride tetrahydrate	JT Baker; 0703801013
Ferrous sulphate heptahydrate	JT Baker; 0632701017

Ferrozine	Sigma Aldrich; MKBD0707
Folin-Ciocalteu Reagent	Sigma Aldrich; BCBD5119
Gallic acid	Fluka; 1126284
Hydrochloric acid (37%)	Sigma Aldrich; SZBA2250
Methanol	Sigma Aldrich; SZE9365S
n-Butanol	Fluka; 52150
n-Hexane	Sigma Aldrich; SZBA0655
Neocuproine	Sigma Aldrich; 120M1890V
N,N-dimethyl-p-phenylendiamine	Sigma Aldrich; 07770
Quercetin dehydrate	Sigma Aldrich; 116K1836
Pancreatin	Sigma Aldrich; P7545
Pepsin	Sigma Aldrich; P0525000
Sodium acetate trihydrate	Riedel de Haen; 33450
Sodium bicarbonate	Sigma Aldrich; S6014
Sodium carbonate	Riedel de Haen; 2217A
Sodium chloride	Sigma Aldrich; S7653
Sodium dodecyl sulphate	Merck; 8.2205.1000
Sodium hydroxide	Riedel de Haen; 60030
Sodium phosphate monobasic	Riedel de Haen; 62840
Sulfuric acid (98%)	Riedel de Haen; 62260
Trichloroacetic acid	Riedel de Haen; 23100
Trolox	Sigma Aldrich; BCBF4547V
Vanillin	Fluka; 1435805

3.1.3. Equipments

Balance	Ohaus Explorer
Beaker	(50, 100, 250 mL)
Centrifuge	Sigma 3-16 PK
Eppendorf tubes	(1.5 mL)
Erlenmayer flask	(50, 100, 250 mL)
Hairdryer	Arçelik
High Performance Thin Layer Chromatography	CAMAG
Lyophilizator	Christ Alpha 2-4 LD
Micropipette	(100-1000 microlt) Isolab
Micropipette	(500-5000 microlt) Rainin
Micropipett	(5-50 microL, 20-200 microlt)
Transferpette	
Microplate reader	Thermo Multiskan Ascent
Milli Q water device	Millipore
Oven	Binder
pH meter	Mettler-Toledo MP220
Refrigerator	Arçelik
Rotatory evaporator	Buchi, Heidolph
Spectrophotometer	Spekol 1300
TLC Tanks	CAMAG
Ultrasonic bath	Sonorex RK156BH
Volumetric flasks	(5, 10, 25, 50, 100, 200, 500, 1000 mL)
Vortex	Heidolph Reax
Waterbath	GFL

3.2.1. Extraction

Fresh fruits (2 kg for each extract) were washed and directly mashed by an electrical blender and then the juicy marc was lyophilized at -80°C. Dried samples were separately extracted with 96% methanol or distilled water at room temperature. 500 mL of each solvent were used for 250 g dried fruit sample, and the extraction process in the shaker device was lasted for 3 hours. This practice was repeated three times for both solvent extractions, resulting with yields are in the table below.

Table 34. Extraction Yields

Plant Name	Part Used	Solvent	Yield (%)
Sambucus nigra	Fruits	MeOH	14.25%
Sambucus nigra	Fruits	H_2O	5.93%
Sambucus ebulus	Fruits	MeOH	5.59%
Sambucus ebulus	Fruits	H_2O	7.75%
Viburnum opulus	Fruits	MeOH	7.49%
Viburnum opulus	Fruits	H_2O	4.67%

3.2.2. *In vitro* Digestion Procedure

The simulation of human gastrointestinal digestion model was employed as previously described (128). Briefly, 2500 µL of sample solution were added to a certain volume of gastric milieu including pepsin enzyme and related electrolytes (pH: 2). The mixture was put in a shaking water bath in order to mimic the peristaltic movement in the gastrointestinal system (2h, 37°C). Later, the samples were placed in an ice bath to terminate the enzymatic process. A sample of 2 mL was set aside as “post-gastric” (PG). After that, the dialysis membrane filled with sufficient amount of $NaHCO_3$ to increase the pH of the acidic environment to neutrality was placed inside the mixture so that the gastrointestinal absorption process was resembled. The medium was left for incubation for another 2 h following the addition of bile acids and pancreatin. Afterwards, the content of the dialysis membrane was taken as the “serum-available”

and “bioavailable” content (IN), while the medium outside the membrane was referred as “colon-available” content (OUT). Each sample was stored at –20°C for further analysis.

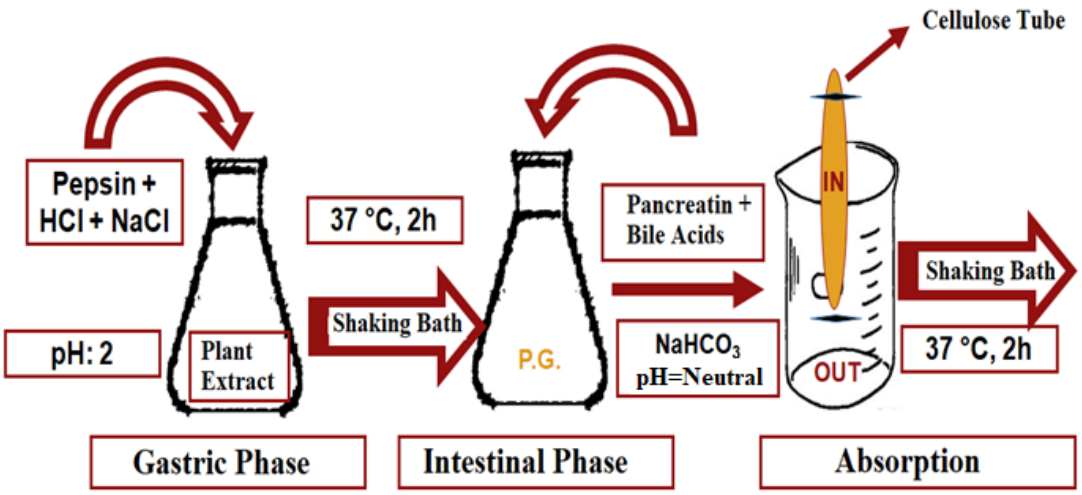

Figure 5. *In vitro* gastric simulation

3.2.3. *In vitro* Studies

3.2.3.1. *In vitro* Determination of Phenolic Profile

3.2.3.1.1. Total Phenolic Content Assay

Total phenolic contents of the samples were calculated as explained earlier in detail (129). After the samples were diluted properly, they were mixed with Folin-Ciocalteu reagent and Na_2CO_3 (20%). The mixture was incubated at 45°C for 30 min, and the absorbances were measured at 765 nm. The results were disclosed as gallic acid equivalents (GAE).

3.2.3.1.2. Total Phenolic Acid Content Assay

The determination of total phenolic acid content was accomplished spectrophotometrically as previously reported (130). According to this method, an interaction between phenolic acids and sodium molybdate–sodium nitrite complex takes place. The absorbance of the formed complex was measured at 490 nm. Results were expressed as caffeic acid equivalents in 1 g dried material.

3.2.3.1.3. Total Flavonoid Content Assay

The spectrophotometrical analysis of total flavonoid content was carried out at 415 according to previously described method (131). Sodium acetate and $AlCl_3$ were mixed with viably diluted samples, and incubated for 30 min. at room temperature. The results were given as quercetin equivalents (QE).

3.2.3.1.4. Total Proanthocyanidin Content Assay

The process explained in our previous study was utilized for the assessment of total proanthocyanidin content (132). Concisely, a certain volume of 1% vanillin and HCl (9 M) were mixed with the dilutions prepared from samples. These mixtures were incubated at 30°C for 20 min. before the measurement of the absorbance at 500 nm. Total proanthocyanidin content was indicated as mg epicatechin equivalents (ECE) in 1 g dry extract.

3.2.3.2. *In vitro* Determination of Antioxidant Capacity

3.2.3.2.1. Free Radical Scavenging Activity Assays

3.2.3.2.1.1. DPPH Radical Scavenging Activity Assay

DPPH radical-scavenging activity test was performed according to the method reported previously (133). Following the dilution of working samples and the addition of 100μM DPPH solution prepared in MeOH, the mixture was incubated 45 min. at room temperature in the dark. The absorbance was read at 517 nm. Butylated hydroxytoluene (BHT) was used as reference substance.

3.2.3.2.1.2. DMPD Radical Scavenging Activity Assay

$DMPD^+$ (N,N-dimethyl-p-phenylendiamine) radical reagent was attained by mixing 100 mM aqueous DMPD solution with 50 mM ferric chloride and acetate buffer (pH 5.25). A certain volume of the solution was added to 50 μL of the diluted samples and incubated for 10 min. Absorbance was read at 505 nm. The results were expressed as mg Trolox equivalent in 1 g dry extract (134).

3.2.3.2.2. Metal Reducing Activity Assays

3.2.3.2.2.1. Ferric Reducing Antioxidant Power Assay

FRAP activity of samples was measured by the method of (135). Aliquots of the samples were mixed with 0.26 mL of FRAP reagent, and diluted with distilled water to

0.3 mL. Following to the period of incubation for 30 min, the absorbance was measured at 593 nm. A standard curve of ferrous chloride (0.25–2 mM) was prepared to assess the results. BHT was used as reference compound. The results were expressed as mM $FeSO_4$ per g sample.

3.2.3.2.2.2. Cupric Reduced Antioxidant Capacity

CUPRAC activity of each sample was measured as reported by Apak et al. (136). Same volumes of neocuproine, ammonium acetate buffer and $CuSO_4$ were mixed with the samples. The mixtures were incubated for 60 min., and the absorbance was calculated at 450 nm. The results were stated as mg ascorbic acid equivalent per g dry sample.

3.2.3.3. Total Antioxidant Activity Assay

The method described by (137) was conducted for the calculation of total antioxidant capacity. Aliquots of sample solutions were added to the reaction mixture composed of sodium phosphate monobasic, sulfuric acid and ammonium molybdate. Then, the mixtures were incubated for 90 min at 95°C. After the incubation process, the absorbance was measured at 695 nm. Total antioxidant capacity was demonstrated as mg ascorbic acid equivalent per 1 g dry sample.

3.2.3.4. Ferrous Ion-Chelating Capacity

The ferrous ion-chelating capacity was determined via method first revealed by according to the method developed by Guo et al. Each sample was mixed with 50 μL 2 mM $FeCL_2.4H_2O$. After 3.7 ml distilled water was added. The reaction was started by the addition of 200 μL of ferrozine (5 mM). Incubation process was 10 min at room temperature. Absorbance was read at 562 nm. EDTA was used as reference (138).

3.2.4. Qualitative and Quantitative Analysis with HPTLC

Qualitative and quantitative analyses of bioactive major metabolites were conducted via a validated method (139). Four fractions of both extracts (Non-digested, post-gastric, colon-available, serum-available) analyzed by using High Performance Thin Layer Chromatography (HPTLC). Sample applicator (Linomat 5), TLC Visualizer, Automated Development Chamber (ADC2) and Immersion Device were the equipments

attached to the device (CAMAG, Muttenz, Switzerland). WinCATS version 1.4.8., CAMAG was used as software. Normal phase glass plates with 60 layers (2 μm thickness) glass-baked silica gel were used for analyses. Plates were pre-washed with MeOH and TLC Plate Heater III was used for drying for 5 minutes at 80 °C. Reference solutions and samples were applied with syringes (100 μl, Hamilton, Bonaduz, Switzerland). Operation conditions were determined as follows: injection volume; 0.5–6 μL, syringe delivery speed; 100 nL/s, distance from bottom; 15 mm, length of chromatogram; 70 mm from the application point band width of analytes; 8 mm. Saturated $MgCl_2 \cdot 6H_2O$ solution was used to set relative humidity to 33% before the development process. The mobile phase used was ethyl acetate:dichloromethane:acetic acid:formic acid:water (100:25:10:10:11 v/v/v/v/v). After the development process, HPTLC plates dried for 3 min. at 100 °C with TLC Plate Heater III, CAMAG. Natural Product Reagent (NPR: 1 g diphenylboric acid 2-aminoethylester dissolved in 200 mL of ethyl acetate) was used by Immersion Device. TLC Visualizer and TLC Scanner were used for determination from derivatized plates under 366 nm UV light. 100 μg/mL was the concentration of standards which were dissolved in MeOH. 10 mg/ml concentration was used for all fractions of sample solution. The calibration curve was established according the linearity between the peak areas and the standard concentrations. Standard concentrations were 100 μg/ml and different volumes of standards were applied (between 1-6 μl). The correlation coefficients (r^2) were found to be >0.98 for the quantification of chlorogenic acid and rutin.

3.2.5. Statistics

Each of the tests and analyses were conducted in triplicate. After the calculation of mean ± standard deviation, the results were statistically compared with ANOVA test. Tukey-Kramer post hoc test was run for multiple comparisons. $p<0.05$ was defined as statistically significant difference.

4. RESULTS

4.1. Results of Total Phenolic Content Assay

4.1.1. Total Phenolic Content of *Sambucus nigra* Fruit Extracts

Total phenolic content of both extracts were affected comparable by human digestion simulation process. After the post-gastric phase amount of total phenolic content ascended significantly for both extracts: 46.37 ± 1.28 to 59.94 ± 2.54 for SNM and 44.91 ± 2.15 to 56.38 ± 3.58 mg GAE/g for SNA. In intestinal phase no significant alteration was observed and total bioavailability resulted 118.76% and 119.66% significantly. Total phenolic content results exhibiting equivalent sequence for both extracts.

Table 35. Total phenolic Content of *Sambucus nigra* Fruit Extracts

Samples	*ND* [A]	*PG*	*OUT*	*IN*	*BAvI (%)*
SNM[**]-TPC[BC]	$46.37^{a} \pm 1.28$	$59.94^{b} \pm 2.54$	$57.61^{bc} \pm 1.20$	$55.07^{c} \pm 0.88$	118.76 %
SNA[***]-TPC	$44.91^{a} \pm 2.15$	$56.38^{b} \pm 3.58$	$57.08^{b} \pm 2.12$	$53.74^{b} \pm 0.17$	119.66 %

[A] The abbreviations for samples are *ND: non-digested, PG: postgastric, OUT: Colon available IN: bioavailable, BAvI: Bioavailability index*

[B] The abbreviation for the analysis is TPC: Total phenolic content

[C] Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg gallic acid equivalents (GAE) in 1 g sample.

* Different letters in the same row indicate significance ($p < 0.05$).

** Methanolic extract of *Sambucus nigra* L. fruit

*** Aqueous extract of *Sambucus nigra* L. fruits

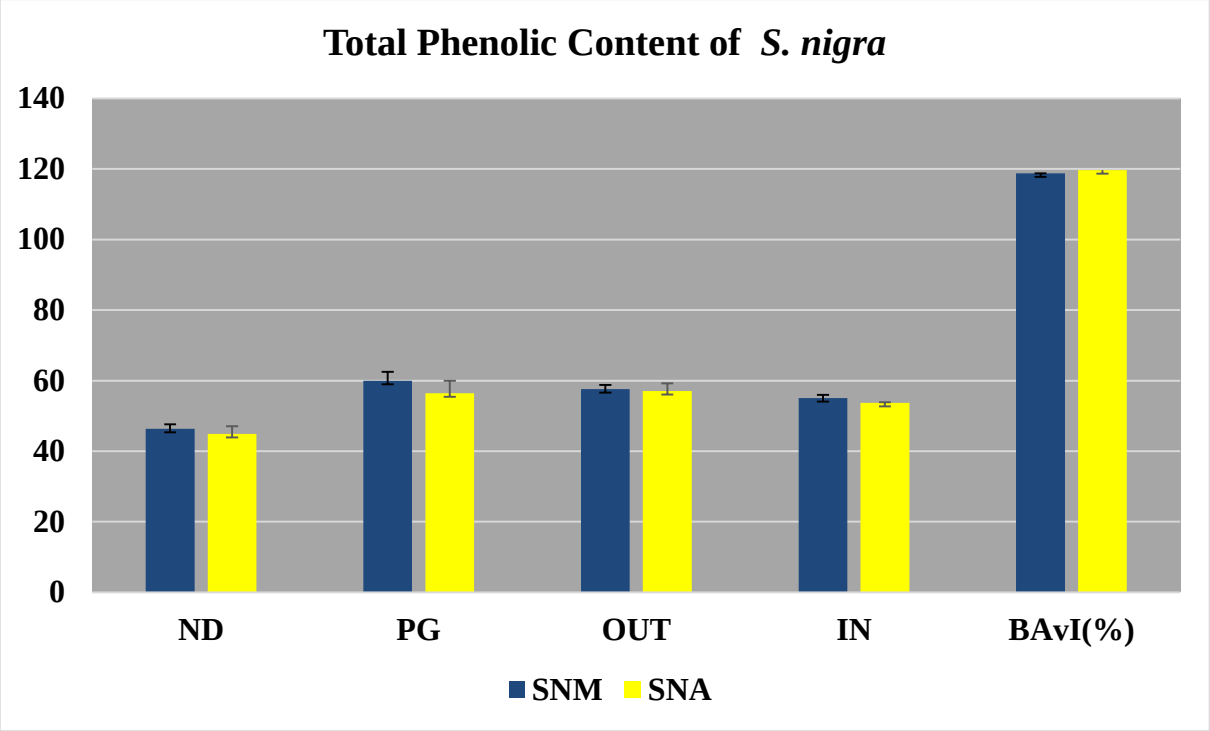

Figure 6. Total Phenolic Content of *Sambucus nigra* Fruit Extracts

4.1.2. Total Phenolic Content of *Sambucus ebulus* Fruit Extracts

Total phenolic content (TPC) of both extracts showed slight but significant elevation, subsequent to the simulation process. TPC of SEM increased from 48.19 ± 0.49 mg/g GAE to 51.13 ± 0.24; meanwhile TPC of SEA correspondingly increased from 44.51 ± 0.35 mg/g GAE to 48.16 ± 1.24, from ND fraction to IN fraction, respectively. Bioavailability of TPC was slightly lesser in SEM than SEA extract (106.10%, 108.20%, respectively).

Table 36. Total phenolic Content of *Sambucus ebulus* Fruit Extracts

Samples	*ND* [A]	*PG*	*OUT*	*IN*	*BAvI (%)*
SEM** -TPC[BC]	$48.19^{a} \pm 0.49$	$54.60^{b} \pm 1.01$	$53.28^{bc} \pm 0.39$	$51.13^{d} \pm 0.24$	106.10%
SEA*** -TPC	$44.51^{a} \pm 0.35$	$52.11^{bc} \pm 0.78$	$50.94^{c} \pm 0.96$	$48.16^{d} \pm 1.24$	108.20%

[A] The abbreviations for samples are *ND: non-digested, PG: postgastric, OUT: Colon available IN: bioavailable, BAvI: Bioavailability index*
[B] The abbreviation for the analysis is TPC: Total phenolic content
[C] Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg gallic acid equivalents (GAE) in 1 g sample.
* Different letters in the same row indicate significance ($p < 0.05$).
** Methanolic extract of *Sambucus ebulus* L.fruit
*** Aqueous extract of *Sambucus ebulus* L. fruits

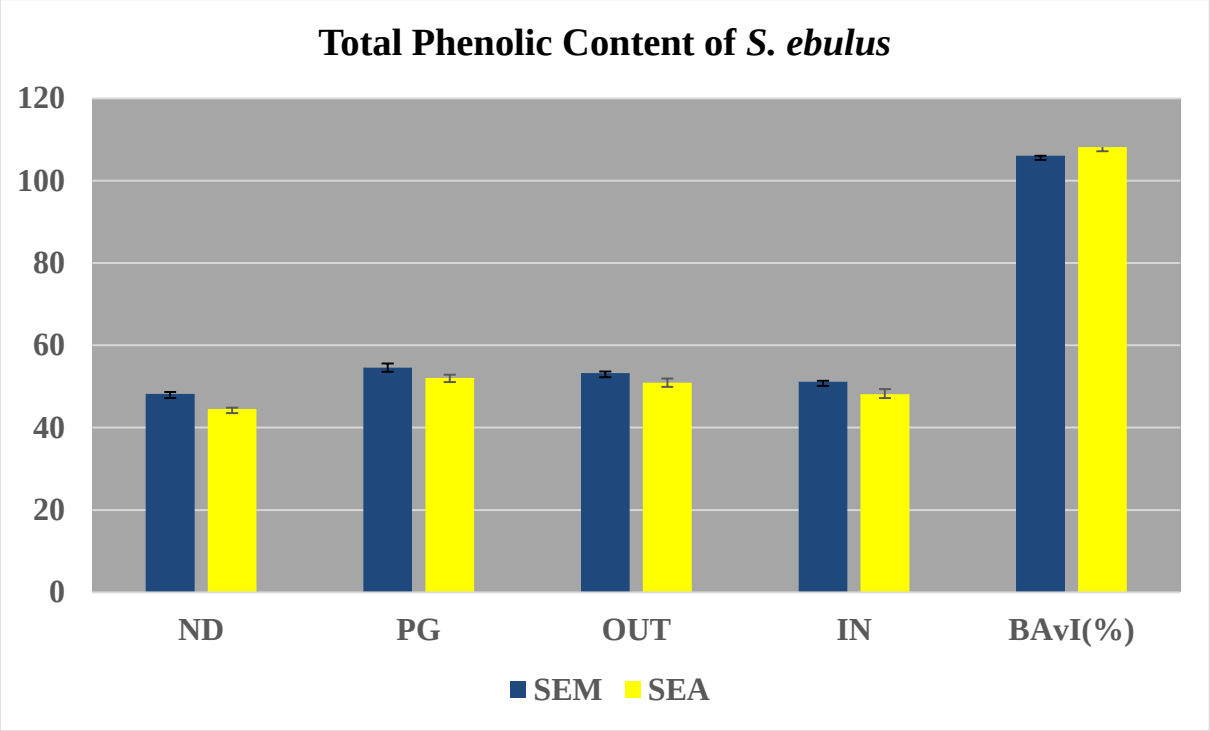

Figure 7. Total Phenolic Content of *Sambucus ebulus* Fruit Extracts

4.1.3. Total Phenolic Content of *Viburnum opulus* Fruit Extracts

Data presented in Table 38 revealed total amounts of phenolic compounds before and after the simulation of human digestion. Amount of total phenolic compounds (TPC) was dramatically decreased after the digestion procedure. TPC of VOM was decreased from 40.17 ± 1.11 mg/g GAE to 14.77 ± 0.18 and TPC of VOA decreased from 25.64 ± 1.06 to 15.23 ± 0.31 in ND fraction and IN fraction, respectively.

Table 37. Total phenolic contents of *V. opulus* Fruit extracts

Samples	*ND* [A]	*PG*	*OUT*	*IN*	*BAvI (%)*
VOM** -TPC[BC]	$40.17^{a} \pm 1.11$	$26.43^{b} \pm 0.91$	$23.77^{c} \pm 0.51$	$14.77^{d} \pm 0.18$	36.77 %
VOA*** -TPC	$25.64^{a} \pm 1.06$	$21.08^{b} \pm 0.81$	$19.28^{c} \pm 0.04$	$15.23^{d} \pm 0.31$	59.40 %

[A] The abbreviations for samples are *ND: non-digested, PG: postgastric, OUT: Colon available IN: bioavailable, BAvI: Bioavailability index*
[B] The abbreviation for the analysis is TPC: Total phenolic content
[C] Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg gallic acid equivalents (GAE) in 1 g sample.
* Different letters in the same row indicate significance ($p < 0.05$).
** Methanolic extract of *Viburnum opulus* L.fruit
*** Aqueous extract of *Viburnum opulus L.* fruits

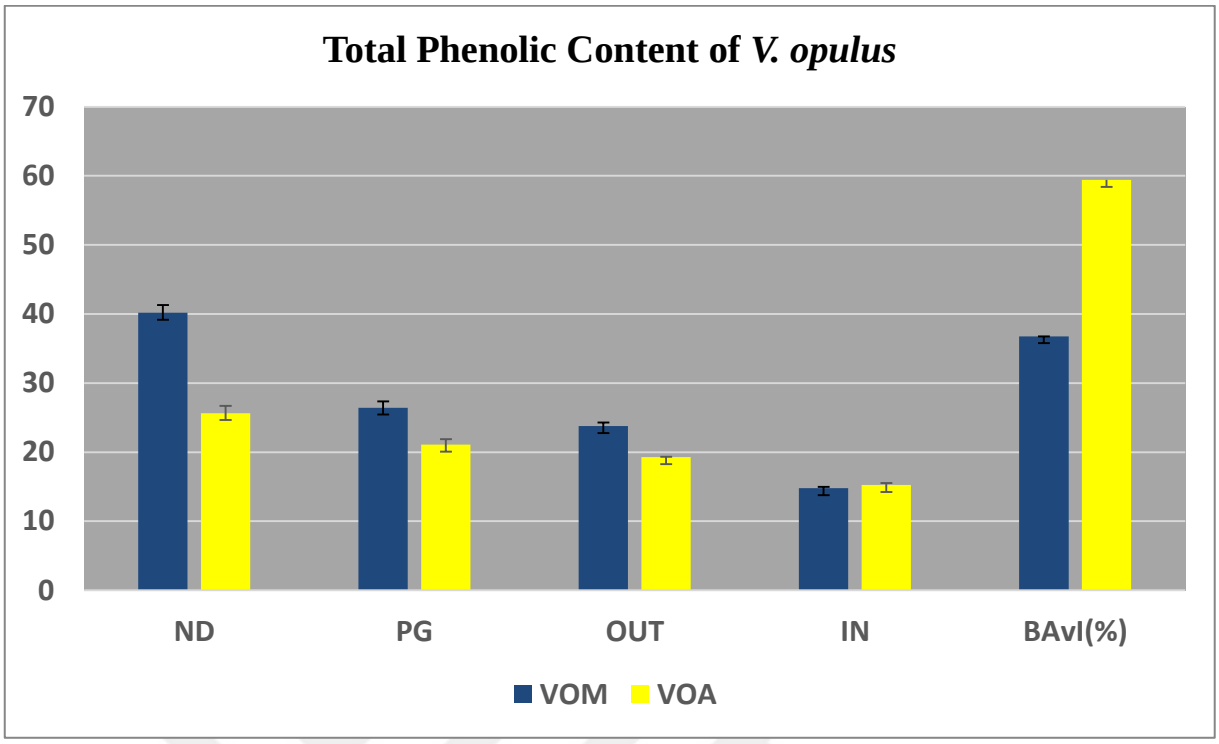

Figure 8. Total phenolic contents of *V. opulus* Fruit extracts

4.2. Results of Total Phenolic Acid Content Assay

4.2.1. Total Phenolic Acid Content of *Sambucus nigra* Fruit Extracts

Unlike total phenolic content, SNM has higher total phenolic acid content than SNA (43.47 ± 0.45 mg CAE/g and 37.16 ± 0.89 respectively). After the gastric phase no significant alteration was observed for both extracts. Even though in colon available phase there were still no significant alterations for both extracts, in serum available phase there was significant reduction for both extracts which resulted 87.72% and 90.04% bioavailability, respectively.

Table 38. Total Phenolic Acid Content of *Sambucus nigra* Fruit Extracts

Samples	*ND* [A]	*PG*	*OUT*	*IN*	*BAvI (%)*
SNM[**]-TPAC[BC]	$43.47^{a} \pm 0.45$	$42.19^{ab} \pm 0.41$	$40.26^{bc} \pm 1.96$	$38.13^{c} \pm 0.94$	87.72 %
SNA[***]-TPAC	$37.16^{a} \pm 0.89$	$36.28^{a} \pm 1.89$	$37.19^{a} \pm 0.02$	$33.46^{b} \pm 0.28$	90.04 %

[A] The abbreviations for samples are *ND: non-digested, PG: postgastric, OUT: Colon available IN: bioavailable, BAvI: Bioavailability index*
[B] The abbreviation for the analysis is TPAC: Total phenolic acid content
[C] Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg caffeic acid equivalents (CAE) in 1 g sample
* Different letters in the same row indicate significance ($p < 0.05$).
** Methanolic extract of *Sambucus nigra* L. fruit
*** Aqueous extract of *Sambucus nigra* L. fruits

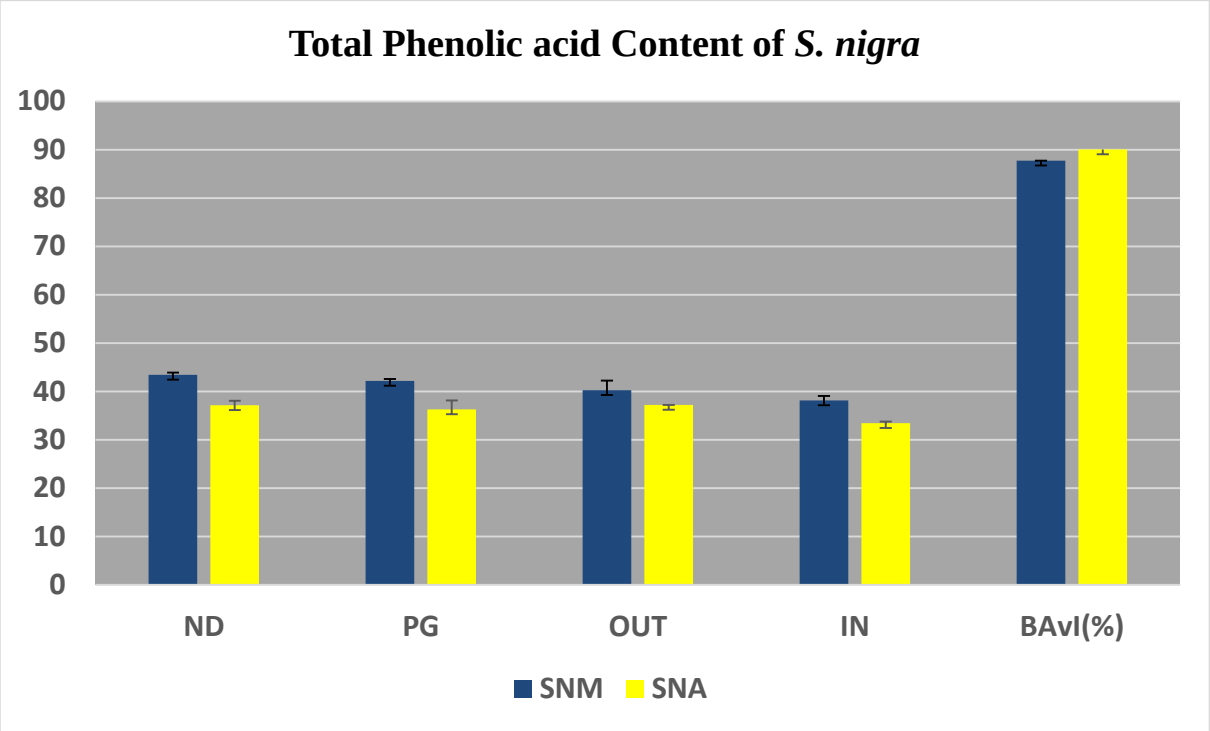

Figure 9. Total Phenolic Acid Content of *Sambucus nigra* Fruit Extracts

4.2.2. Total Phenolic Acid Content of *Sambucus ebulus* Fruit Extracts

Total phenolic acid content (TPAC) was similarly higher in non-digested SEM extract. On the contrary, TPAC bioavailability of SEA was higher. TPAC of SEM reduced in every fraction significantly, while TPAC of SEA remained stable after modest alteration in the post-gastric phase. Bioavailability of total phenolic acids was higher in SEA extract.

Table 39. Total Phenolic Acid Content of *Sambucus ebulus* Fruit Extracts

Samples	*ND* [A]	*PG*	*OUT*	*IN*	*BAvI (%)*
SEM** -TPAC[BC]	$44.27^{a} \pm 0,10$	$43.18^{b} \pm 0,07$	$36.13^{c} \pm 0,56$	$31.47^{d} \pm 0,14$	71.09%
SEA*** -TPAC	$28.13^{a} \pm 0.36$	$23.27^{b} \pm 1.28$	$24.16^{b} \pm 0.74$	$22.28^{b} \pm 0.08$	79.20%

[A] The abbreviations for samples are *ND: non-digested, PG: postgastric, OUT: Colon available IN: bioavailable, BAvI: Bioavailability index*
[B] The abbreviation for the analysis is TPAC: Total phenolic acid content
[C] Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg caffeic acid equivalents (CAE) in 1 g sample.
* Different letters in the same row indicate significance ($p < 0.05$).
** Methanolic extract of *Sambucus ebulus* L.fruit
*** Aqueous extract of *Sambucus ebulus* L. fruits

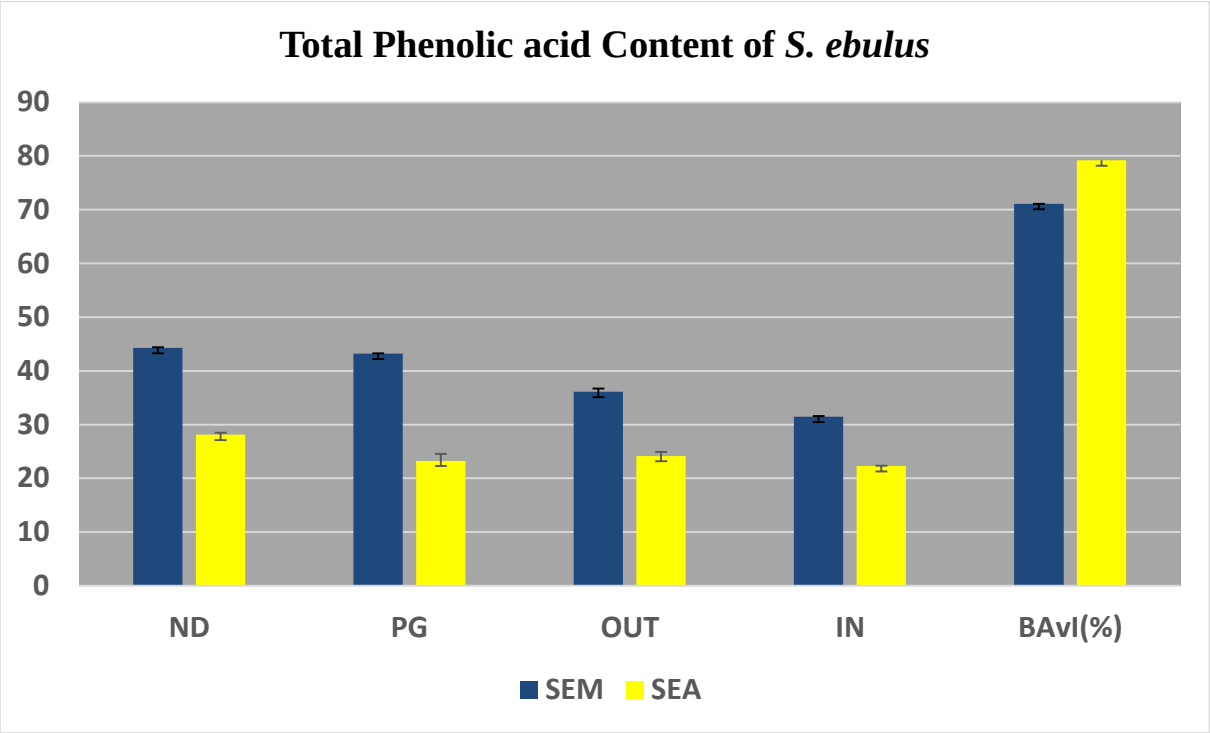

Figure 10. Total Phenolic Acid Content of *Sambucus ebulus* Fruit Extracts

4.2.3. Total Phenolic Acid Content of *Viburnum opulus* Fruit Extracts

Total phenolic acids (TPA) in VOM were strikingly reduced, while reduction in VOA was modest. TPA of VOM was decreased from 50.98 ± 1.46 mg/g CAE to 36.87 ± 2.21 and TPA of VOA was decreased 42.54 ± 1.13 to 38.56 ± 0.26 in ND fraction and IN fraction, respectively.

Table 40. Total Phenolic Acid Content of *Viburnum opulus* Fruit Extracts

Samples	*ND* [A]	*PG*	*OUT*	*IN*	*BAvI (%)*
VOM[**]-TPAC[BC]	$50.98^{a} \pm 1.46$	$44.24^{b} \pm 3.41$	$43.75^{b} \pm 0.96$	$36.87^{c} \pm 2.21$	72.32 %
VOA[***]-TPAC	$42.54^{a} \pm 1.13$	$40.86^{ab} \pm 0.46$	$40.28^{b} \pm 0.98$	$38.56^{bc} \pm 0.26$	90.64 %

[A] The abbreviations for samples are *ND: non-digested, PG: postgastric, OUT: Colon available IN: bioavailable, BAvI: Bioavailability index*
[B] The abbreviation for the analysis is TPAC: Total phenolic acid content
[C] Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg caffeic acid equivalents (CAE) in 1 g sample
* Different letters in the same row indicate significance ($p < 0.05$).
** Methanolic extract of *Viburnum opulus* L.fruit
*** Aqueous extract of *Viburnum opulus L.* fruits

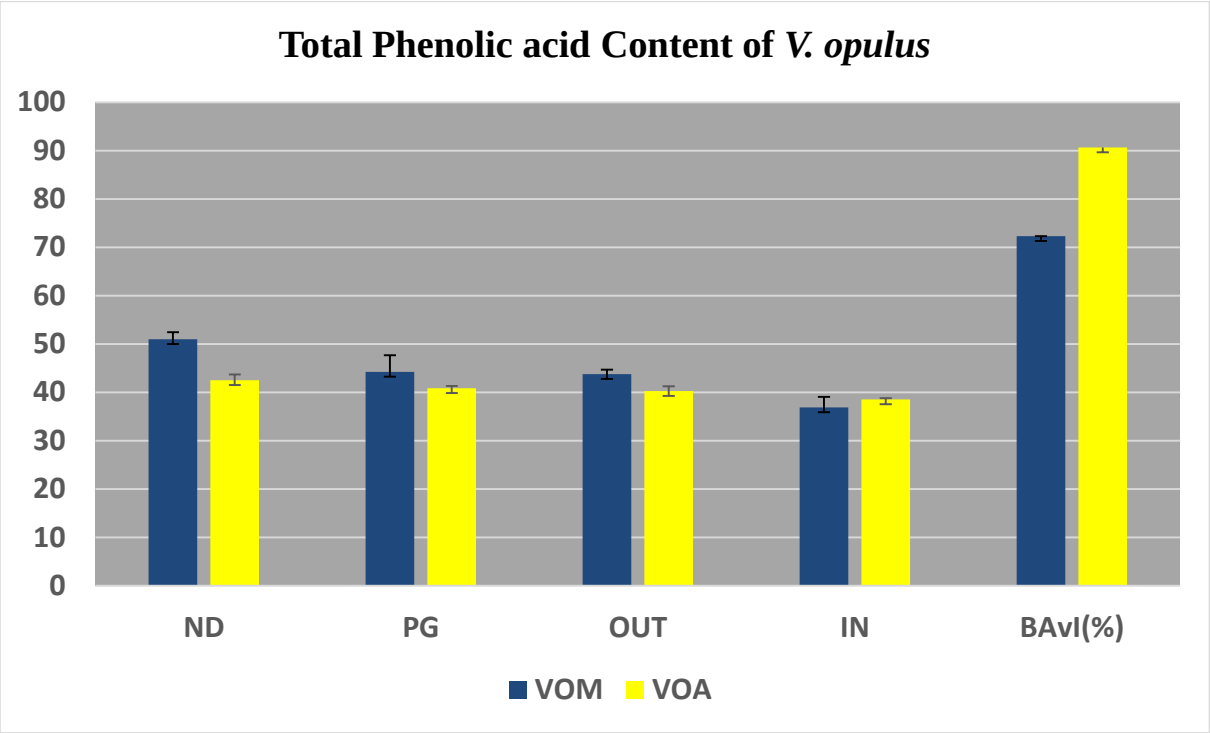

Figure 11. Total Phenolic Acid Content of *Viburnum opulus* Fruit Extracts

4.3. Results of Total Flavonoid Content Assay

4.3.1. Total Flavonoid Content of *Sambucus nigra* Fruit Extract

Total flavonoid content of SNM was relatively higher than SNA (32.80 ± 1.94 mg QE/g and 25.53 ± 0.91 respectively). Total flavonoid content of SNM was not affected from gastric medium and intestinal medium, however bioavailability rate was lower than SNA (77.59% and 93.58% respectively). TFC of SNA was not effected in PG and OUT phase, also bioavailability index was higher than SNM thus amounts in serum available phase were corresponding for both extracts.

Table 41. Total Flavonoid Content of *Sambucus nigra* Fruit Extract

Samples	*ND* [A]	*PG*	*OUT*	*IN*	*BAvI (%)*
SNM** -TFC[BC]	$32.80^{a} \pm 1.94$	$31.34^{a} \pm 2.59$	$33.25^{a} \pm 0.47$	$25.45^{b} \pm 0.86$	77.59 %
SNA***-TFC	$25.53^{ab} \pm 0.91$	$26.73^{a} \pm 1.60$	$25.59^{ab} \pm 0.28$	$23.89^{b} \pm 0.18$	93.58 %

[A] The abbreviations for samples are *ND: non-digested, PG: postgastric, OUT: Colon available IN: bioavailable, BAvI: Bioavailability index*
[B] The abbreviation for the analysis is TFC: Total flavonoid content
[C] Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg quercetin equivalents (QE) in 1 g sample
* Different letters in the same row indicate significance ($p < 0.05$).
** Methanolic extract of *Sambucus ebulus* L. fruit
*** Aqueous extract of *Sambucus ebulus* L. fruits

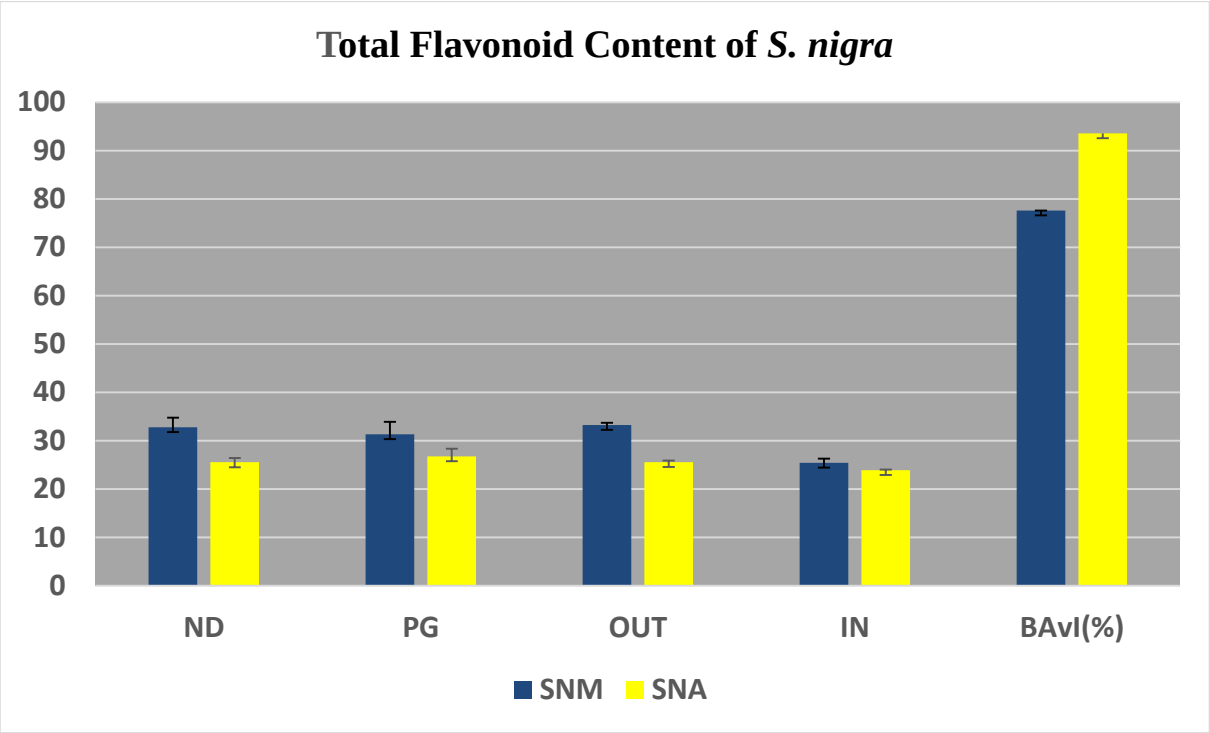

Figure 12. Total Flavonoid Content of *Sambucus nigra* Fruit Extract

4.3.2. Total Flavonoid Content of *Sambucus ebulus* Fruit Extract

Total flavonoid content of both extracts reduced significantly from ND to PG phase. After PG phase, no significant changes were observed in SEM extract while reduction in SEA extract was modest. Flavonoids bioavailability of SEA was higher than SEM extract.

Table 42. Total Flavonoid Content of *Sambucus ebulus* Fruit Extract

Samples	*ND* [A]	*PG*	*OUT*	*IN*	*BAvI (%)*
SEM** -TFC[BC]	$37.62^{a} \pm 1.43$	$29.05^{b} \pm 0.46$	$27.36^{b} \pm 0.24$	$26.87^{b} \pm 1.59$	71.42%
SEA*** -TFC	$28.44^{a} \pm 1.93$	$25.14^{b} \pm 0.52$	$26.26^{ab} \pm 0.83$	$24.42^{bc} \pm 1.16$	85.86%

[A] The abbreviations for samples are *ND: non-digested, PG: postgastric, OUT: Colon available IN: bioavailable, BAvI: Bioavailability index*
[B] The abbreviation for the analysis is TFC: Total flavonoid content
[C] Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg quercetin equivalents (QE) in 1 g sample.
* Different letters in the same row indicate significance ($p < 0.05$).
** Methanolic extract of *Sambucus ebulus* L.fruit
*** Aqueous extract of *Sambucus ebulus* L. fruits

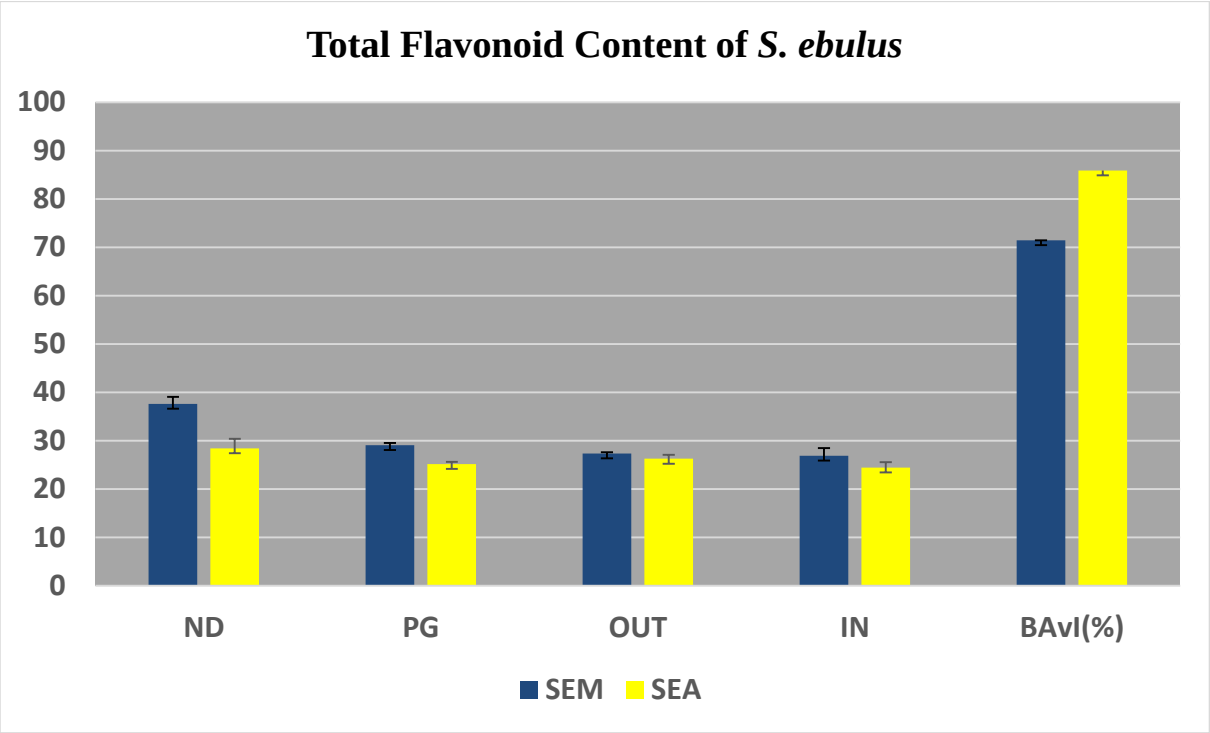

Figure 13. Total Flavonoid Content of *Sambucus ebulus* Fruit Extract

4.3.3. Total Flavonoid Content of *Viburnum opulus* Fruit Extract

No extreme alterations were measured in both extracts in total flavonoid content assay. Total flavonoid content was slightly decreased in VOM from 25.09 mg/g QE ± 0.77 to 23.35 ± 0.37 and in VOA from 24.57 ± 0.47 to 22.02 ± 0.44.

Table 43. Total Flavonoid Content of *Viburnum opulus* Fruit Extract

Samples	*ND* [A]	*PG*	*OUT*	*IN*	*BAvI (%)*
VOM[**]-TFC[BC]	25.09[a] ± 0.77	25.61[a] ± 0.37	25.54[a] ± 0.88	23.35[b] ± 0.37	93.06 %
VOA[***]-TFC	24.57[a] ± 0.47	24.18[a] ± 0.55	24.57[a] ± 0.41	23.02[b] ± 0.44	93.69 %

[A] The abbreviations for samples are *ND: non-digested, PG: postgastric, OUT: Colon available IN: bioavailable, BAvI: Bioavailability index*
[B] The abbreviation for the analysis is TFC: Total flavonoid content
[C] Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg quercetin equivalents (QE) in 1 g sample
* Different letters in the same row indicate significance ($p < 0.05$).
** Methanolic extract of *Viburnum opulus* L.fruit
*** Aqueous extract of *Viburnum opulus L.* fruits

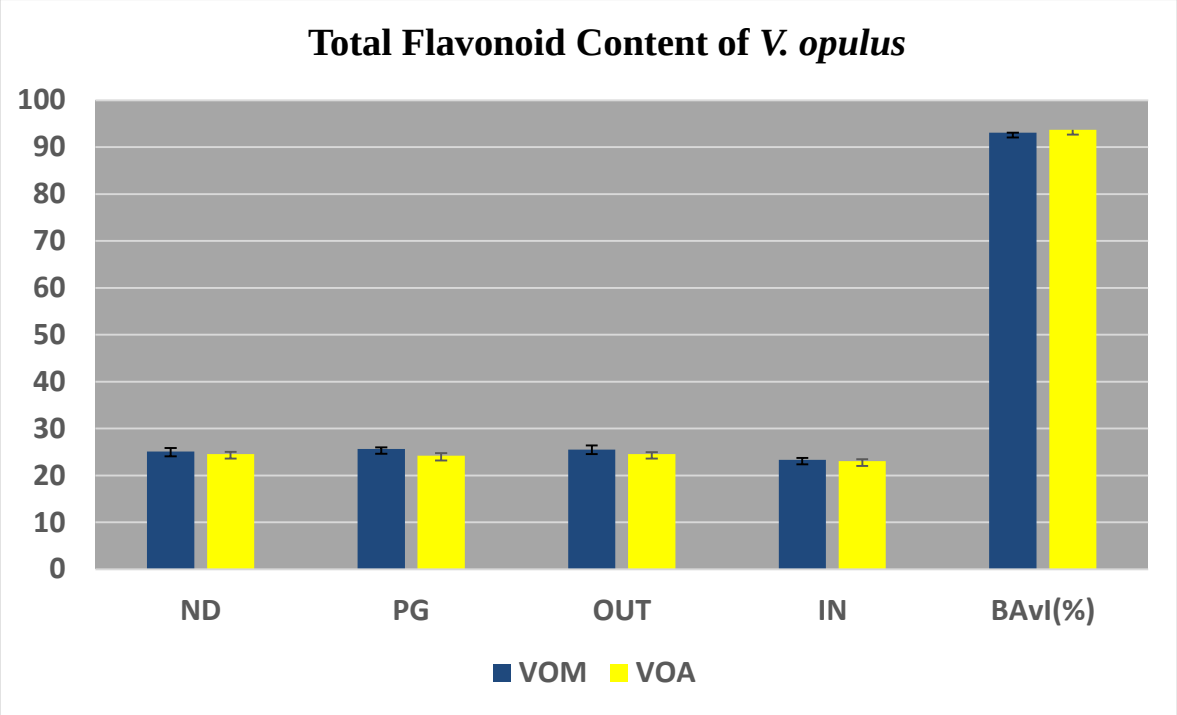

Figure 14. Total Flavonoid Content of *Viburnum opulus* Fruit Extract

4.4. Results of Total Proanthocyanidin Content Assay

4.4.1. Total Proanthocyanidin Content of *Sambucus nigra* Fruit Extracts

Total proanthocyanidin content of SNM was adequately higher than SNA (105.94 ± 3.21 mg ECE/g and 81.93 ± 2.56 respectively). In every step of digestion amounts of proanthocyanidin showed descent substantially. Total bioavailability of extracts was very low when compared other phenolic compounds (29.79% and 27.15% respectively).

Table 44. Total Proanthocyanidin Content of *Sambucus nigra* Fruit Extracts

Samples	*ND* [A]	*PG*	*OUT*	*IN*	*BAvI (%)*
SNM** – TPACC[BC]	$105.64^{a} \pm 3.21$	$82.66^{b} \pm 1.65$	$64.27^{c} \pm 1.11$	$31.47^{d} \pm 0.05$	29.79 %
SNA*** - TPACC	$81.93^{a} \pm 2.56$	$59.27^{b} \pm 2.18$	$41.42^{c} \pm 2.90$	$22.24^{c} \pm 2.13$	27.15 %

[A] The abbreviations for samples are *ND: non-digested, PG: postgastric, OUT: Colon available IN: bioavailable, BAvI: Bioavailability index*
[B] The abbreviation for the analysis is TPACC: Total proanthocyanidin content
[C] Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg epicatechin equivalents (ECE) in 1 g sample.
* Different letters in the same row indicate significance ($p < 0.05$).
** Methanolic extract of *Sambucus nigra* L. fruits
*** Aqueous extract of *Sambucus nigra* L. fruits

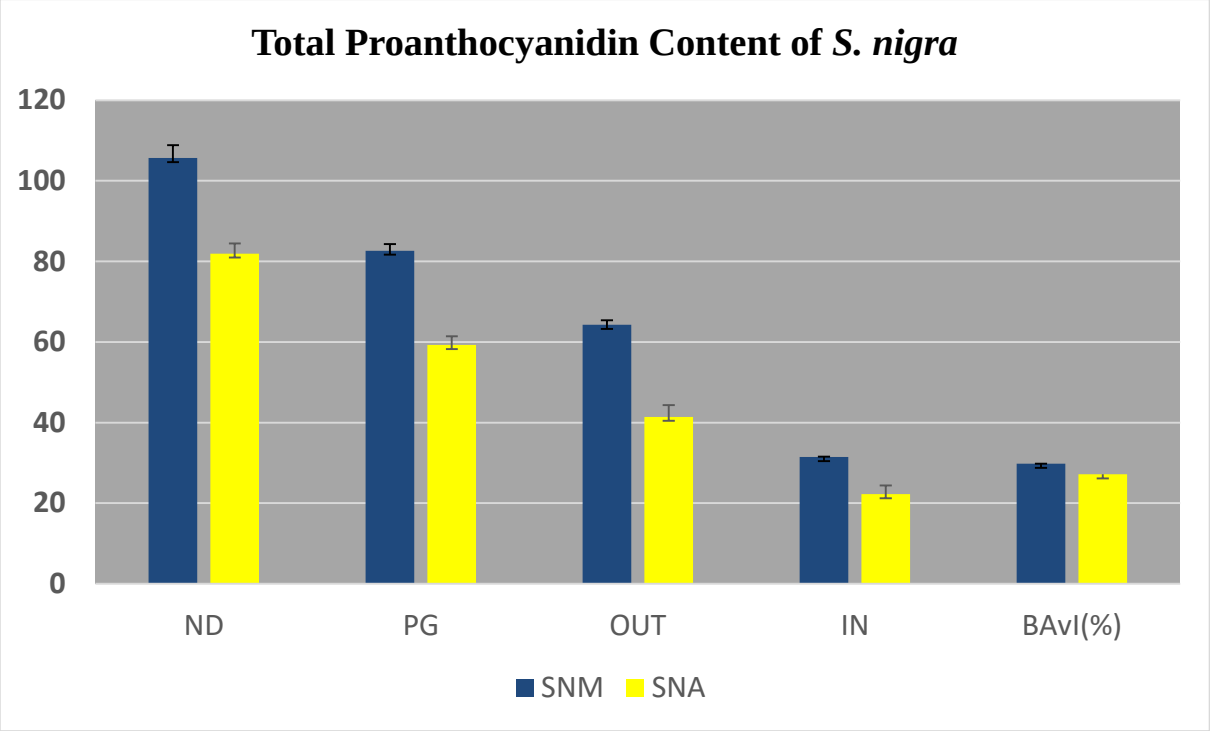

Figure 15. Total Proanthocyanidin Content *Sambucus nigra* Fruit Extracts

4.4.2. Total Proanthocyanidin Content of *Sambucus ebulus* Fruit Extracts

Total proanthocyanidin content (TPACC) of extracts exerted lowest bioavailability among all phenolic assays. Although non-digested SEM extract possessed higher proanthocyanidin content, quantity was decreased in every further step of human digestion significantly. SEA extract had lower TPACC but higher proanthocyanidin bioavailability. Non-digested SEA had 59.66 ± 2.56 TPACC, but it decreased considerably to 16.28 ± 0.96 at IN fraction. Proanthocyanidin bioavailability of SEA was slightly higher than SEM (27.29%, 24.75% respectively).

Table 45. Total Proanthocyanidin Content of *Sambucus ebulus* Fruit Extracts

Samples	*ND* [A]	*PG*	*OUT*	*IN*	*BAvI (%)*
SEM-TPACC[BC]	98.43[a] ± 3.74	60.28[b] ± 1.42	44.48[c] ± 2.16	24.36[d] ± 1.16	24.75%
SEA-TPACC	59.66[a] ± 2.56	41.27[b]± 1.98	33.62[c] ± 0.65	16.28[d] ± 0.96	27.29%

[A] The abbreviations for samples are *ND: non-digested, PG: postgastric, OUT: Colon available IN: bioavailable, BAvI: Bioavailability index*
[B] The abbreviation for the analysis is TFC: Total flavonoid content
[C] Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg epicatechin equivalents (ECE) in 1 g sample.
* Different letters in the same row indicate significance ($p < 0.05$).
** Methanolic extract of *Sambucus ebulus* L. fruits
*** Aqueous extract of *Sambucus ebulus* L. fruits

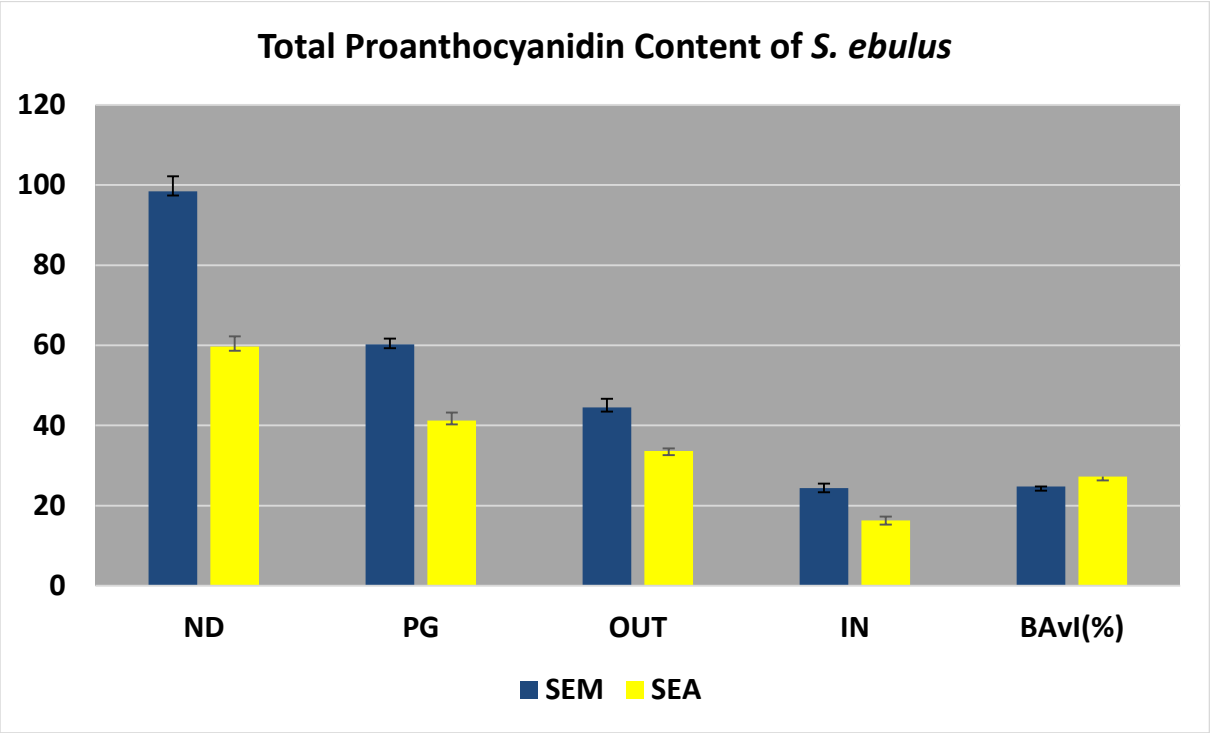

Figure 16. Total Proanthocyanidin Content of *Sambucus ebulus* Fruit Extracts

4.4.3. Total Proanthocyanidin Content of *Viburnum opulus* Fruit Extracts

Total proanthocyanidin content assay was performed on *V. opulus* fruits. However no significant result was obtained from assay. This result indicates that proanthocyanidin content of *V. opulus* has lower concentration than LOQ.

4.5. Results of HPTLC Analysis

4.5.1. HPTLC Analysis of *Sambucus nigra* Fruit Extracts

Chlorogenic acid amount of SNM was notably higher than SNA (12.44 ± 0.42 mg/g dry extract and 5.44 ± 0.05 respectively). Mild reduction in every step of digestion resulted similar bioavailability index results (75.32% and 74.82% respectively). Rutin amount was significantly higher in methanolic extract (SNM: 12.44 ± 0.42 and SNA: 5.44 ± 0.05). In post-gastric phase rutin amount was showed a significant increase, in contrary to SNA which showed extreme decline. These results caused variance in bioavailability index (70.04% and 36.31% respectively).

Table 46. HPTLC Analysis of *Sambucus nigra* Fruit Extracts

Samples	*ND* [A]	*PG*	*OUT*	*IN*	*BAvI (%)*
SNM- Chlorogenic acid[B]	12.44 ± 0.42	12.00 ± 0.35	11.67 ± 0.31	9.37 ± 0.41	75.32%
SNA- Chlorogenic acid	5.44 ± 0.05	5.46 ± 0.09	4.10 ± 0.11	4.07 ± 0.01	74.82%
SNM – Rutin	15.99 ± 2.40	22.06 ± 0.23	21.39 ± 0.06	11.20 ± 0.85	70.04%
SNA – Rutin	9.06 ± 0.08	4.26 ± 0.08	7.80 ± 0.17	3.29 ± 0.24	36.31%

[A] The abbreviations for samples are *ND: non-digested, PG: postgastric, OUT: Colon available IN: bioavailable, BAvI: Bioavailability index*
[B] Results are expressed as mg/g dry extract with standart deviations
* Different letters in the same row indicate significance ($p < 0.05$).
** Methanolic extract of *Sambucus nigra* L. fruit
*** Aqueous extract of *Sambucus nigra* L. fruits

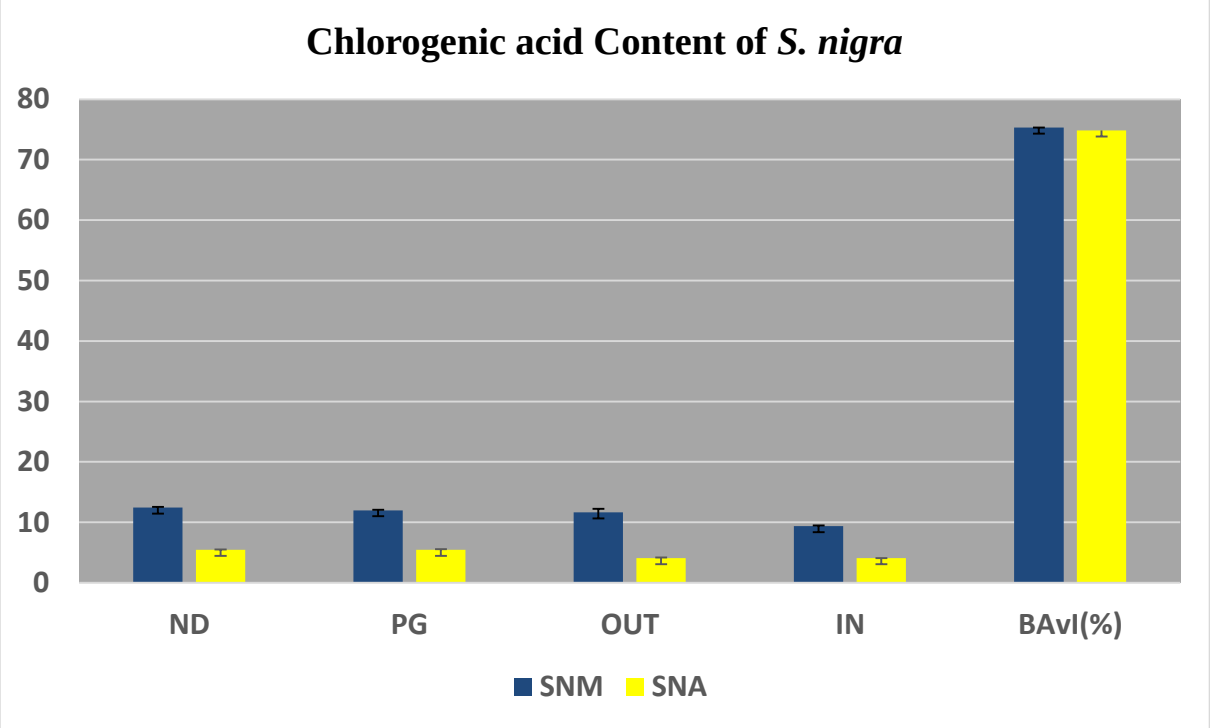

Figure 17. Chlorogenic acid content of *Sambucus nigra* Fruit Extracts

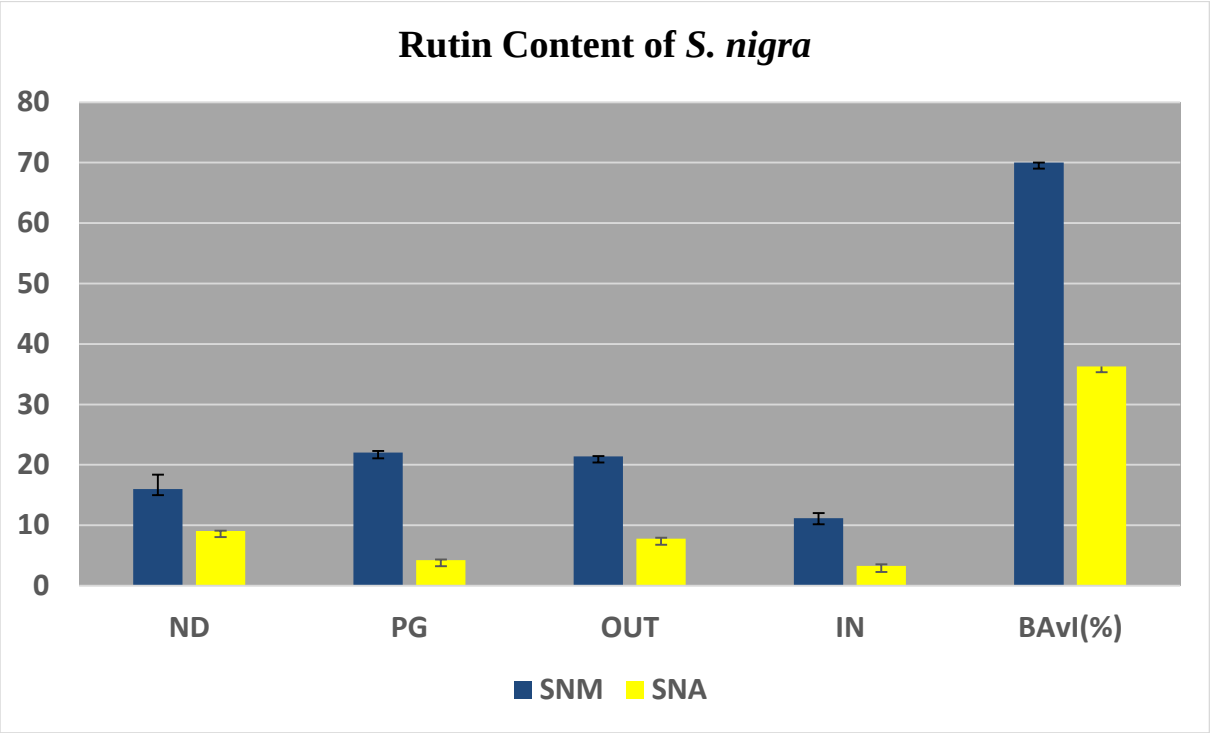

Figure 18. Rutin content of *Sambucus nigra* Fruit Extracts

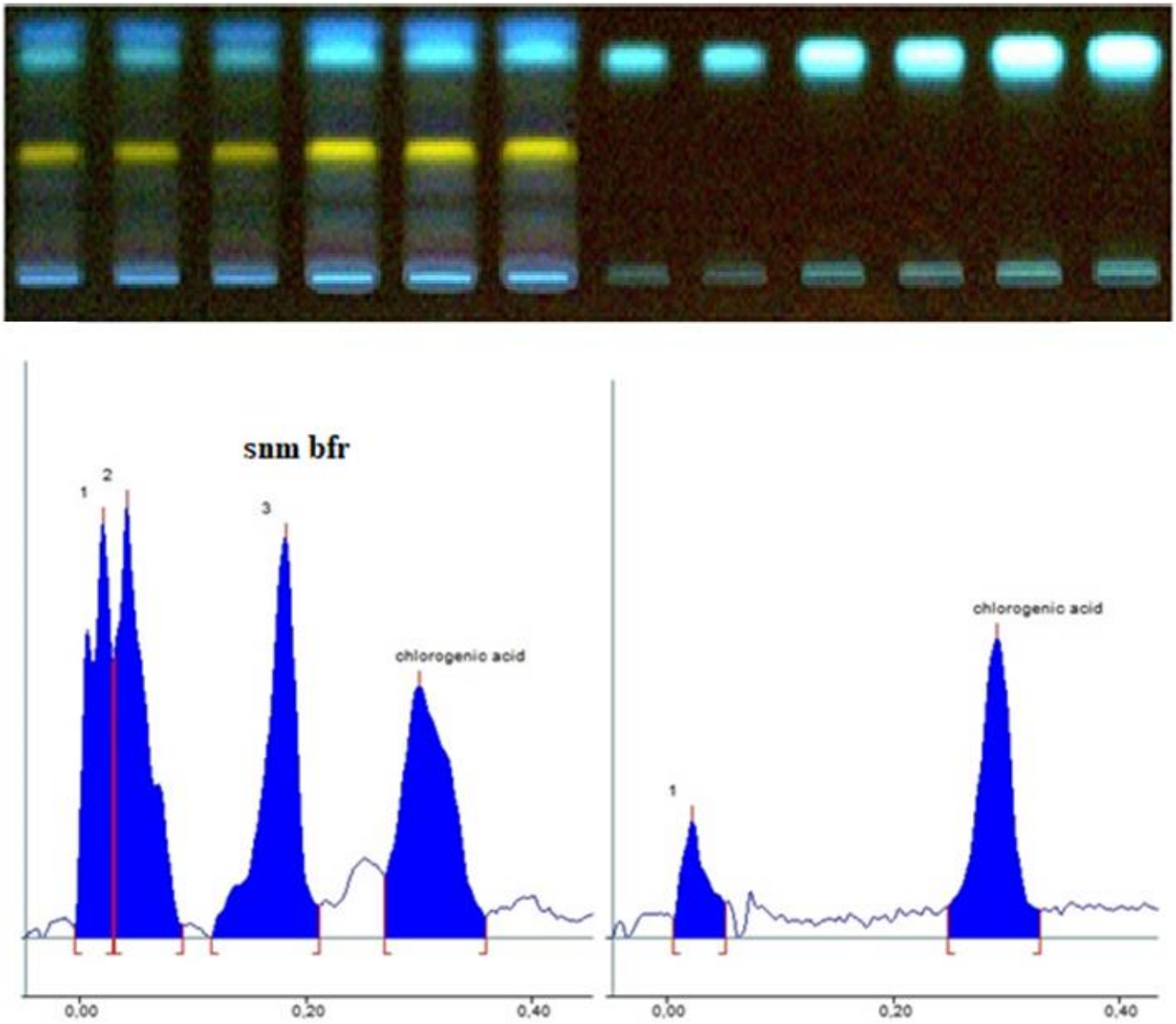

Figure 19. Chlorogenic acid analysis of not-digested phase of SNM on HPTLC.

Spectrums of Chlorogenic acid standard and SNM BFR at 366 nm. Mobile phase: AcOEt/ CH_2Cl_2/ CH_3COOH/ HCOOOH/ H_2O (100:25:10:10:11); Derivatization: NPR reagent. SNM bfr: Not-digested SNM

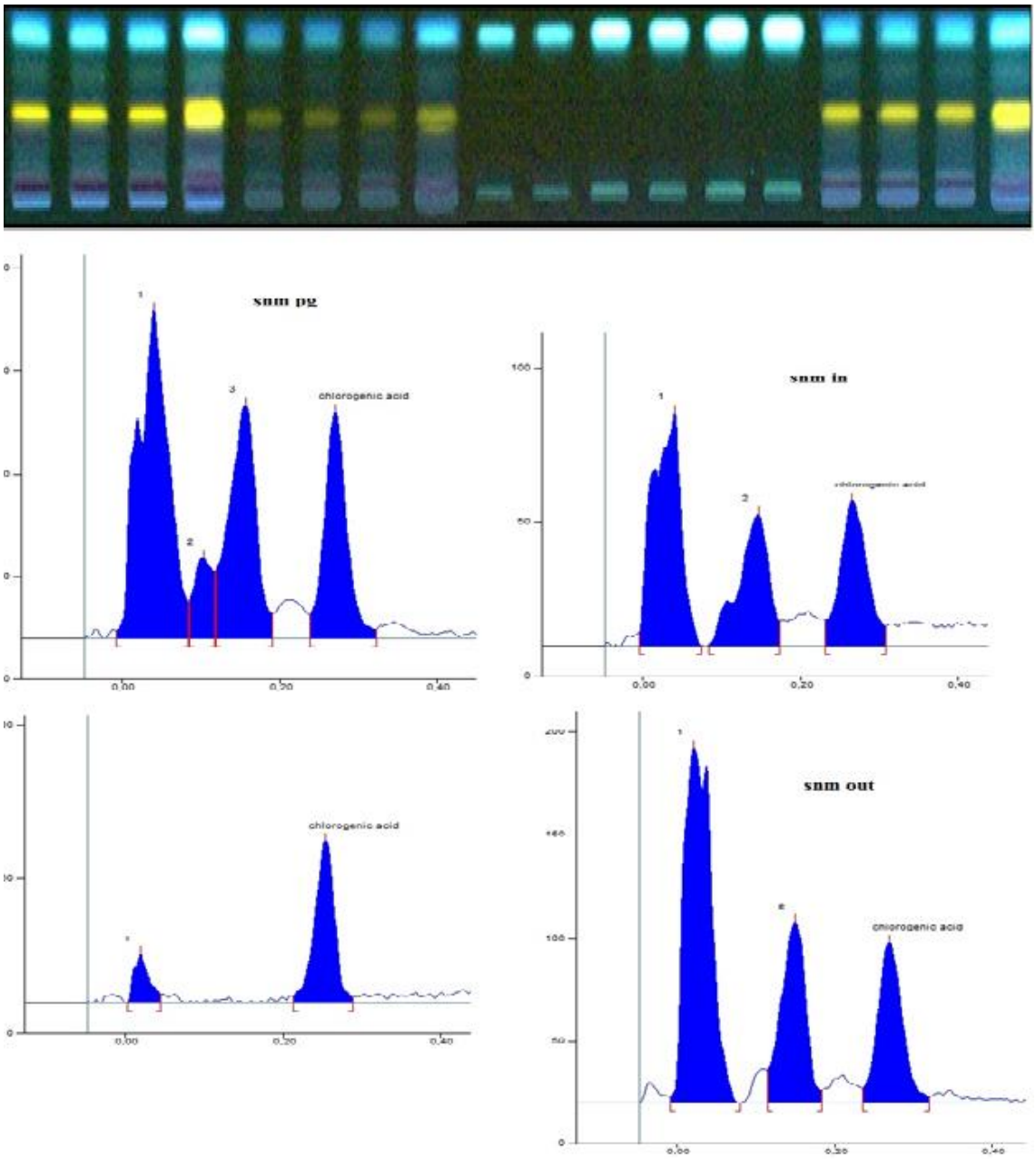

Figure 20. Chlorogenic acid analysis of post gastric, colon available and serum available phases of SNM on HPTLC

Spectrums of Chlorogenic acid standard and SNM PG, SNM OUT, SNM IN at 366 nm. Mobile phase: AcOEt/ CH_2Cl_2/ CH_3COOH/ HCOOOH/ H_2O (100:25:10:10:11); Derivatization: NPR reagent. SNM pg: Post-gastric phase of SNM, SNM out: colon-available phase of SNM. SNM in: Serum-available phase of SNM

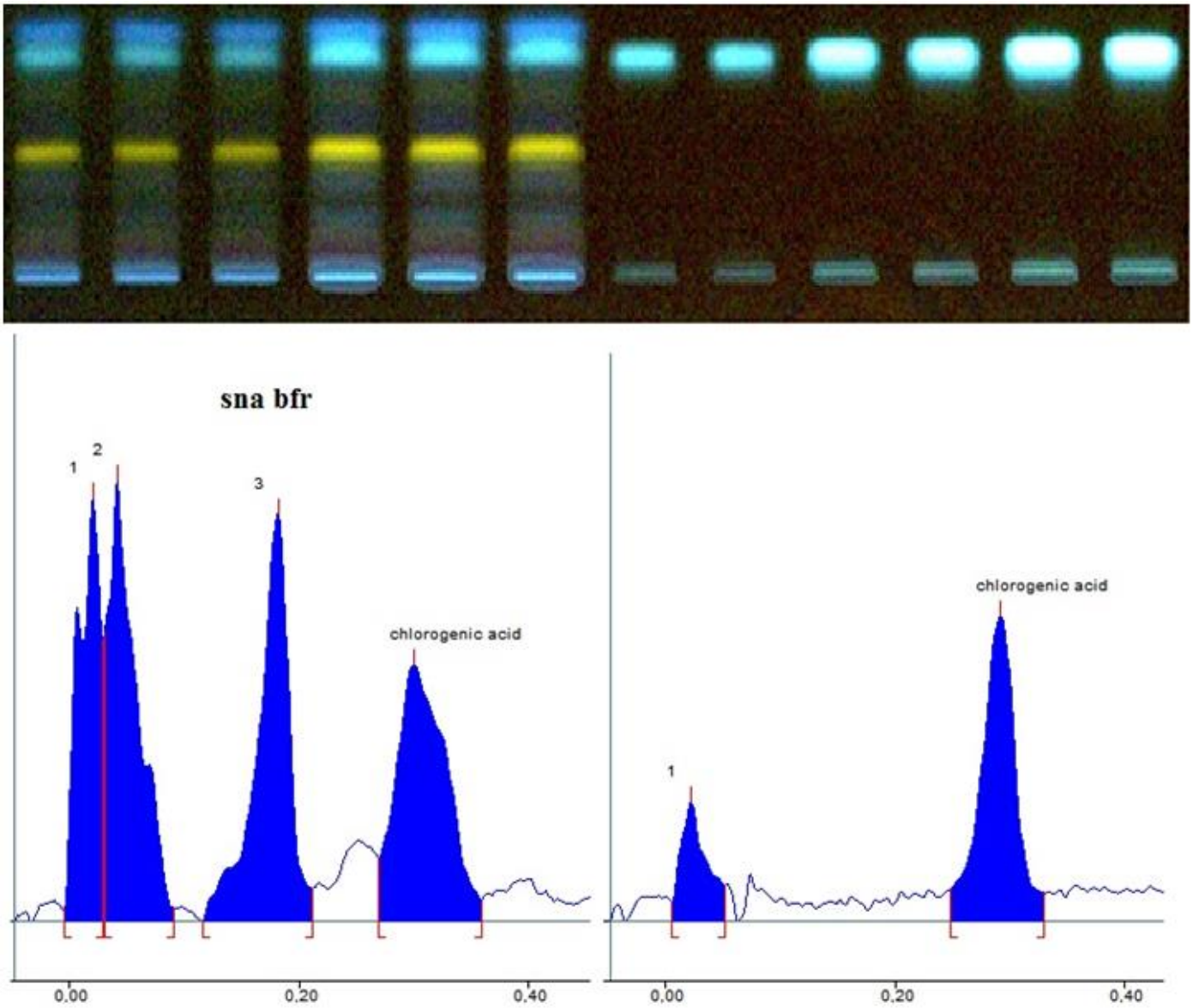

Figure 21. Chlorogenic acid analysis of not-digested phase of SNA on HPTLC

Spectrums of Chlorogenic acid standard and SNA BFR at 366 nm. Mobile phase: AcOEt/ CH_2Cl_2/ CH_3COOH/ HCOOOH/ H_2O (100:25:10:10:11); Derivatization: NPR reagent. Not-digested SNA

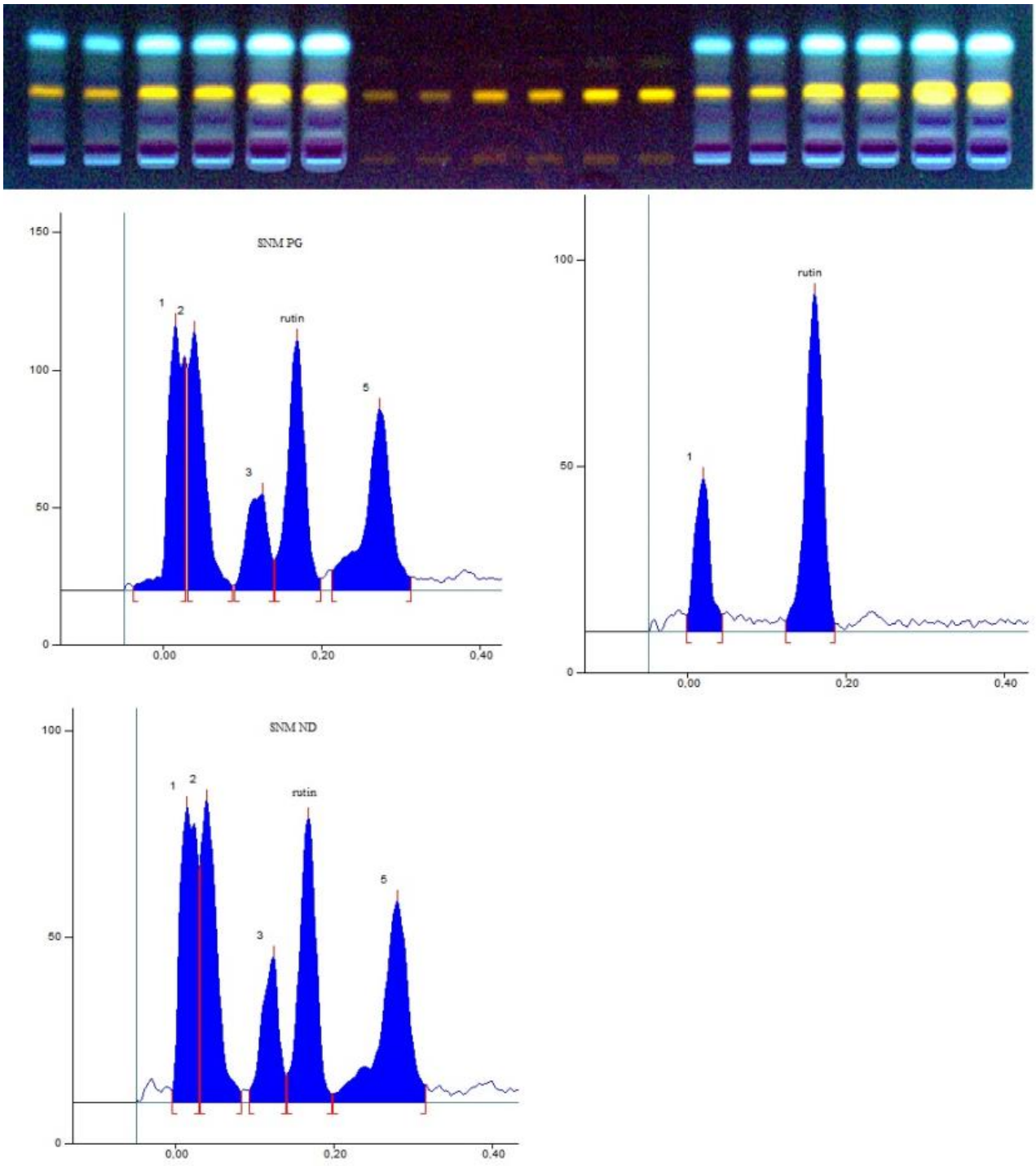

Figure 22. Rutin analysis of not-digested and post gastric phase of SNM on HPTLC

Spectrums of Rutin standard and SNM BFR and SNM PG at 366 nm. Mobile phase: AcOEt/ CH_2Cl_2/ CH_3COOH/ HCOOOH/ H_2O (100:25:10:10:11); Derivatization: NPR reagent. Not-digested SNM. SNM pg: Post-gastric phase of SNM

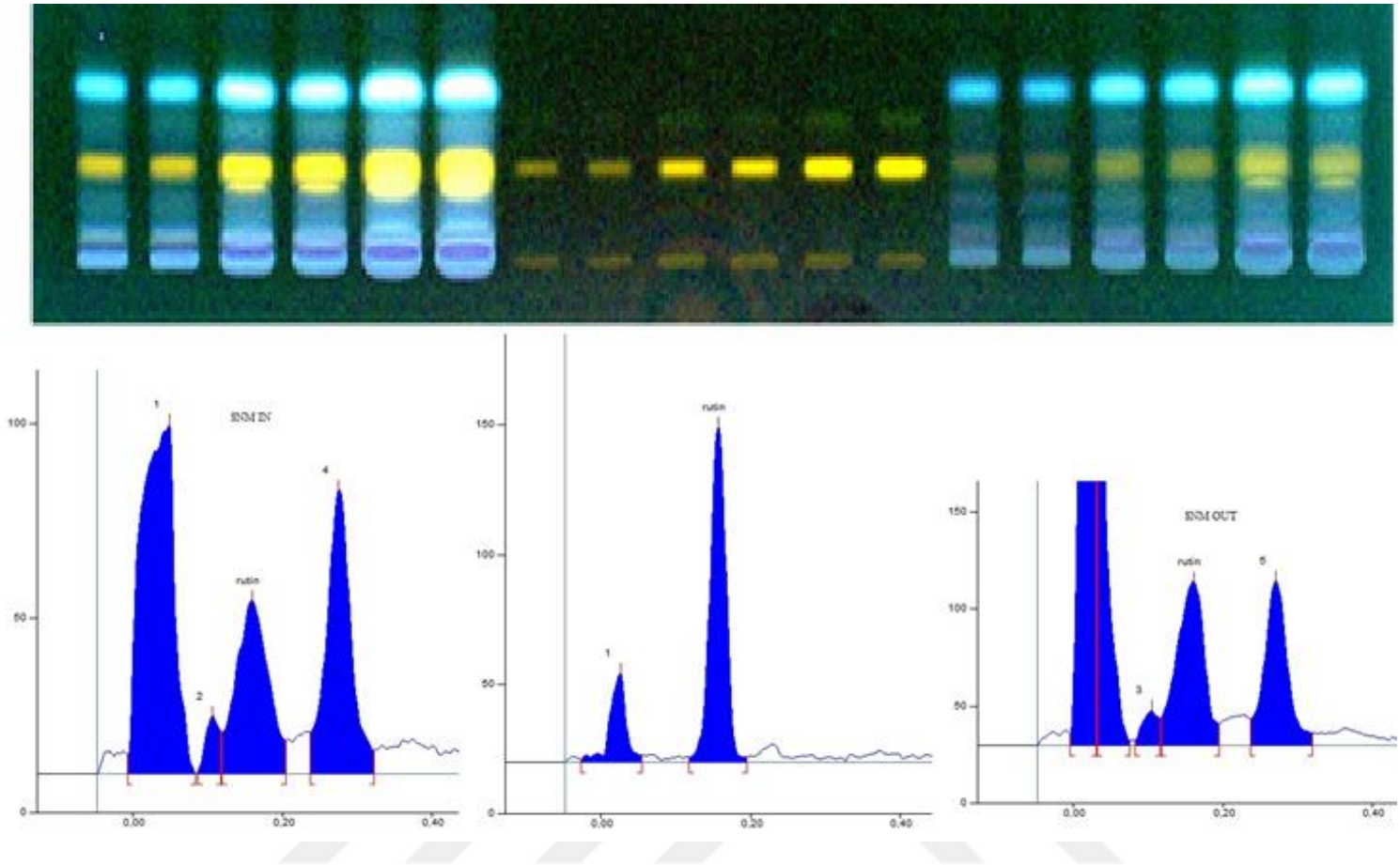

Figure 23. Rutin analysis of colon available and serum available phases of SNM

Spectrums of Rutin standard and SNM IN and SNM OUT at 366 nm. Mobile phase: AcOEt/ CH_2Cl_2/ CH_3COOH/ HCOOOH/ H_2O (100:25:10:10:11); Derivatization: NPR reagent. SNM out: colon-available phase of SNM. SNM in: Serum-available phase of SNM

4.5.2. HPTLC Analysis of *Sambucus ebulus* Fruit Extracts

Amount of chlorogenic acid was higher in SEM, however bioavailability of chlorogenic acid in SEA was higher. Chlorogenic acid content in non-digested SEM was 16.67 ± 0.17 mg/g dry extract, while its concentration decreased in every step of digestion significantly (PG: 10.37 ± 0.60, OUT: 9.70 ± 0.40, IN: 7.63 ± 0.06) and bioavailability index was found as 45.77%. SEA showed expressively lower amount of chlorogenic acid in non-digested phase; 2.39 ± 0.07. A slow but significant decrease was observed (PG: 1.60 ± 0.09, OUT 1.36 ± 0.05, IN: 1.24 ± 0.04) to yield a bioavailability rate of 51.88%.

Table 47. HPTLC Analysis of *Sambucus ebulus* Fruit Extracts

Samples	*ND* [A]	*PG*	*OUT*	*IN*	*BAvI (%)*
SEM-Chlorogenic acid[B]	$16.67^{a} \pm 0.17$	$10.37^{b} \pm 0.60$	$9.70^{c} \pm 0.40$	$7.63^{d} \pm 0.06$	45.77%
SEA-Chlorogenic acid	$2.39^{a} \pm 0.07$	$1.60^{b} \pm 0.09$	$1.36^{c} \pm 0.05$	$1.24^{c} \pm 0.04$	51.88%

[A] The abbreviations for samples are *ND: non-digested, PG: postgastric, OUT: Colon available IN: bioavailable, BAvI: Bioavailability index*
[B] Results are expressed as mg/g dry extract with standard deviations
* Different letters in the same row indicate significance ($p < 0.05$).
** Methanolic extract of *Sambucus ebulus* L. fruits
*** Aqueous extract of *Sambucus ebulus* L. fruits

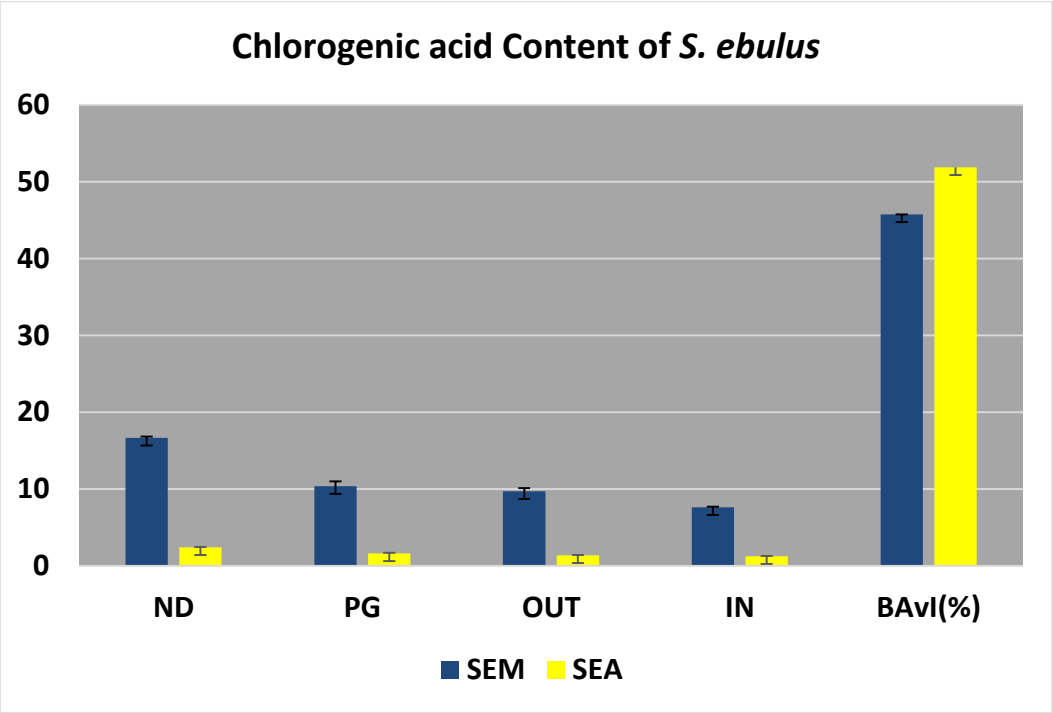

Figure 24. Chlorogenic acid content of *Sambucus ebulus* Fruit Extracts

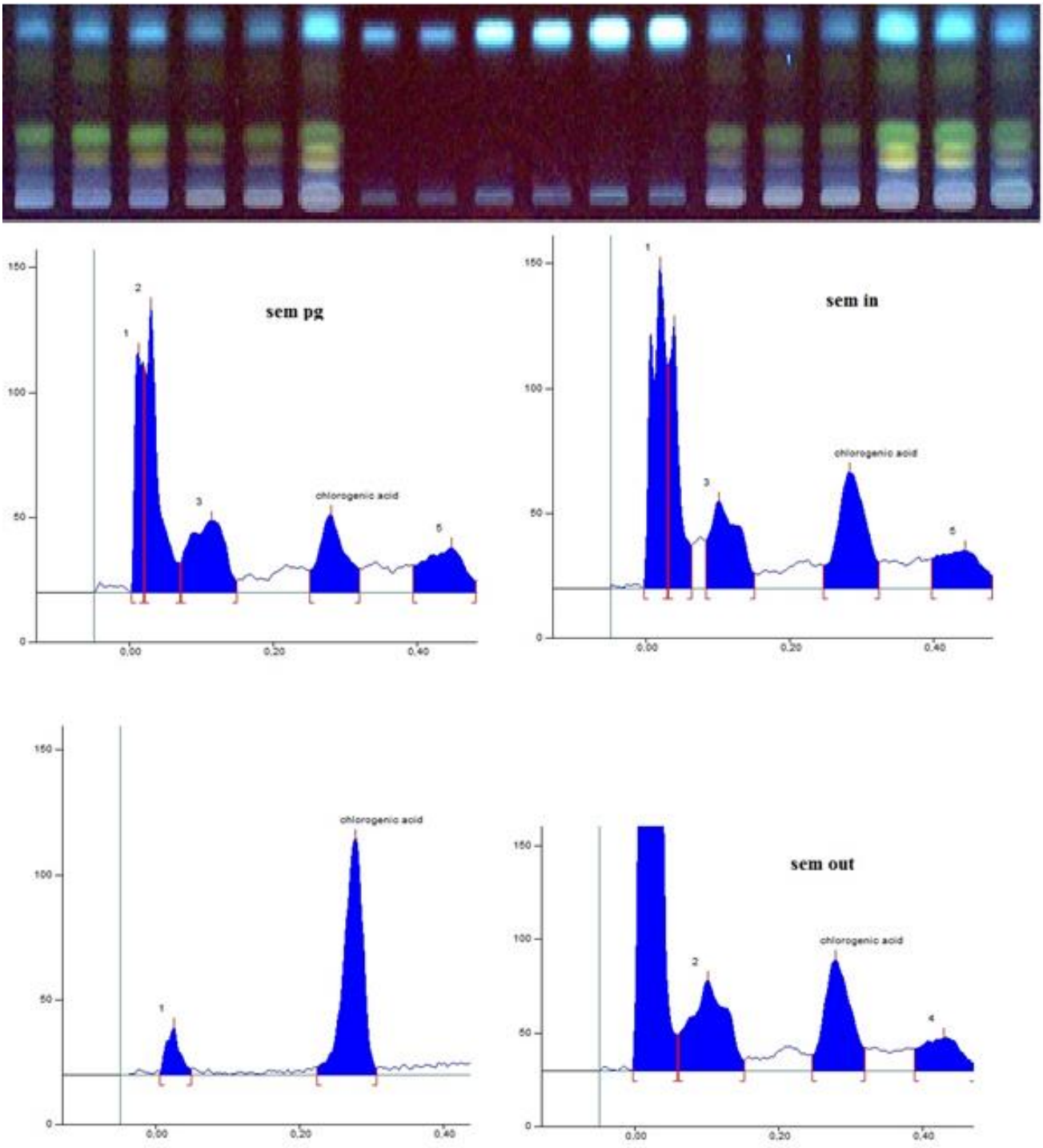

Figure 25. Chlorogenic acid analysis of post gastric, colon available and serum available phases of SEM on HPTLC

Spectrums of Chlorogenic acid standard and SEM PG, SEM OUT, SEM IN at 366 nm. Mobile phase: AcOEt/ CH_2Cl_2/ CH_3COOH/ HCOOOH/ H_2O (100:25:10:10:11); Derivatization: NPR reagent. SEM out: colon-available phase of SEM. SEM in: Serum-available phase of SEM. SEM pg: Post-gastric phase of SEM

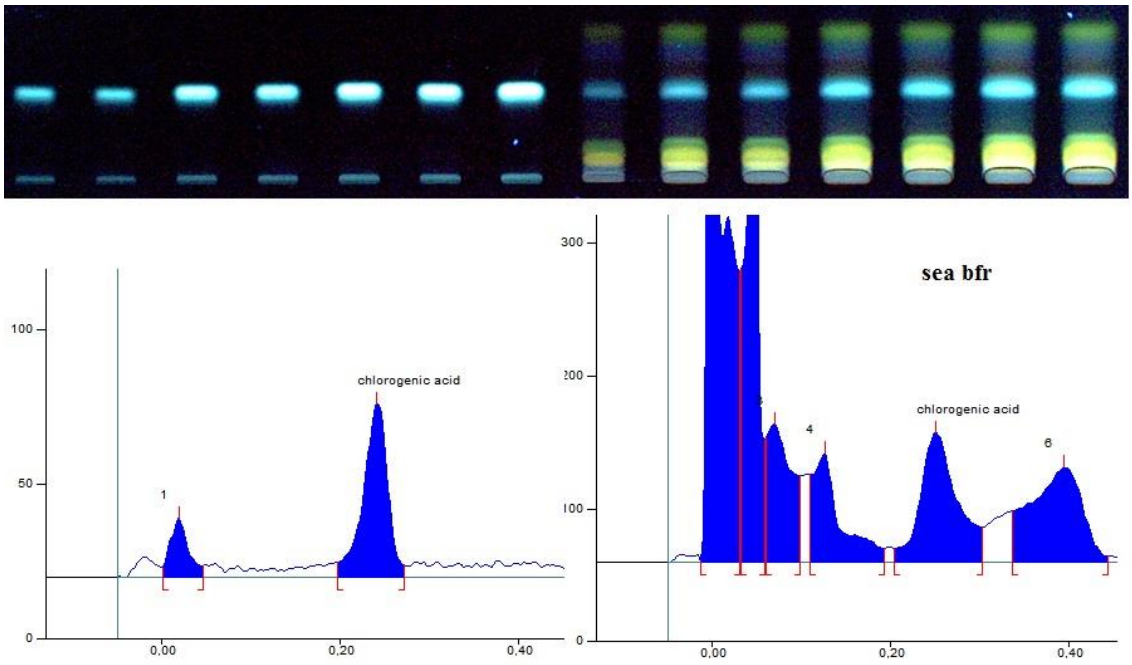

Figure 26. Chlorogenic acid analysis of non-digested phase of SEA on HPTLC

Spectrums of Chlorogenic acid standard and SEA BFR at 366 nm. Mobile phase: AcOEt/ CH_2Cl_2/ CH_3COOH/ HCOOOH/ H_2O (100:25:10:10:11); Derivatization: NPR reagent. SEA bfr: Not-digested SEA

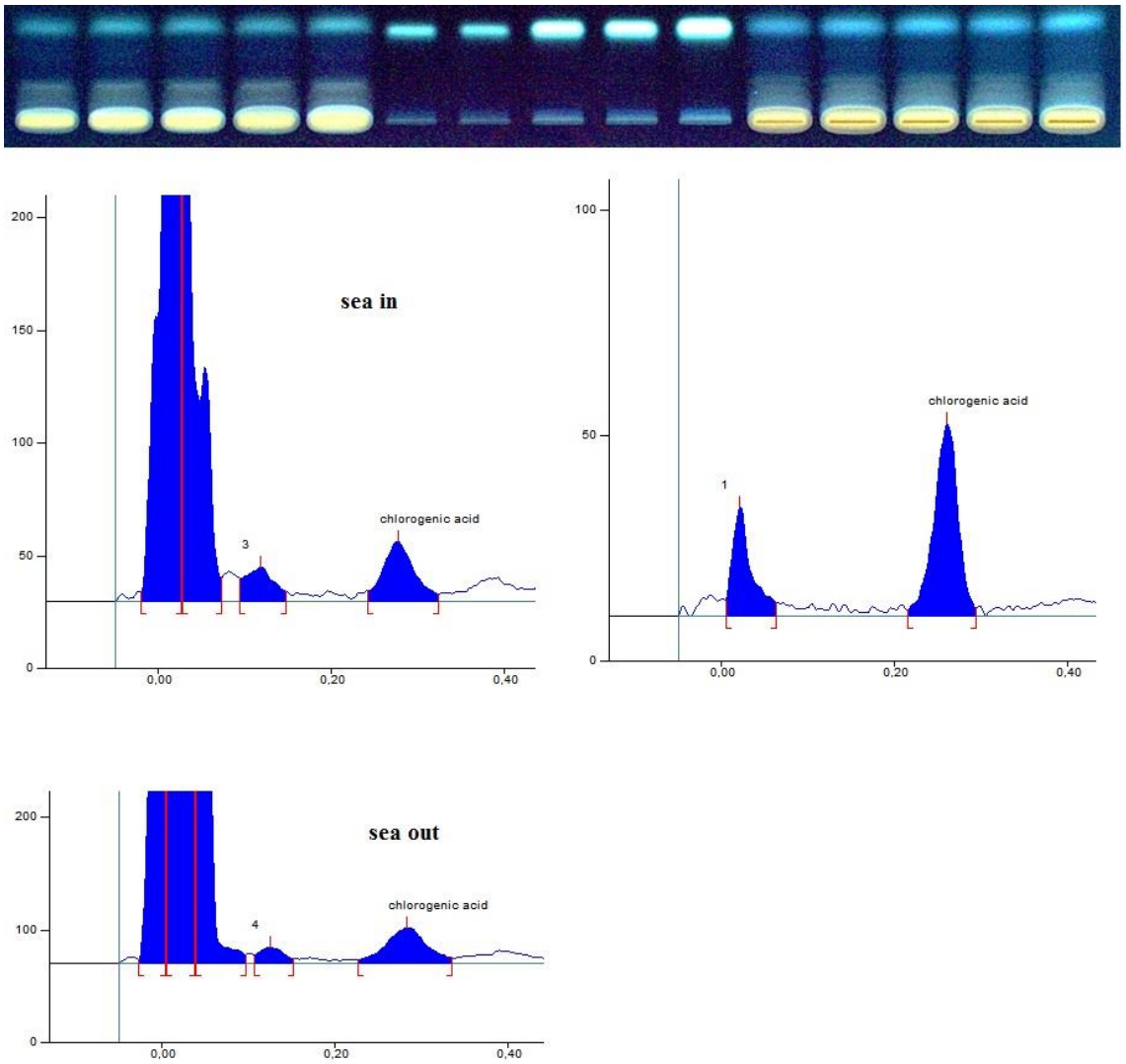

Figure 27. Chlorogenic acid analysis of colon available and serum available phases of SEA on HPTLC

Spectrums of Chlorogenic acid standard and SEA IN and SEA OUT at 366 nm. Mobile phase: AcOEt/ CH_2Cl_2/ CH_3COOH/ HCOOOH/ H_2O (100:25:10:10:11); Derivatization: NPR reagent. SEA out: Colon-available SEA, SEA in: Serum-available SEA

4.5.3. HPTLC Analysis of *Viburnum opulus* Fruit Extracts

Chlorogenic acid quantity of non-digested VOM extract was determined as 34.42 ± 1.22 mg/g dry extract, which is higher than non-digested VOA extract, 26.76 ± 0.91. Both extracts were demonstrated decline in post-gastric phase (25.50 ± 0.45, 18.61 ± 0.07 respectively). Even though modest enhancement was measured in colon-available phase (31.56 ± 1.00 and 21.91 ± 0.43, respectively), a notable decrease was detected in serum available phase (15.09 ± 0.32 and 13.39 ± 0.74, respectively). Total bioavailability of the active metabolite in VOM extract was measured as 38.90% in the meantime in VOA extract total bioavailability was measured 56.40%.

Table 48. HPTLC Analysis of *Viburnum opulus* Fruit Extracts

Samples	*ND* [A]	*PG*	*OUT*	*IN*	*BAvI (%)*
VOM-Chlorogenic acid[B]	34.42^{a} ± 1.22	25.50^{b} ± 0.45	31.56^{c} ± 1.00	13.39^{d} ± 0.74	38.90 %
VOA-Chlorogenic acid	26.76^{a} ± 0.91	18.61^{b} ± 0.07	21.91^{c} ± 0.43	15.09^{d} ± 0.32	56.40 %

[A] The abbreviations for samples are *ND: non-digested, PG: postgastric, OUT: Colon available IN: bioavailable, BAvI: Bioavailability index*

[B] Results are expressed as mg/g dry extract with standard deviations

* Different letters in the same row indicate significance ($p < 0.05$).

** Methanolic extract of *Viburnum opulus* L. fruits

*** Aqueous extract of *Viburnum opulus L.* fruits

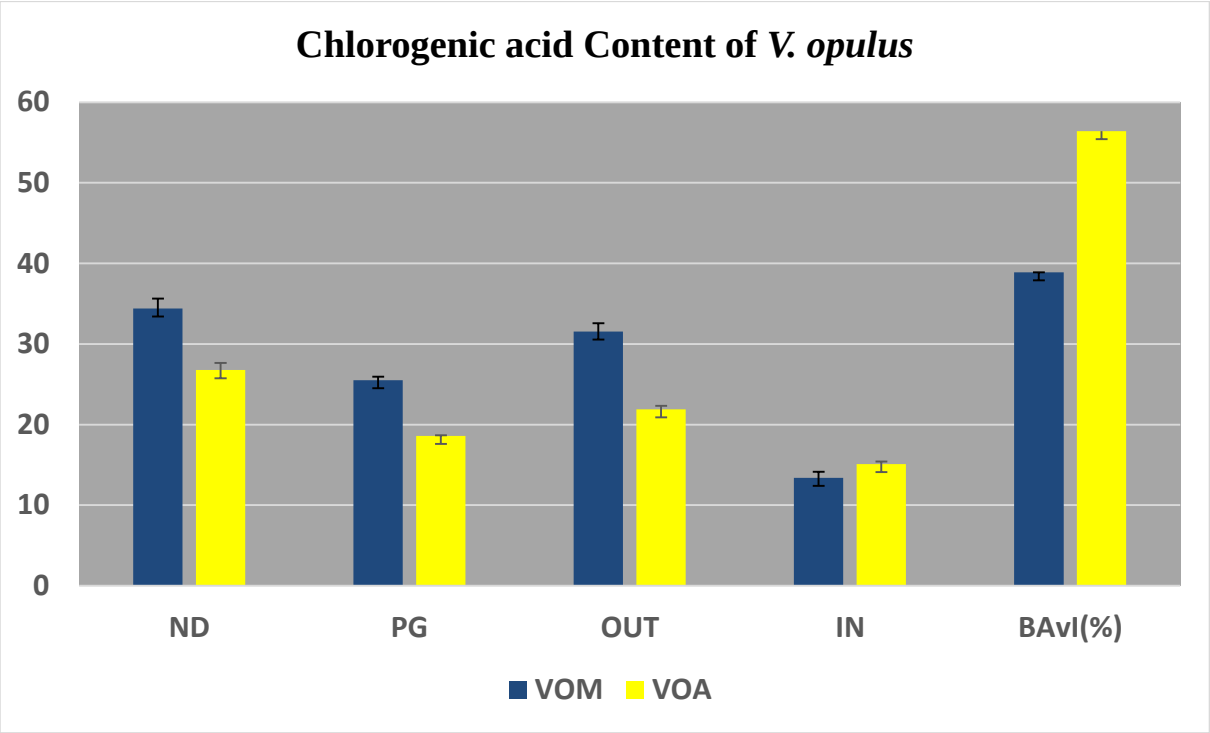

Figure 28. Chlorogenic acid content of *Viburnum opulus* Fruit Extracts

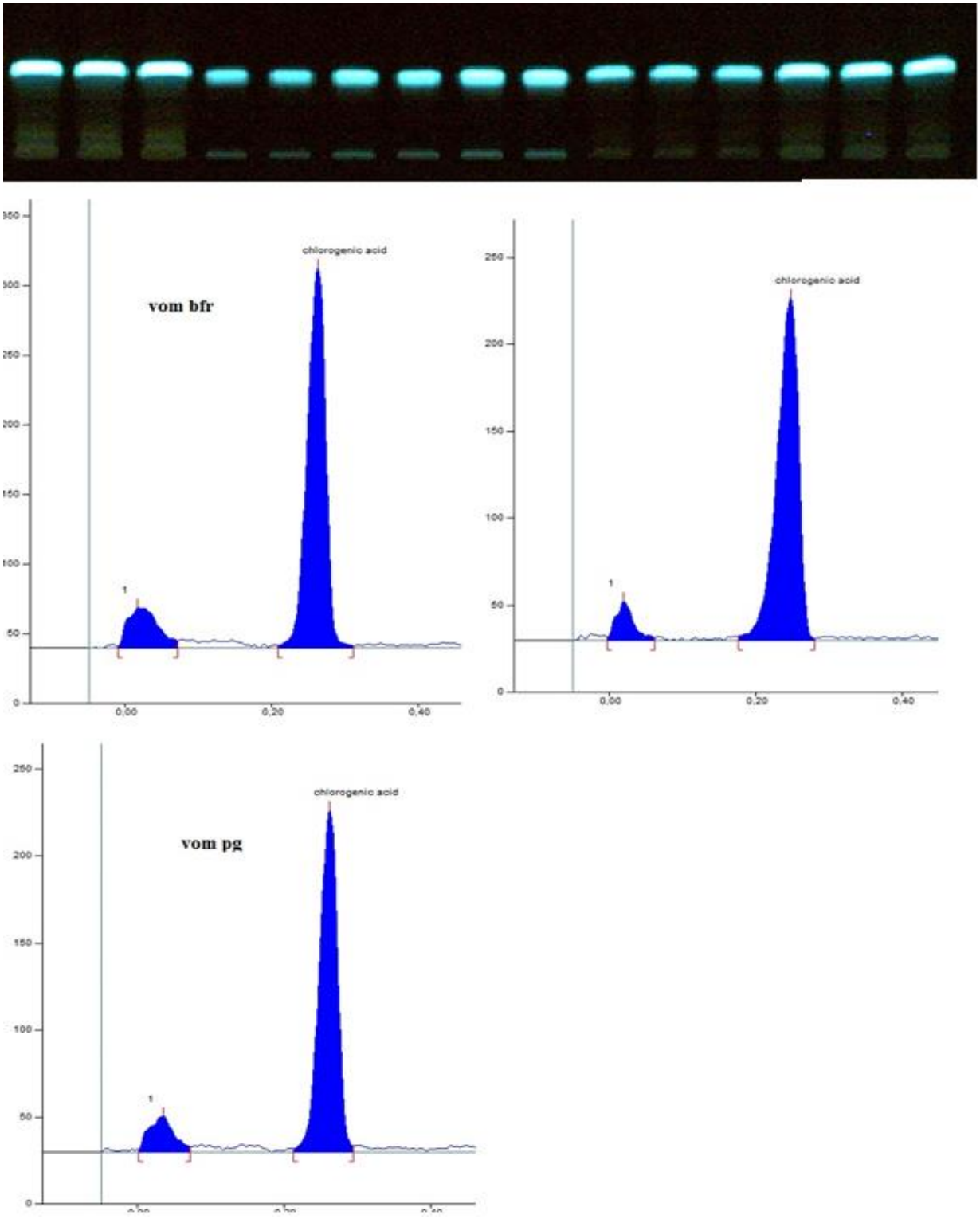

Figure 29. Chlorogenic acid analysis of non-digested and post gastric phases of VOM on HPTLC

Spectrums of Chlorogenic acid standard and VOM BFR and VOM PG at 366 nm. Mobile phase: AcOEt/ CH_2Cl_2/ CH_3COOH/ HCOOOH/ H_2O (100:25:10:10:11); Derivatization: NPR reagent. VOM bfr: Not-digested VOM, VOM pg: Post-gastric phase of VOM

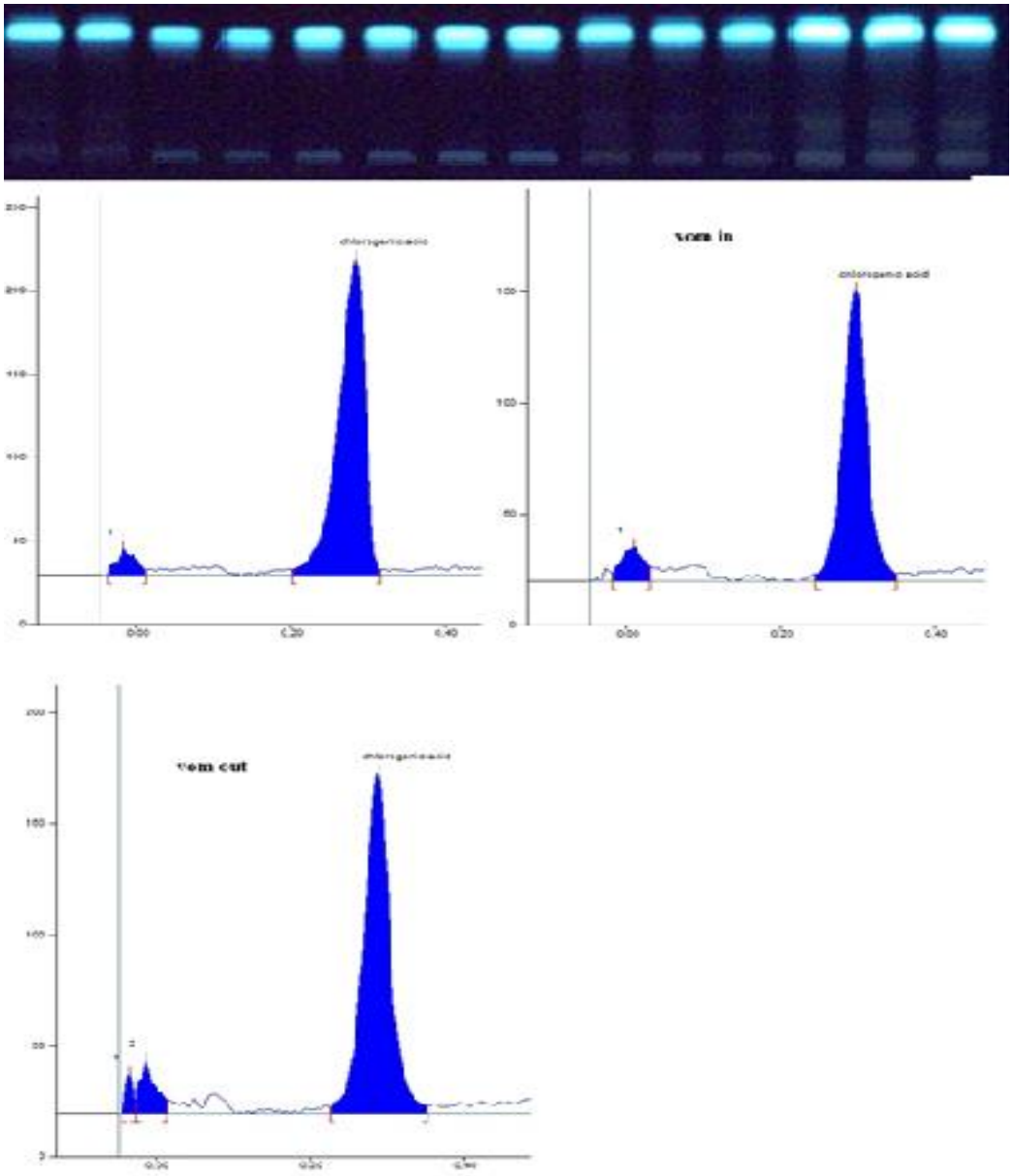

Figure 30. Chlorogenic acid analysis of colon available and serum available phases of VOM on HPTLC

Spectrums of Chlorogenic acid standard and VOM IN and VOM OUT at 366 nm. Mobile phase: AcOEt/ CH_2Cl_2/ CH_3COOH/ HCOOOH/ H_2O (100:25:10:10:11); Derivatization: NPR reagent. VOM out: Colon-available phase of VOM, VOM in: Serum-available phase of VOM

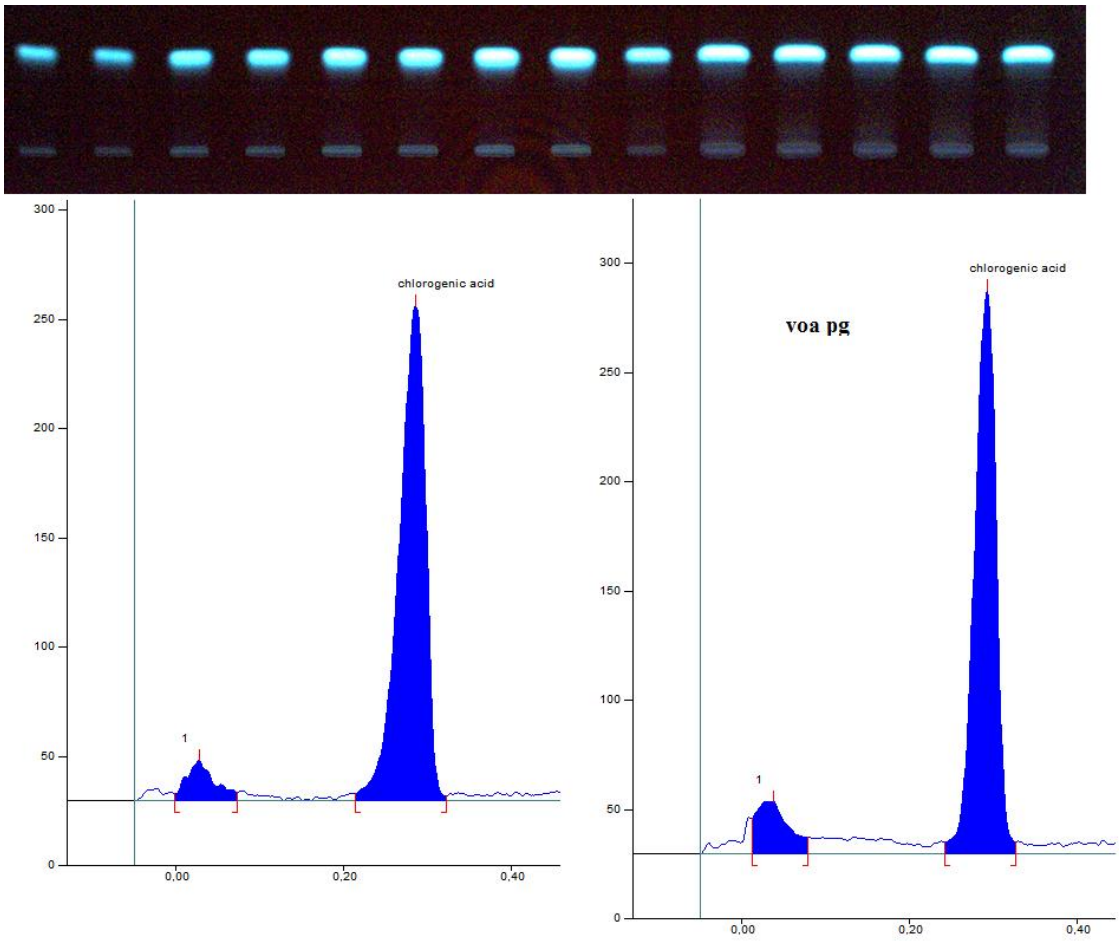

Figure 31. Chlorogenic acid analysis of Post-gastric phases of VOA on HPTLC

Spectrums of Chlorogenic acid standard and VOA PG at 366 nm. Mobile phase: AcOEt/ CH_2Cl_2/ CH_3COOH/ HCOOOH/ H_2O (100:25:10:10:11); Derivatization: NPR reagent. VOA pg: Post gastric phase of VOA

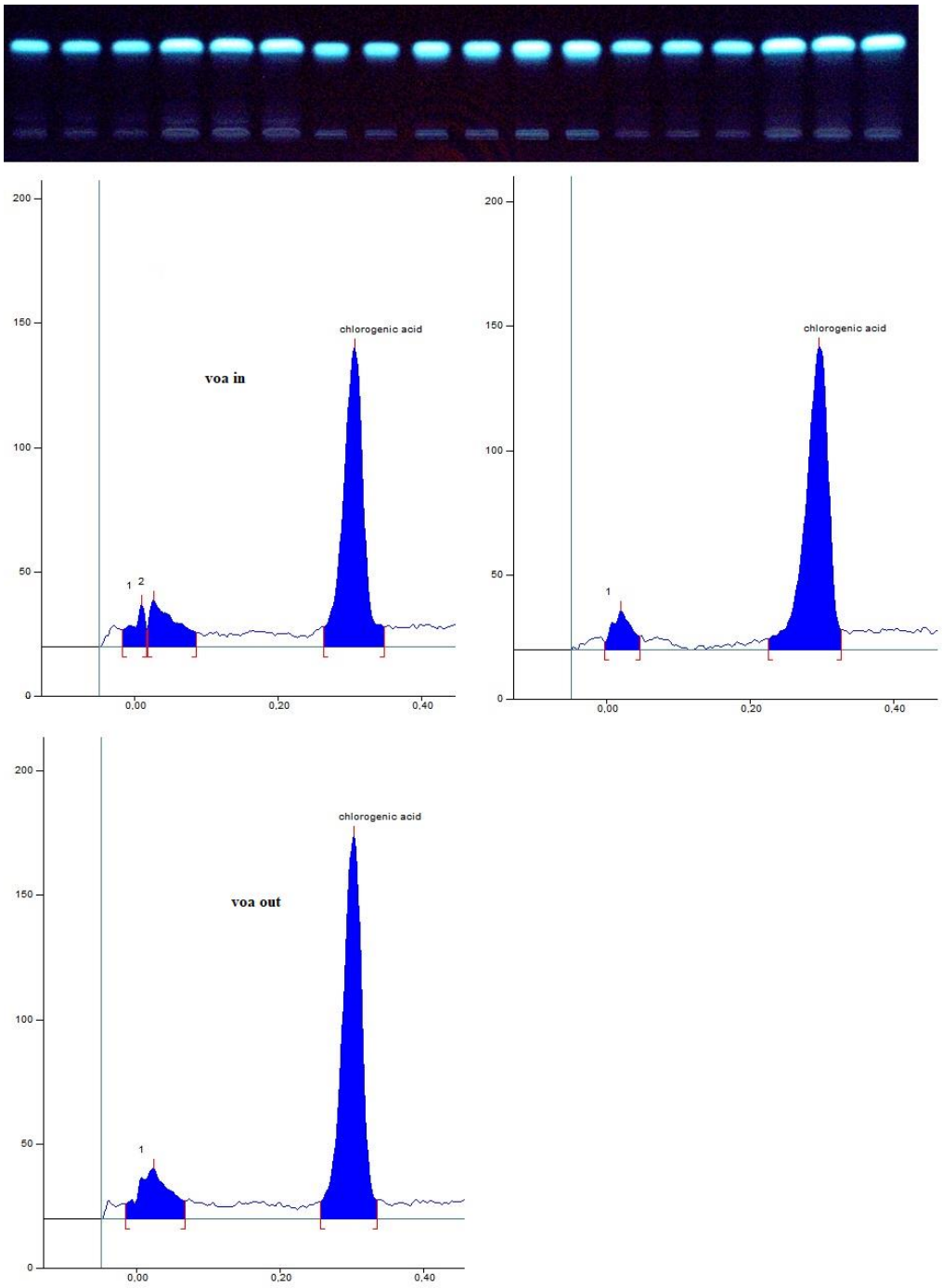

Figure 32. Chlorogenic acid analysis of colon available and serum available phases of VOA on HPTLC

Spectrums of Chlorogenic acid standard and VOA OUT and VOA IN at 366 nm. Mobile phase: AcOEt/ CH_2Cl_2/ CH_3COOH/ HCOOOH/ H_2O (100:25:10:10:11); Derivatization: NPR reagent. VOA out: Colon-available phase of VOA, VOA in: Serum-available phase of VOA

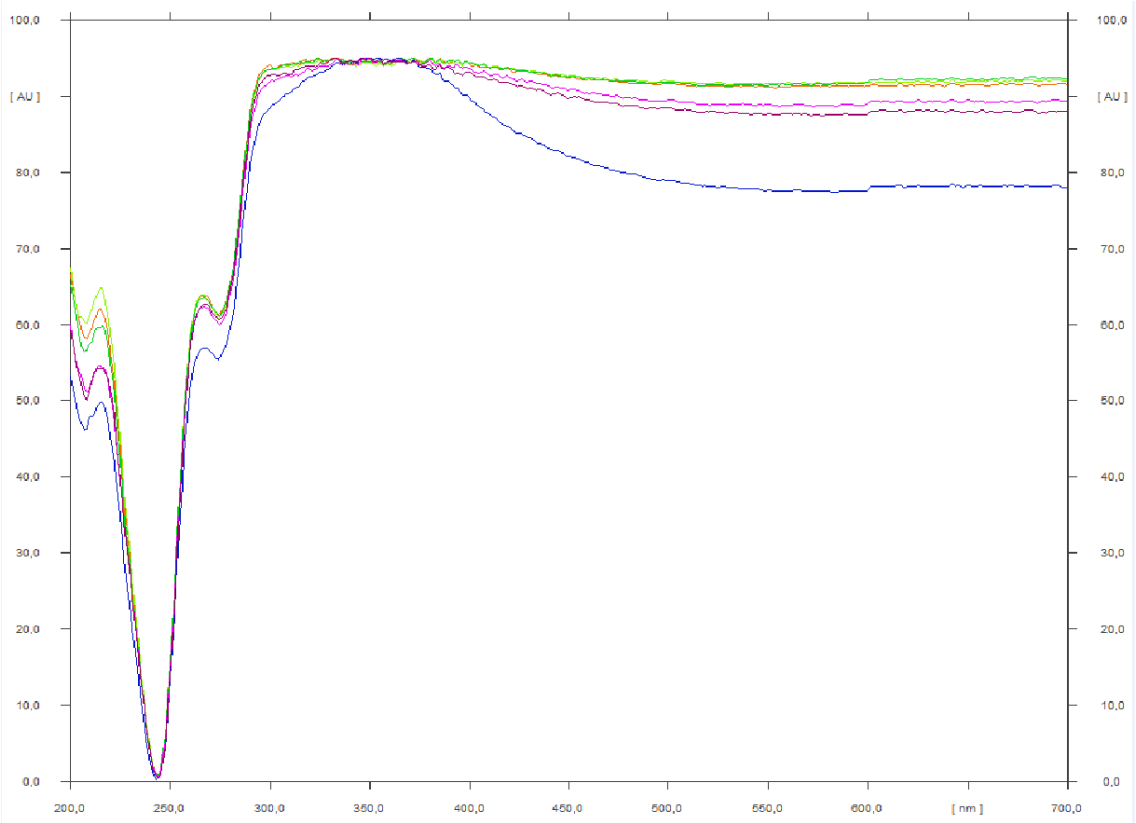

Figure 33. Overlaid UV spectra of chlorogenic acid in all tracks

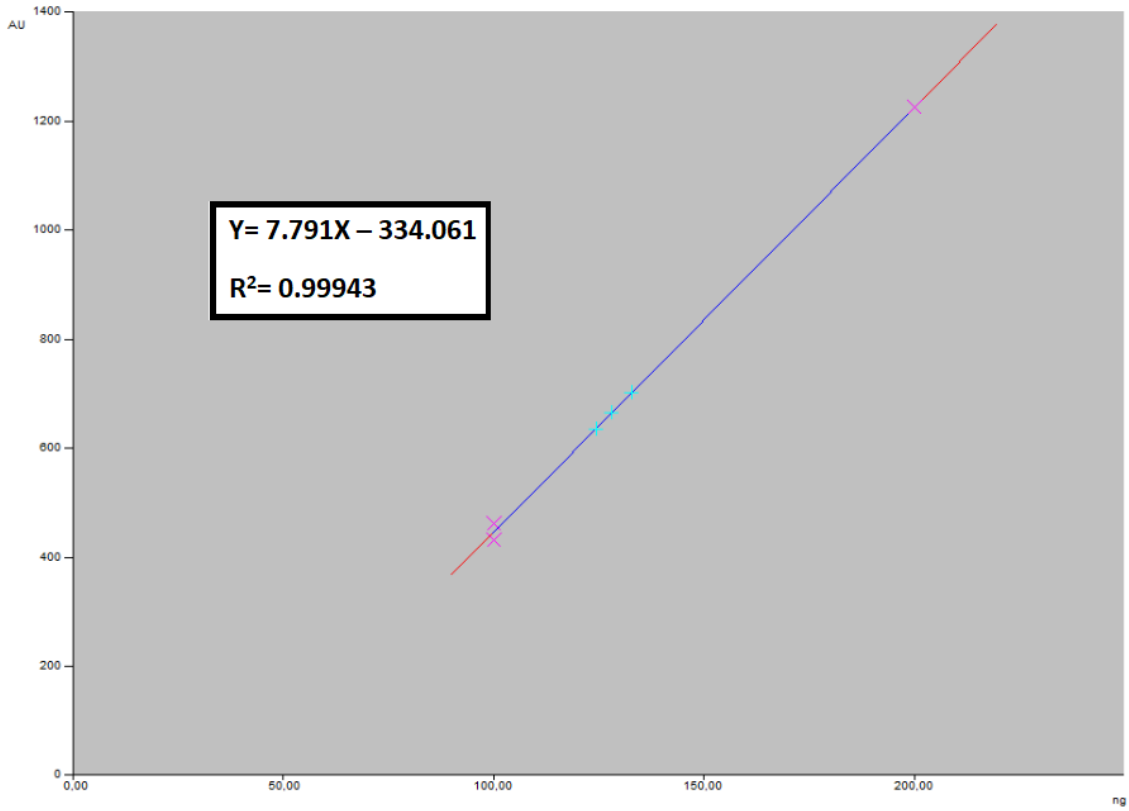

Figure 34. The calibration curve for chlorogenic acid

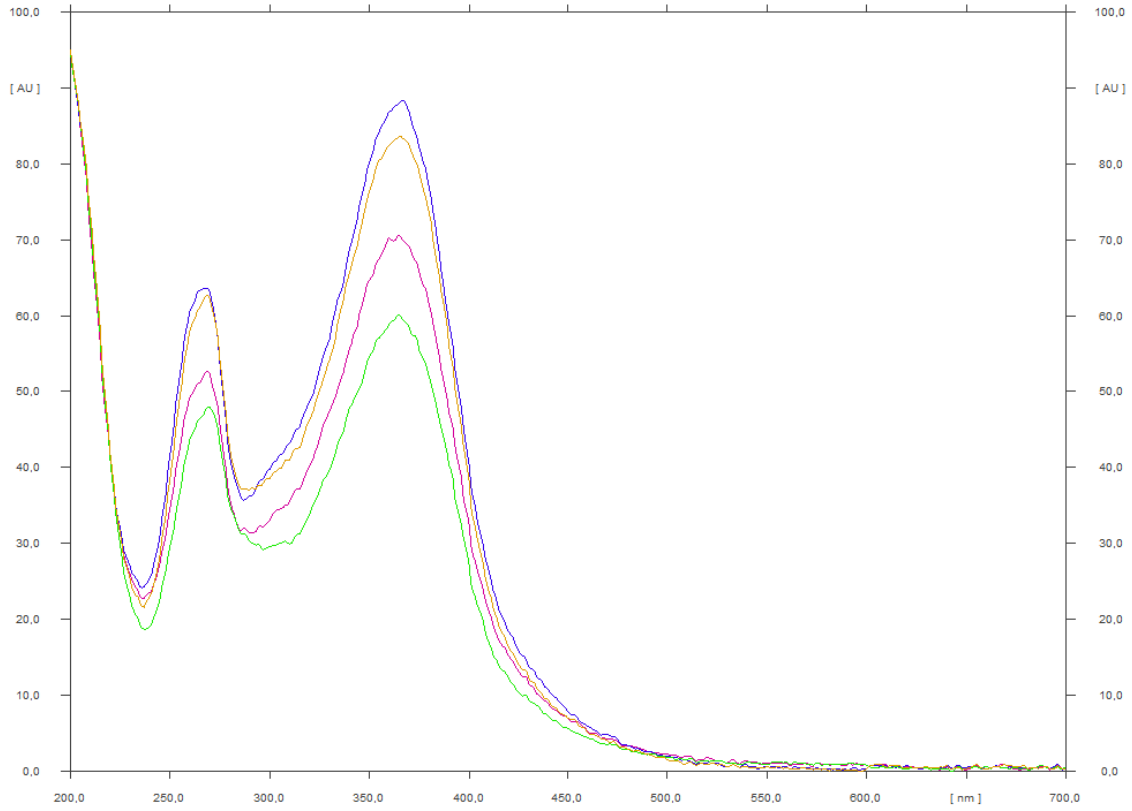

Figure 35. Overlaid UV spectra of rutin in all tracks

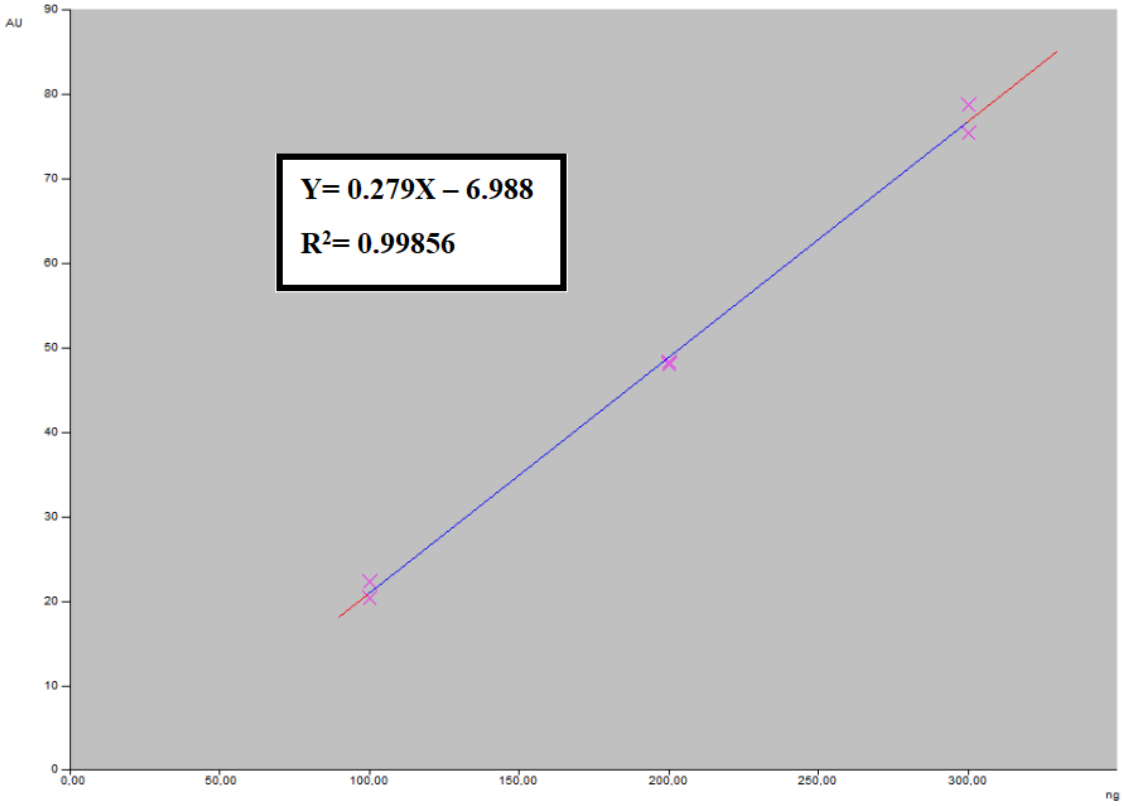

Figure 36. The calibration curve for rutin

4.6. Results of Cupric Reducing Antioxidant Capacity (CUPRAC) Assay

4.6.1. Cupric Reducing Antioxidant Capacity of *Sambucus nigra* Fruit Extracts

Likewise in CUPRAC assay SNM showed higher metal reducing activity. SNA demonstrated no significant alteration in bioactivity however; colon available phase of SNM exhibited a minor induction. But serum available phase of both extracts exhibited substantial reduction in Cupric reducing activity

Table 49. Cupric Reducing Antioxidant Capacity of *Sambucus nigra* Fruit Extracts

Name of the analysis	*ND* [A]	*PG*	*OUT*	*IN*
SNM-CUPRAC [BC]	$194.23^{a} \pm 6.79$	$201.95^{a} \pm 4.40$	$212.16^{b} \pm 3.13$	$101.58^{c} \pm 4.44$
SNA-CUPRAC	$114.62^{a} \pm 0.20$	$103.77^{a} \pm 8.95$	$98.84^{a} \pm 4.48$	$66.63^{b} \pm 1.08$

[A] The abbreviations for samples are *ND: non-digested, PG: postgastric, OUT: Colon available IN: bioavailable, BAvI: Bioavailability index*
[B] The abbreviation for the analysis is CUPRAC: Cupric reducing antioxidant capacity
[C] Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg ascorbic acid equivalents (AAE) in 1 g sample.
* Different letters in the same row indicate significance ($p < 0.05$).
** Methanolic extract of *Sambucus nigra* L. fruits
*** Aqueous extract of *Sambucus nigra* L. fruits

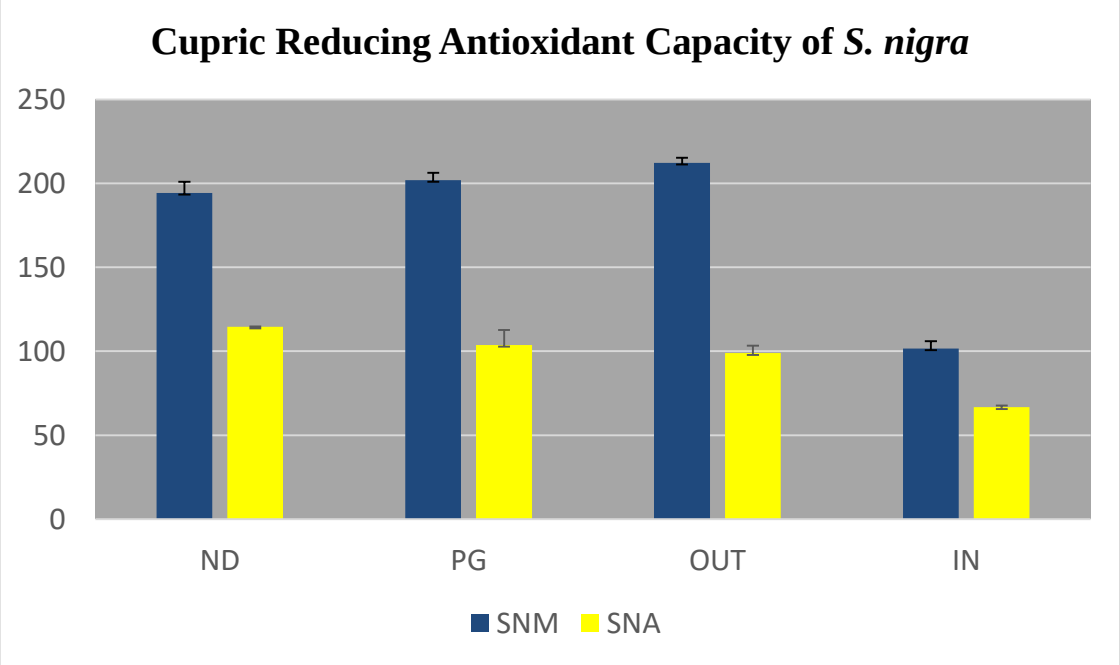

Figure 37. Cupric Reducing Antioxidant Capacity of *Sambucus nigra* Fruit Extracts

4.6.2. Cupric Reducing Antioxidant Capacity of *Sambucus ebulus* Fruit Extracts

In CUPRAC assay both extracts showed higher activity in non-digested phase. Both extracts had downtrend of cupric reducing activity in every step significantly, resulted at serum available fraction.

Table 50. Cupric Reducing Antioxidant Capacity of *Sambucus ebulus* Fruit Extracts

Name of the analysis	*ND* [A]	*PG*	*OUT*	*IN*
SEM-CUPRAC [BC]	$162.92^{a} \pm 6.99$	$147.38^{b} \pm 6.82$	$130.60^{c} \pm 1.93$	$113.53^{d} \pm 2.51$
SEA-CUPRAC	$132.15^{a} \pm 0.82$	$99.34^{b} \pm 0.79$	$105.66^{c} \pm 1.34$	$60.85^{d} \pm 0.60$

[A] The abbreviations for samples are *ND: non-digested, PG: post-gastric, OUT: colon-available IN: bioavailable*
[B] The abbreviation for the analysis is CUPRAC: Cupric reducing antioxidant capacity
[C] Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg ascorbic acid equivalents (AAE) in 1 g sample.
* Different letters in the same row indicate significance ($p < 0.05$).
** Methanolic extract of *Sambucus ebulus* L. fruits
*** Aqueous extract of *Sambucus ebulus* L. fruits

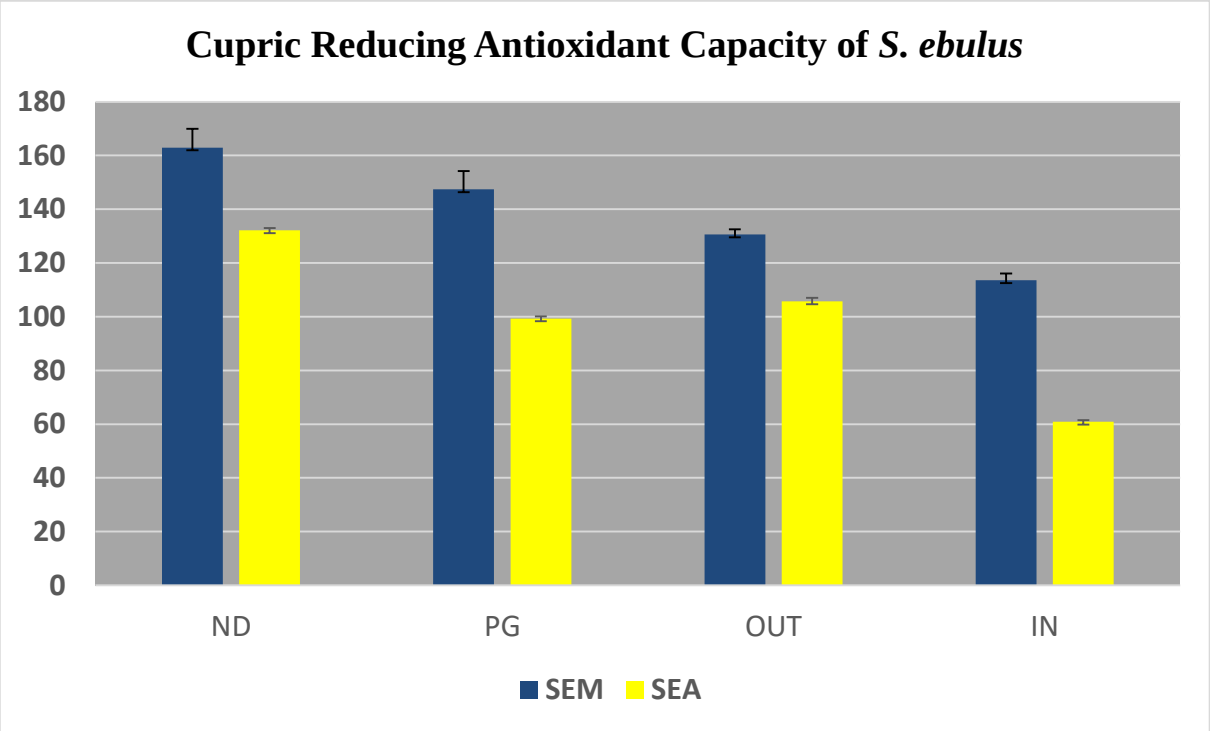

Figure 38. Cupric Reducing Antioxidant Capacity of *Sambucus ebulus* Fruit Extracts

4.6.3. Cupric Reducing Antioxidant Capacity of *Viburnum opulus* Fruit Extracts

In CUPRAC assay, non-digested VOM showed higher activity than non-digested VOA. After the gastric phase, cupric reducing capacity of both extracts were reduced significantly. Eventhough colon-available (OUT) fractions have similar activity with post-gastric (PG) fractions (164.01 ± 4.75, 100.93 ± 2.03 respectively), cupric reducing capacity of both extracts were diminished significantly in the serum available phase (IN).

Table 51. Cupric Reducing Antioxidant Capacity of *Viburnum Opulus* Fruit Extracts

Name of the analysis	*ND* [A]	*PG*	*OUT*	*IN*
VOM-CUPRAC [BC]	$208.87^{a} \pm 9.32$	$156.36^{b} \pm 3.00$	$164.01^{b} \pm 4.75$	$96.50^{c} \pm 1.68$
VOA-CUPRAC	$156.49^{a} \pm 4.32$	$107.85^{b} \pm 11.48$	$100.93^{bc} \pm 2.03$	$90.72^{c} \pm 2.82$

[A] The abbreviations for samples are *ND: non-digested, PG: postgastric ,OUT:colon-available IN: bioavailable*
[B] The abbreviation for the analysis is CUPRAC: Cupric reducing antioxidant capacity
[C] Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg ascorbic acid equivalents (AAE) in 1 g sample.
* Different letters in the same row indicate significance ($p < 0.05$).
** Methanolic extract of *Viburnum opulus* L. fruits
*** Aqueous extract of *Viburnum opulus* L. fruits

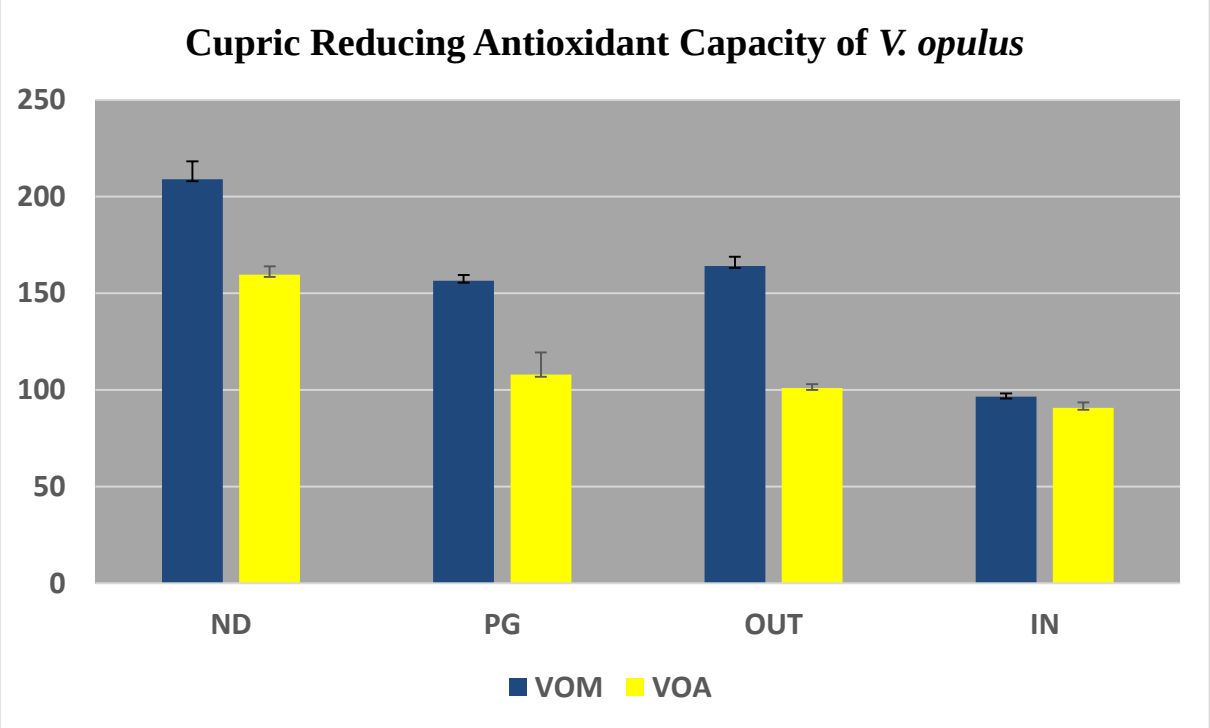

Figure 39. Cupric Reducing Antioxidant Capacity of *Viburnum opulus* Fruit Extracts

4.7. Results of Ferric Reducing Antioxidant Power Assay (FRAP)

4.7.1. Ferric Reducing Antioxidant Power of *Sambucus nigra* Fruit extracts

In FRAP assay SNM extract had higher activity when compared to SNA in non-digested phase (0.74 ± 0.02 Fe^{2+} µmol/g and 0.66 ± 0.05 respectively). There were no significant changes in post-gastric and colon-available phase. Serum available phase exhibited minor but significant reduction for both extracts (0.59 ± 0.04 and 0.41 ± 0.01 respectively).

Table 52. Ferric Reducing Antioxidant Power of *Sambucus nigra* Fruit extracts

Name of the analysis	*ND* [A]	*PG*	*OUT*	*IN*
SNM-FRAP [BC]	$0.74^{a} \pm 0.02$	$0.78^{a} \pm 0.04$	$0.77^{a} \pm 0.01$	$0.59^{a} \pm 0.04$
SNA-FRAP	$0.66^{a} \pm 0.05$	$0.63^{a} \pm 0.02$	$0.65^{a} \pm 0.06$	0.41 ± 0.01

[A] The abbreviations for samples are *ND: non-digested, PG: postgastric, OUT: Colon available IN: bioavailable, BAvI: Bioavailability index*
[B] The abbreviation for the analysis is FRAP: Ferric Reducing Antioxidant Power
[C] Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as Mm $FeSO_4$ equivalents in 1 g sample.
* Different letters in the same row indicate significance ($p < 0.05$).
** Methanolic extract of *Sambucus nigra* L. fruits
*** Aqueous extract of *Sambucus nigra* L. fruits
P.S FRAP activity of the reference compound "butylated hydroxytoluene (BHT)" is found to be 4.18 ± 0.26 mM $FeSO_4$ eq. in 1 g sample.

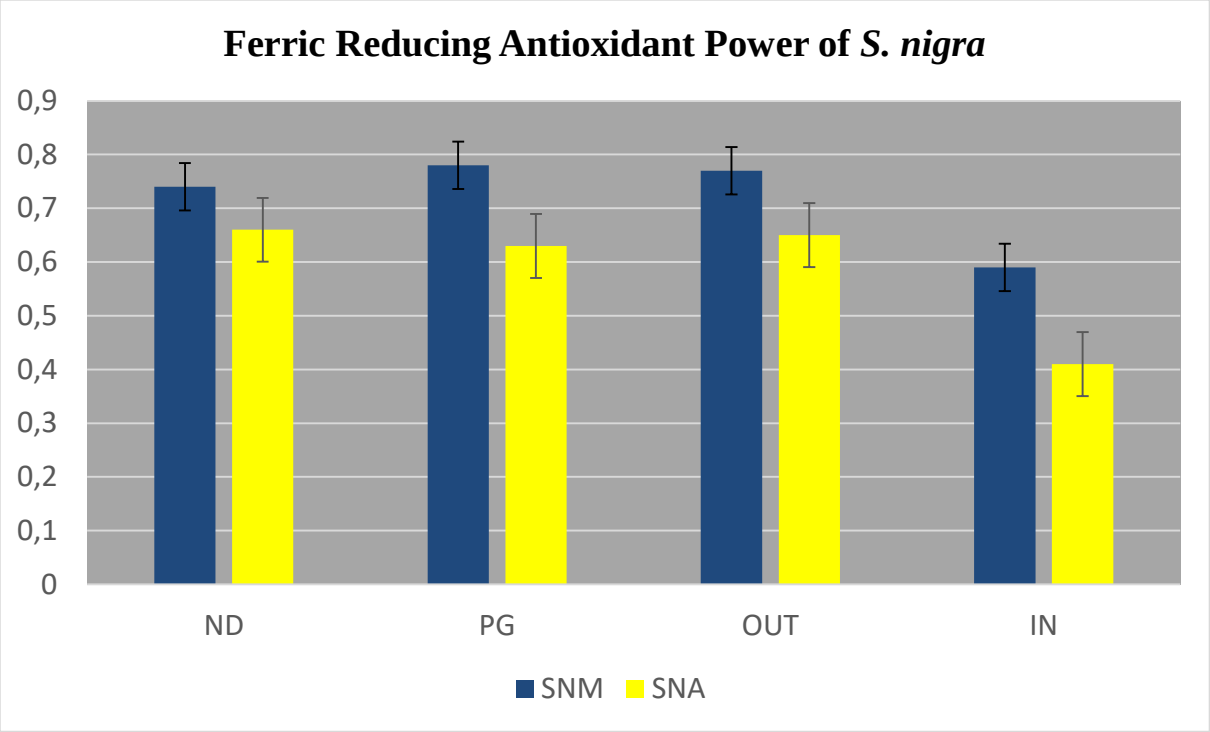

Figure 40. Ferric Reducing Antioxidant Power of *Sambucus nigra* Fruit extracts

4.7.2. Ferric Reducing Antioxidant Power of *Sambucus ebulus* Fruit extracts

In FRAP assay, results were analogous with CUPRAC assay. Ferric reducing capacity of SEM extract reduced from 0.66 ± 0.03 Fe^{2+} µmol/g to 0.38 ± 0.01 from ND to serum available fraction. Similarly, SEA extract showed reduction from ND to IN fraction, such as from 0.51 ± 0.01 to 0.14 ± 0.01, respectively.

Table 53. Ferric Reducing Antioxidant Power of *Sambucus ebulus* Fruit extracts

Name of the analysis	*ND* [A]	*PG*	*OUT*	*IN*
SEM-FRAP [BC]	$0.66^{a} \pm 0.03$	$0.53^{b} \pm 0.06$	$0.44^{bc} \pm 0.02$	$0.38^{c} \pm 0.01$
SEA-FRAP	$0.51^{a} \pm 0.01$	$0.36^{b} \pm 0.02$	$0.34^{b} \pm 0.04$	$0.14^{c} \pm 0.01$

[A] The abbreviations for samples are *ND: non-digested, PG: postgastric, OUT: Colon available IN: bioavailable, BAvI: Bioavailability index*
[B] The abbreviation for the analysis is FRAP: Ferric Reducing Antioxidant Power
[C] Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mM $FeSO_4$ equivalents in 1 g sample.
* Different letters in the same row indicate significance ($p < 0.05$).
** Methanolic extract of *Sambucus ebulus* L. fruits
*** Aqueous extract of *Sambucus ebulus* L. fruits
P.S FRAP activity of the reference compound "butylated hydroxytoluene (BHT)" is found to be 4.18 ± 0.26 mM $FeSO_4$ eq. in 1 g sample.

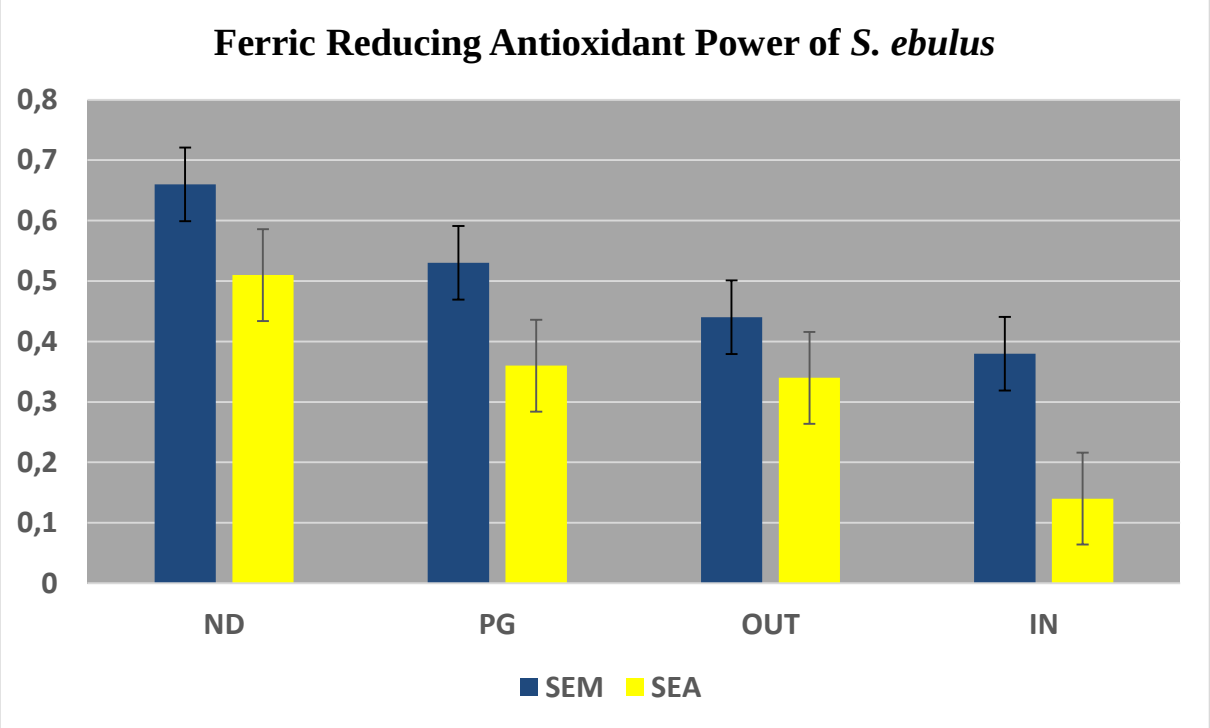

Figure 41. Ferric Reducing Antioxidant Power of *Sambucus ebulus* Fruit extracts

4.7.3. Ferric Reducing Antioxidant Power of *Viburnum opulus* Fruit extracts

In FRAP assay, both extracts showed similar activities for all phases. Non-digested VOM and VOA displayed similar ferric reducing activity. Downtrend of reducing activity was analogous in both extracts. Post-gastric activities of VOM and VOA were not significantly different with non-digested phases. Likewise, colon-available phases of both extracts exhibited parallel activity with non-digested and post-gastric phases. Serum-available phases of both extracts indicated slight but significant decline, ultimately both of them showed similar activity.

Table 54. Ferric Reducing Antioxidant Power of *Viburnum opulus* Fruit extracts

Name of the analysis	*ND* [A]	*PG*	*OUT*	*IN*
VOM-FRAP [BC]	$0.46^{a} \pm 0.05$	$0.45^{a} \pm 0.08$	$0.36^{ab} \pm 0.01$	$0.29^{b} \pm 0.04$
VOA-FRAP	$0.41^{a} \pm 0.09$	$0.40^{ab} \pm 0.01$	$0.38^{ab} \pm 0.03$	$0.28^{b} \pm 0.02$

[A] The abbreviations for samples are *ND: non-digested, PG: postgastric, OUT: Colon available IN: bioavailable, BAvI: Bioavailability index*
[B] The abbreviation for the analysis is FRAP: Ferric Reducing Antioxidant Power
[C] Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mM $FeSO_4$ equivalents in 1 g sample.
* Different letters in the same row indicate significance ($p < 0.05$).
** Methanolic extract of *Viburnum opulus* L. fruits
*** Aqueous extract of *Viburnum opulus* L. fruits
P.S. FRAP activity of the reference compound "butylated hydroxytoluene (BHT)" is found to be 4.18 ± 0.26 mM $FeSO_4$ eq. in 1 g sample.

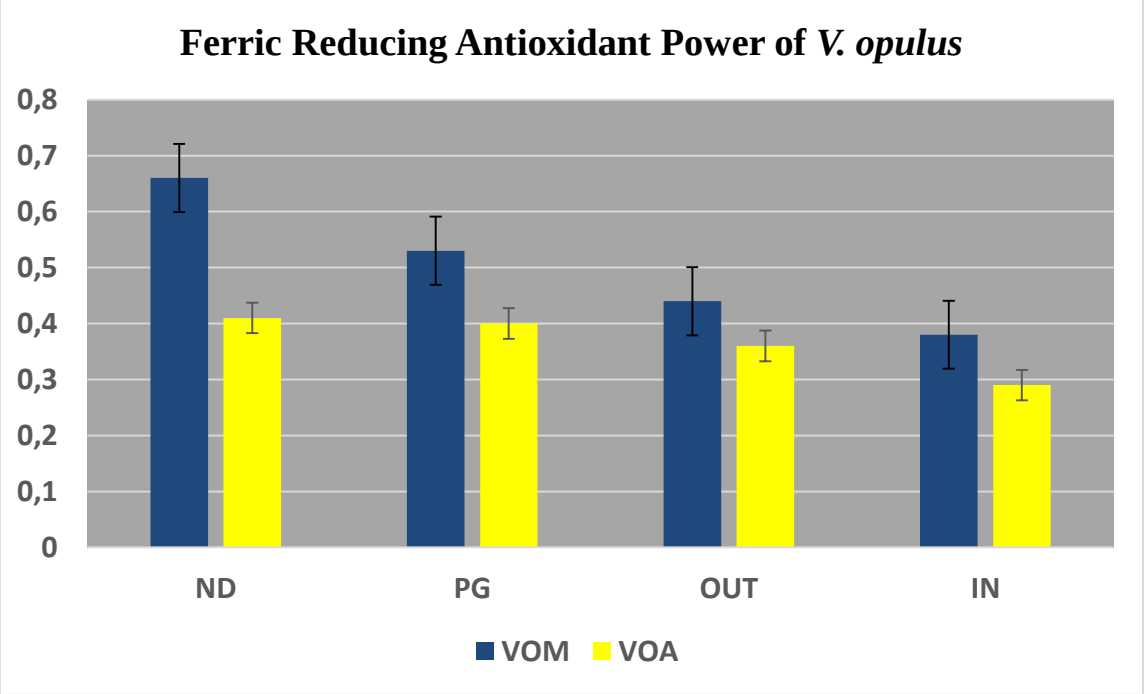

Figure 42. Ferric Reducing Antioxidant Power of *Viburnum opulus* Fruit extracts

4.8. Results of DPPH Radical Scavenging Activity Assay

4.8.1. DPPH Radical Scavenging Activity of *Sambucus nigra* Fruit Extracts

Non-digested SNM showed higher activity than SNA (129.11 ± 5.69 and 99.86 ± 3.51 respectively). In serum available phase both SNM showed higher decrease in DPPH radical scavenging activity ever then, had still higher bioactivity (99.96 mg AAE/g ± 1.96 and 92.56 ± 1.58 respectively).

Table 55. DPPH Radical Scavenging Activity of *Sambucus nigra* Fruit Extracts

Name of the analysis	*ND* [A]	*PG*	*OUT*	*IN*
SNM-DPPH scavenging act. [BC]	$129.11^{a} \pm 5.69$	$121.74^{b} \pm 2.47$	$141.55^{b} \pm 4.82$	$99.96^{c} \pm 1.96$
SNA-DPPH scavenging act.	$99.86^{a} \pm 3.51$	$101.29^{a} \pm 2.57$	$96.55^{ab} \pm 1.29$	$92.56^{b} \pm 1.58$

[A] The abbreviations for samples are *ND: non-digested, PG: postgastric, OUT: Colon available IN: bioavailable, BAvI: Bioavailability index*
[B] The abbreviation for the analysis is DPPH: 2,2-diphenyl-1-picrylhydrazyl
[C]Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg butylated hydroxyl toluene equivalents (BHTE) in 1 g sample.
* Different letters in the same row indicate significance ($p < 0.05$).
** Methanolic extract of *Sambucus nigra* L. fruits
*** Aqueous extract of *Sambucus nigra* L. fruits

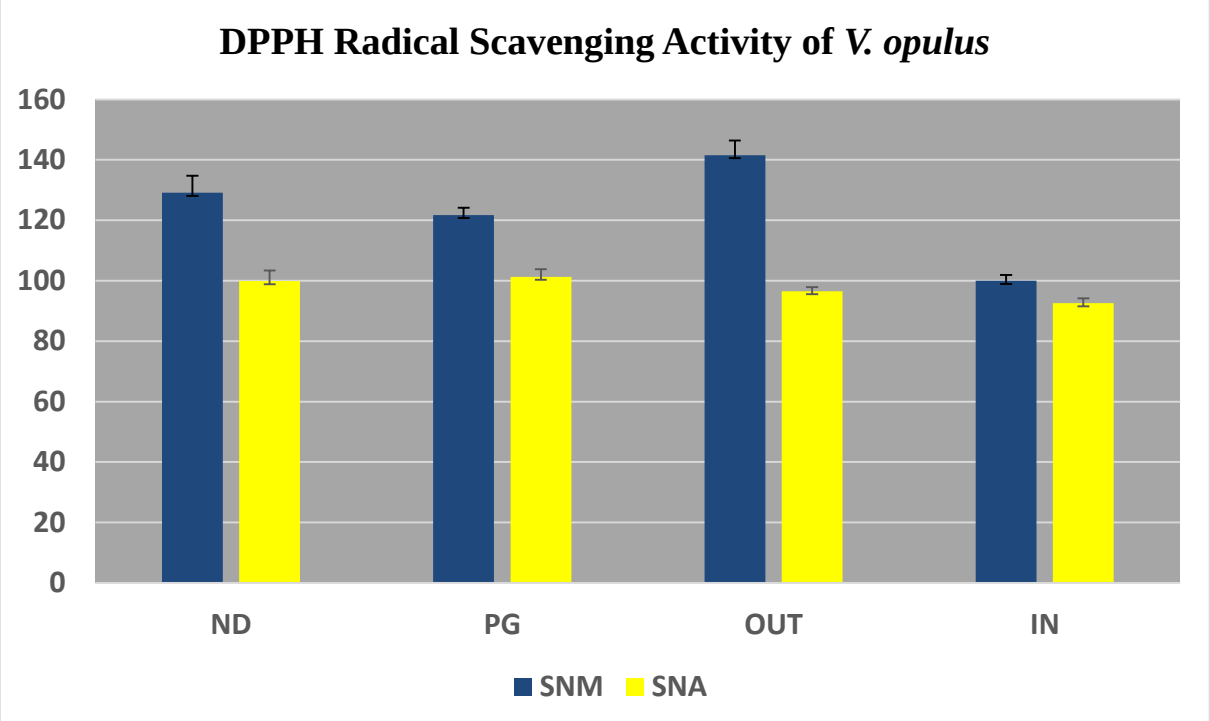

Figure 43. DPPH Radical Scavenging Activity of *Sambucus nigra* Fruit Extracts

4.8.2. DPPH Radical Scavenging Activity of *Sambucus ebulus* Fruit Extracts

DPPH radical scavenging activity of SEM showed no significant changes between ND and IN fractions. On the other hand, DPPH radical scavenging activity of SEA exerted minor but significant decline.

Table 56. DPPH Radical Scavenging Activity of *Sambucus ebulus* Fruit Extracts

Name of the analysis	*ND* [A]	*PG*	*OUT*	*IN*
SEM-DPPH scavenging act. [B#]	$98.25^{a} \pm 2.57$	$104.10^{b} \pm 1.74$	$105.06^{b} \pm 0.28$	$93.55^{a} \pm 2.29$
SEA-DPPH scavenging act.	$97.46^{a} \pm 0.88$	$101.50^{b} \pm 1.08$	$91.93^{c} \pm 1.15$	$90.43^{c} \pm 2.49$

[A] The abbreviations for samples are *ND: non-digested, PG: postgastric, OUT: Colon available IN: bioavailable, BAvI: Bioavailability index*
[B] The abbreviation for the analysis is DPPH: 2,2-diphenyl-1-picrylhydrazyl
[C] Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg butylated hydroxyl toluene equivalents (BHTE) in 1 g sample.
* Different letters in the same row indicate significance ($p < 0.05$).
** Methanolic extract of *Sambucus ebulus* L. fruits
*** Aqueous extract of *Sambucus ebulus* L. fruits

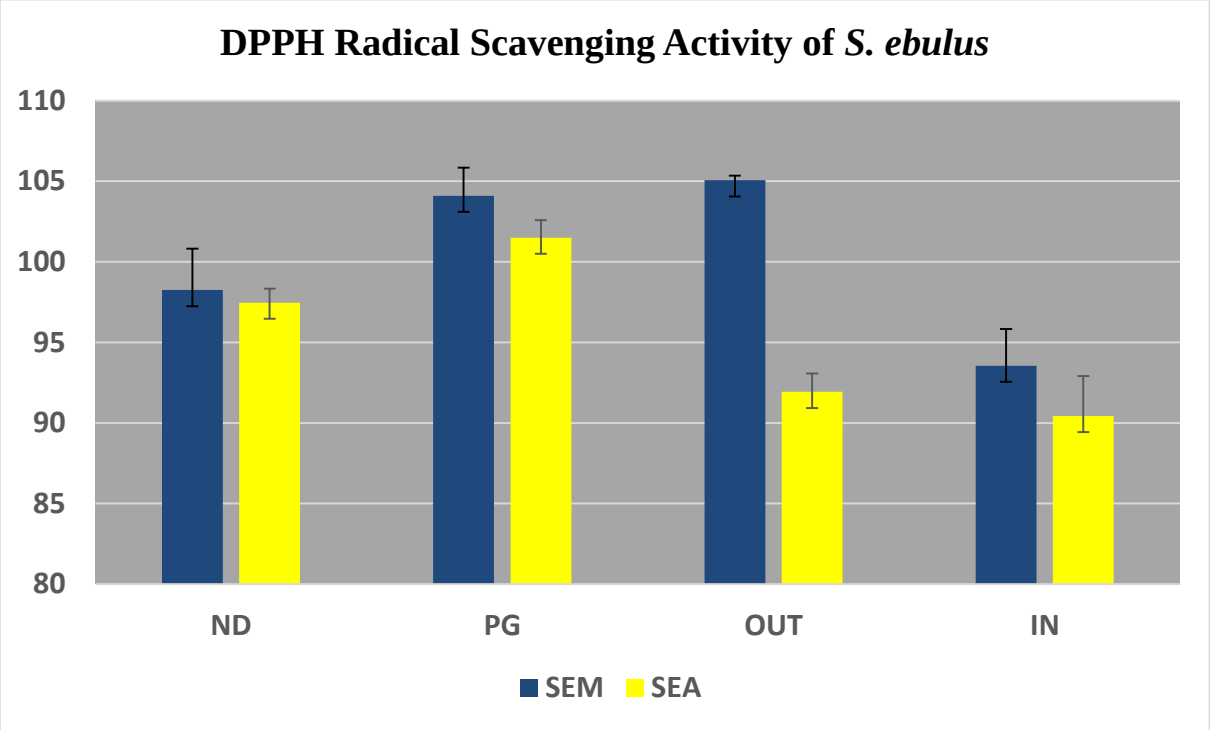

Figure 44. DPPH Radical Scavenging Activity of *Sambucus ebulus* Fruit Extracts

4.8.3. DPPH Radical Scavenging Activity of *Viburnum opulus* Fruit Extracts

In DPPH (1,1-diphenyl-2-picrylhydrazyl) assay, post-gastric phases of both extracts had negligible changes with non-digested phase. In addition, both extracts demonstrated alleviation in small quantities in DPPH radical scavenging activity at serum available fraction.

Table 57. DPPH Radical Scavenging Activity of *Viburnum opulus* Fruit Extracts

Name of the analysis	*ND* [A]	*PG*	*OUT*	*IN*
VOM-DPPH scavenging act. [BC]	$103.59^{a} \pm 5.26^{**}$	$106.02^{a} \pm 2.79$	$92.28^{b} \pm 0.96$	$88.60^{b} \pm 3.18$
VOA-DPPH scavenging act.	$96.74^{a} \pm 4.15$	$92.88^{ab} \pm 4.75$	$93.41^{ab} \pm 2.11$	$87.22^{b} \pm 1.09$

[A] The abbreviations for samples are *ND: non-digested, PG: postgastric, OUT: Colon available IN: bioavailable, BAvI: Bioavailability index*
[B] The abbreviation for the analysis is DPPH: 2,2-diphenyl-1-picrylhydrazyl
[C] Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg butylated hydroxyl toluene equivalents (BHTE) in 1 g sample.
* Different letters in the same row indicate significance ($p < 0.05$).
** Methanolic extract of *Viburnum opulus* L. fruits
*** Aqueous extract of *Viburnum opulus* L. fruits

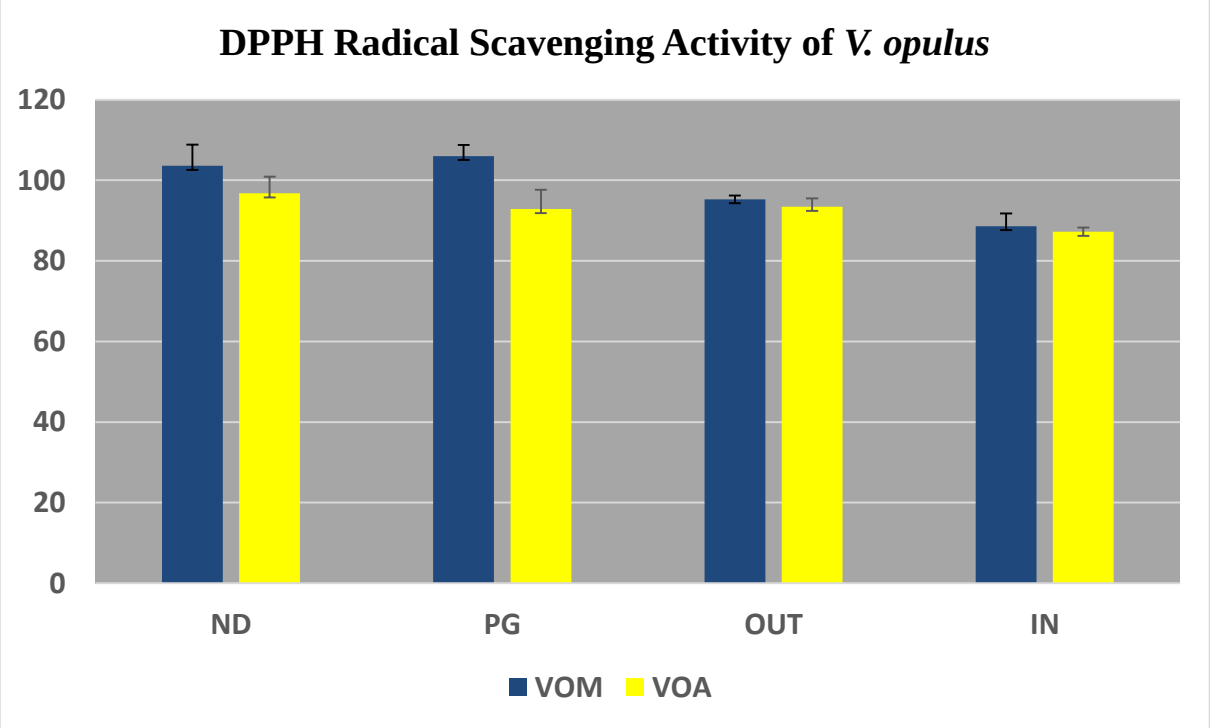

Figure 45. DPPH Radical Scavenging Activity of *Viburnum opulus* Fruit Extracts

4.9. Results of DMPD Radical Scavenging Activity Assay

4.9.1. DMPD Radical Scavenging Activity of *Sambucus nigra* Fruits

Same trends were observed in DMPD assay. SNM had higher DMPD radical scavenging activity and higher reduction rate in serum available phase. Nonetheless in all phases SNM had higher bioactivity when compared to SNA.

Table 58. DMPD Radical Scavenging Activity of *Sambucus nigra* Fruits

Name of the analysis	*ND* [A]	*PG*	*OUT*	*IN*
SNM-DMPD scavenging act. [BC]	$168.17^{a} \pm 3.89$	$161.90^{a} \pm 1.54$	$151.27^{b} \pm 5.87$	$148.64^{b} \pm 0.49$
SNA-DMPD scavenging act.	$126.26^{a} \pm 1.71$	$121.74^{ab} \pm 2.47$	$118.56^{b} \pm 0.76$	$113.11^{c} \pm 2.35$

[A] The abbreviations for samples are *ND: non-digested, PG: postgastric, OUT: Colon available IN: bioavailable, BAvI: Bioavailability index*
[B] The abbreviation for the analysis is DMPD: N,N-dimethyl-p-phenylendiamine
[C] Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg Trolox equivalents (TE) in 1 g sample.
* Different letters in the same row indicate significance ($p < 0.05$).
** Methanolic extract of *Sambucus nigra* L. fruit
*** Aqueous extract of *Sambucus nigra* L. fruits

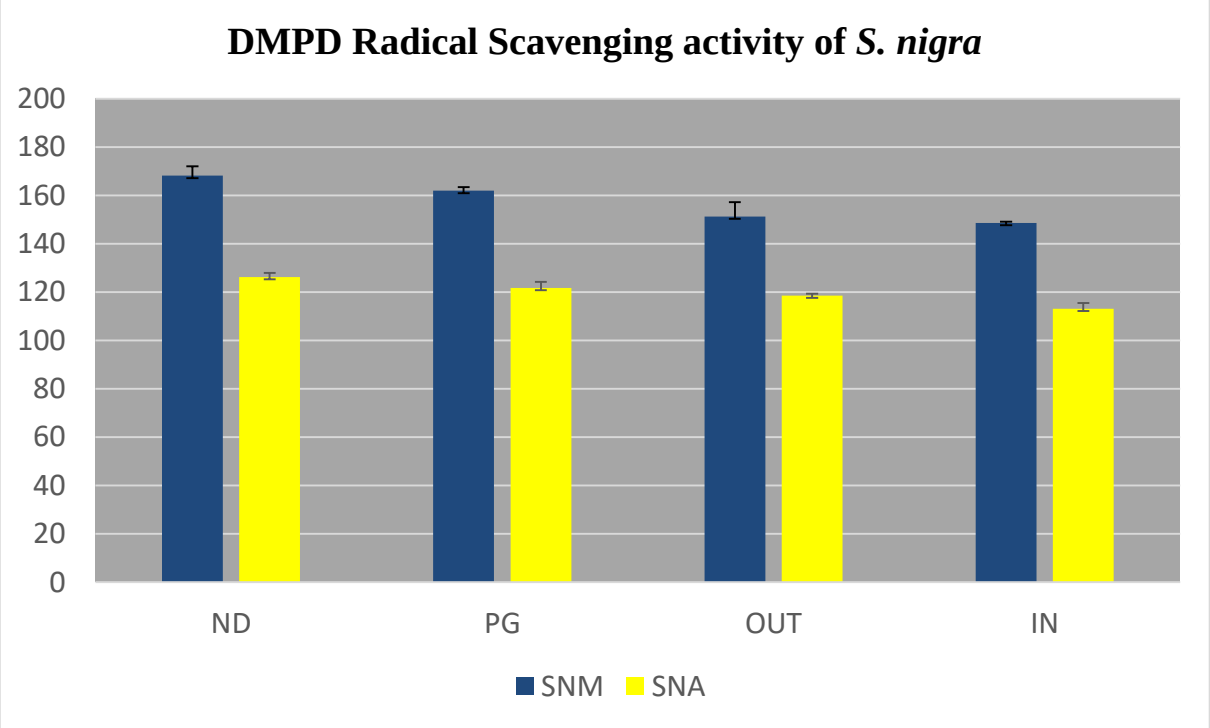

Figure 46. DMPD Radical Scavenging Activity of *Sambucus nigra* Fruits

4.9.2. DMPD Radical Scavenging Activity of *Sambucus ebulus* Fruits

SEA demonstrated no significant alterations from ND to IN fraction in DMPD radical scavenging activity assay, while SEM exhibited minor but significant reduction. Free radical scavenging activity of both extracts was found to be similar in-serum available fraction.

Table 59. DMPD Radical Scavenging Activity of *Sambucus ebulus* Fruits

Name of the analysis	*ND* [A]	*PG*	*OUT*	*IN*
SEM-DMPD scavenging act. [BC]	$48.15^{a} \pm 0.59$	$49.26^{ab} \pm 0.17$	$45.19^{bc} \pm 1.09$	$45.68^{c} \pm 0.88$
SEA-DMPD scavenging act.	$39.56^{a} \pm 0.47$	$38.88^{a} \pm 0.79$	$38.64^{a} \pm 1.22$	$37.96^{a} \pm 0.27$

[A] The abbreviations for samples are *ND: non-digested, PG: postgastric, OUT: Colon available IN: bioavailable, BAvI: Bioavailability index*
[B] The abbreviation for the analysis is DMPD: N,N-dimethyl-p-phenylendiamine
[C] Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg Trolox equivalents (TE) in 1 g sample.
* Different letters in the same row indicate significance ($p < 0.05$).
** Methanolic extract of *Sambucus ebulus* L.fruit
*** Aqueous extract of *Sambucus ebulus* L. fruits

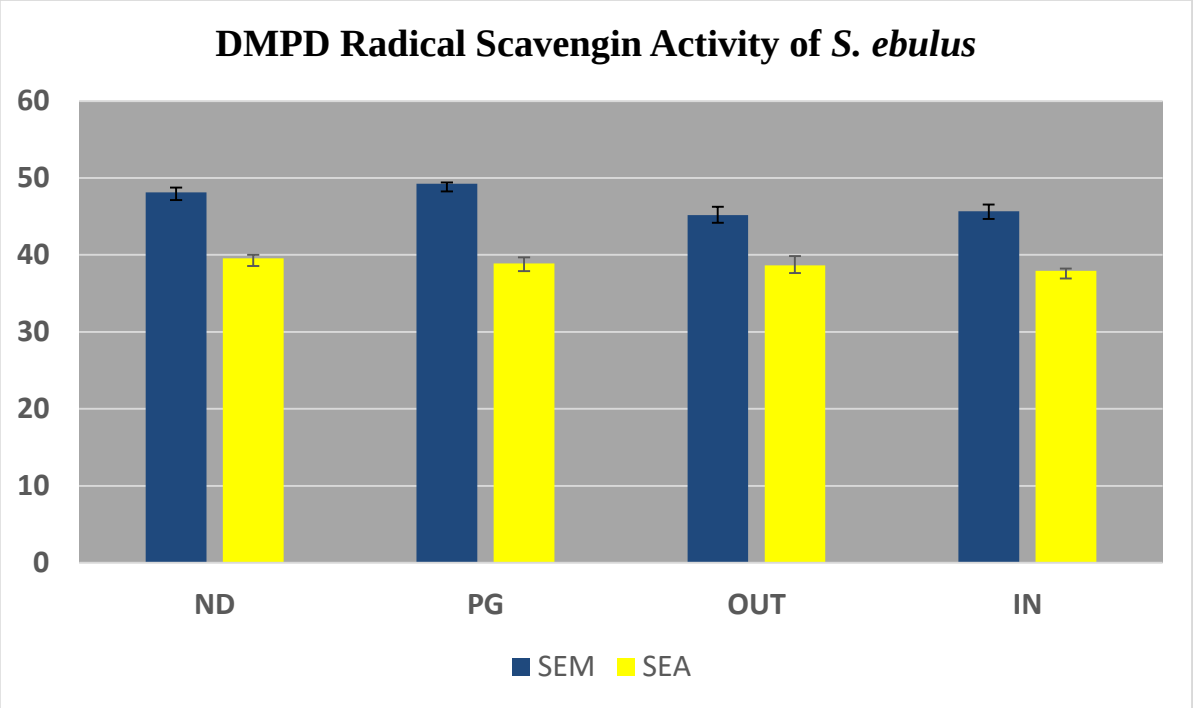

Figure 47. DMPD Radical Scavenging Activity of *Sambucus ebulus* Fruits

4.9.3. DMPD Radical Scavenging Activity of *Viburnum opulus* Fruits

In DMPD (Dimethyl-4-phenylenediamine) assay, both extracts showed no significant changes for all phases. Non-digested, post-gastric, colon available and serum-available phases possessed resembling DMPD radical activity.

Table 60. DMPD Radical Scavenging Activity of *Viburnum opulus* Fruits

Name of the analysis	*ND* [A]	*PG*	*OUT*	*IN*
VOM-DMPD scavenging act. [B§]	52.55[a] ± 4.87	46.41[a] ± 9.27	47.64[a] ± 7.67	42.11[a] ± 1.15
VOA-DMPD scavenging act.	55.00[a] ± 2.81	45.39[a] ± 6.64	49.48[a] ± 2.62	52.55[a] ± 6.47

[A] The abbreviations for samples are *ND: non-digested, PG: postgastric, OUT: Colon available IN: bioavailable, BAvI: Bioavailability index*
[B] The abbreviation for the analysis is DMPD: N,N-dimethyl-p-phenylendiamine
[C] Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg Trolox equivalents (TE) in 1 g sample.
* Different letters in the same row indicate significance ($p < 0.05$).
** Methanolic extract of *Viburnum opulus* L fruits
*** Aqueous extract of *Viburnum opulus* L. fruits

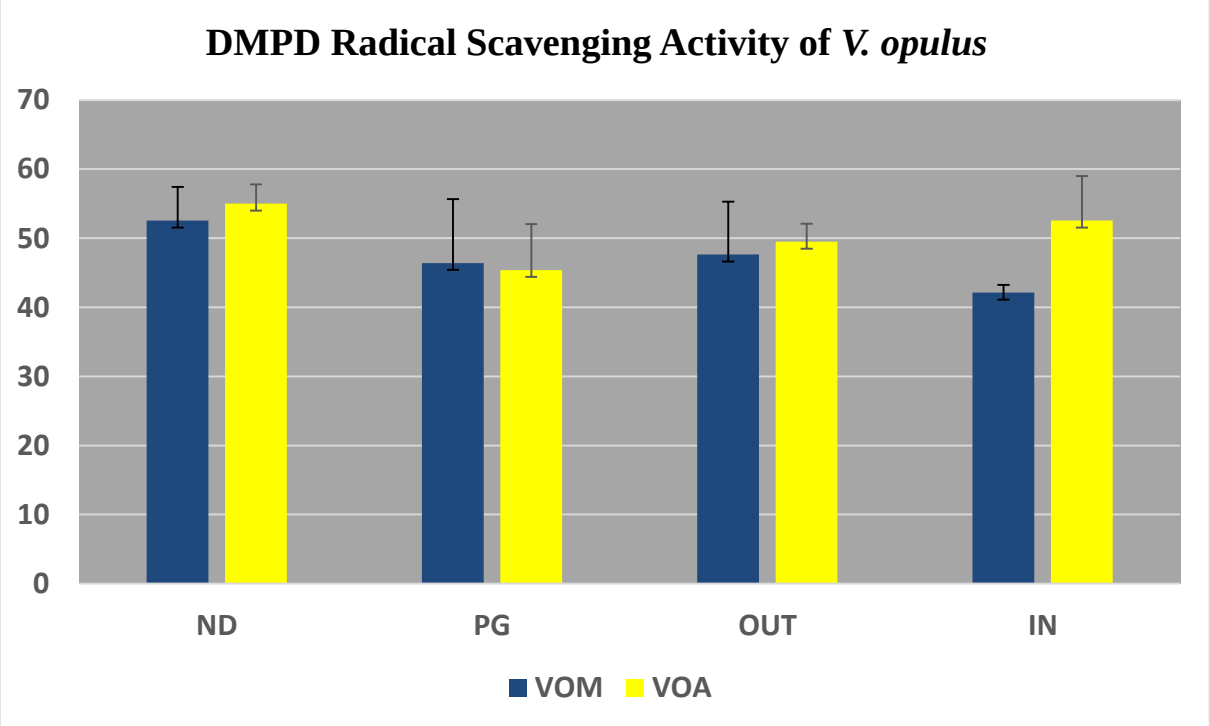

Figure 48. DMPD Radical Scavenging Activity of *Viburnum opulus* Fruits

4.10. Results of Total Antioxidant Activity (TOAC) Assay

4.10.1. Total Antioxidant Activity of *Sambucus nigra* Fruit Extracts

Methanolic extract exhibited higher activity in non-digested samples (SNM ND: 55.88 ± 6.85 mg AAE/g, SNA ND: 40.38 ± 4.88). Same trend was observed for both extracts in every step of digestion process. Post-gastric and colon-available phases showed no alteration nonetheless; bioactivity of both extracts strongly declined in serum-available phase.

Table 61. Total Antioxidant Activity of *Sambucus nigra* Fruit Extracts

Name of the analysis	*ND* [A]	*PG*	*OUT*	*IN*
SNM-Total antioxidant capacity[B]	$55.88^{a} \pm 6.85$	$55.56^{a} \pm 0.55$	$53.67^{a} \pm 4.31$	$35.94^{b} \pm 5.03$
SNA- Total antioxidant capacity	$40.38^{a} \pm 4.88$	$38.31^{a} \pm 1.25$	$35.47^{a} \pm 4.35$	$27.55^{b} \pm 4.80$

[A] The abbreviations for samples are *ND: non-digested, PG: postgastric, OUT: Colon available IN: bioavailable, BAvI: Bioavailability index*

[B] Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg ascorbic acid equivalents (AAE) in 1 g sample.

* Different letters in the same row indicate significance ($p < 0.05$).

** Methanolic extract of *Sambucus nigra* L. fruits

*** Aqueous extract of *Sambucus nigra* L. fruits

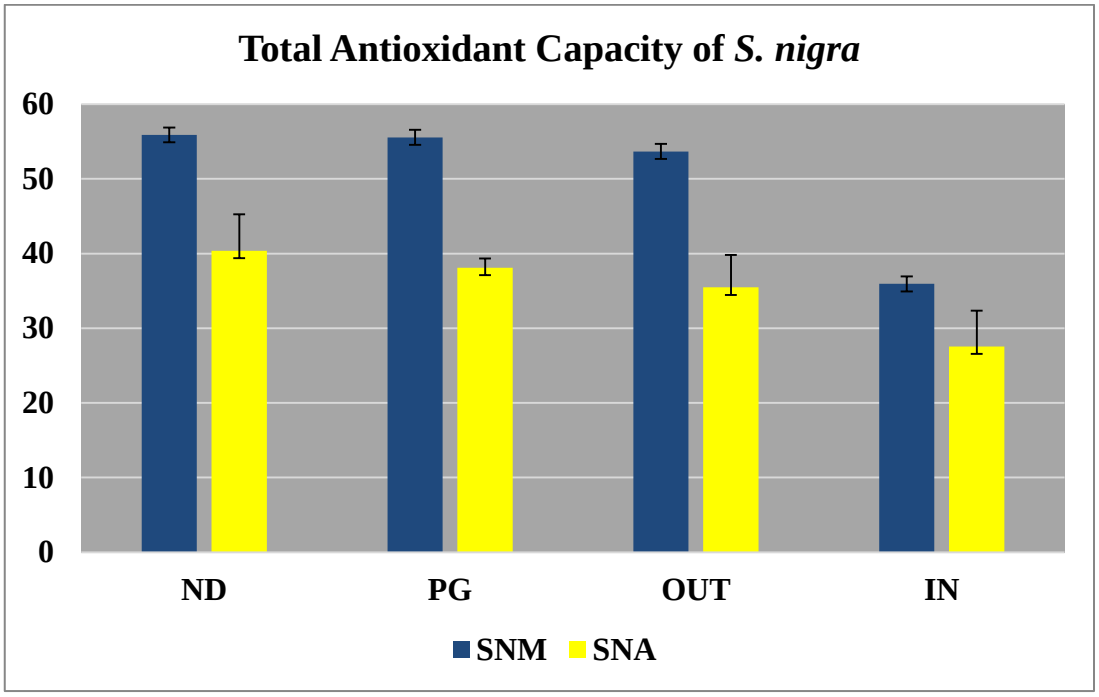

Figure 49. Total Antioxidant Activity of *Sambucus nigra* Fruit Extracts

4.10.2. Total Antioxidant Activity of *Sambucus ebulus* Fruit Extracts

SEM extract showed considerably higher activity than SEA extract. In post-gastric phase, both extracts showed significant reduction in activity. However, after gastric phase SEM exhibited significant reduction, while SEA exhibited small but significant alteration in serum-available phase.

Table 62. Total Antioxidant Activity of *Sambucus ebulus* Fruit Extracts

Name of the analysis	*ND* [A]	*PG*	*OUT*	*IN*
SEM-Total antioxidant capacity[B]	$82.31^{a} \pm 4.09$	$45.27^{b} \pm 2.90$	$34.99^{c} \pm 1.65$	$37.37^{d} \pm 0.48$
SEA- Total antioxidant capacity	$51.13^{a} \pm 7.37$	$32.77^{b} \pm 2.44$	$31.19^{b} \pm 1.25$	$25.18^{b} \pm 0.72$

[A] The abbreviations for samples are *ND: non-digested, PG: postgastric, OUT: Colon available IN: bioavailable, BAvI: Bioavailability index*
[B] Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg ascorbic acid equivalents (AAE) in 1 g sample.
* Different letters in the same row indicate significance ($p < 0.05$).
** Methanolic extract of *Sambucus ebulus* L. fruits
*** Aqueous extract of *Sambucus ebulus* L. fruits

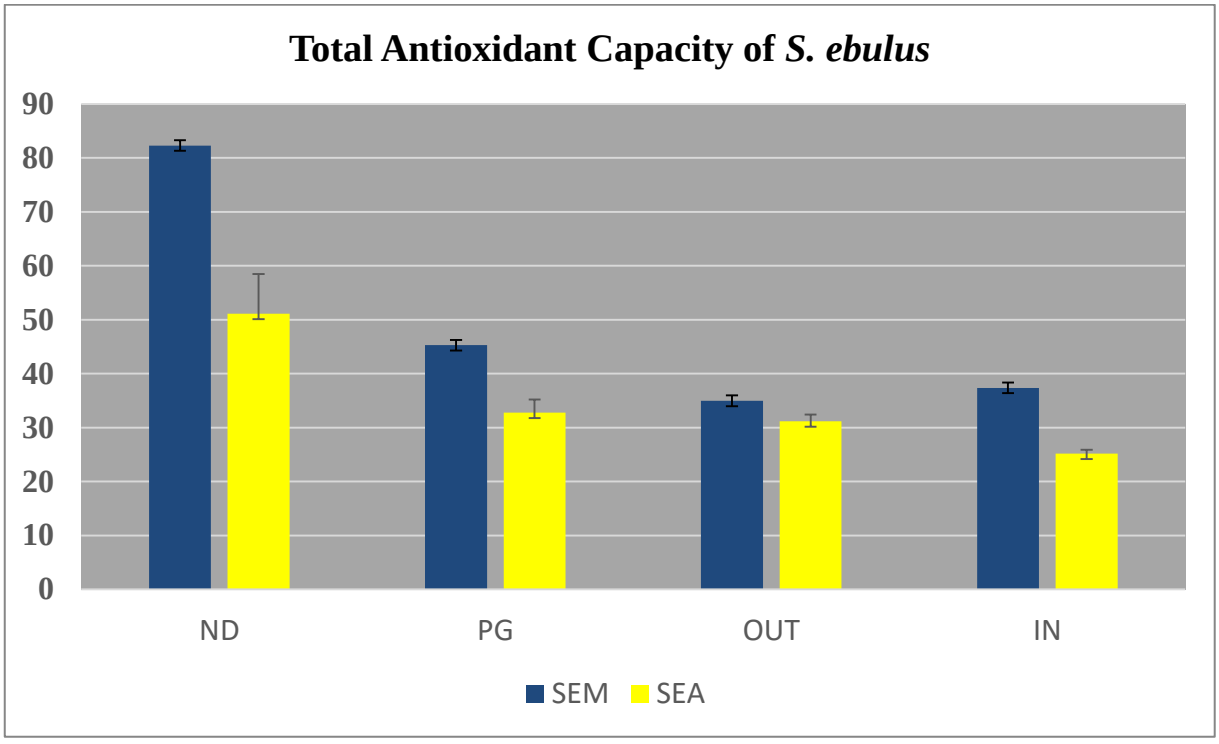

Figure 50. Total Antioxidant Activity of *Sambucus ebulus* Fruit Extracts

4.10.3. Total Antioxidant Activity of *Viburnum opulus* Fruit Extracts

In TOAC assay VOM extract demonstrated higher activity than VOA extracts. After gastric simulation phase activity of both extracts dropped significantly. In VOM extract, serum-available phase had lesser activity than post-gastric and colon-available phase, while VOA extract showed no significant changes after post-gastric phase.

Table 63. Total Antioxidant Activity of *Viburnum opulus* Fruit Extracts

Name of the analysis	*ND* [A]	*PG*	*OUT*	*IN*
VOM-Total antioxidant capacity [B]	$56.89^{a} \pm 5.14$	$40.69^{b} \pm 0.48$	$36.73^{bc} \pm 2.14$	$30.72^{c} \pm 2.89$
VOA- Total antioxidant capacity	$49.07^{a} \pm 6.20$	$33.09^{b} \pm 0.82$	$31.35^{b} \pm 1.37$	$30.40^{b} \pm 1.93$

[A] The abbreviations for samples are *ND: non-digested, PG: postgastric, OUT: Colon available IN: bioavailable, BAvI: Bioavailability index*

[B]Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg ascorbic acid equivalents (AAE) in 1 g sample.

* Different letters in the same row indicate significance ($p < 0.05$).

** Methanolic extract of *Viburnum opulus* L fruits

*** Aqueous extract of *Viburnum opulus* L. fruits

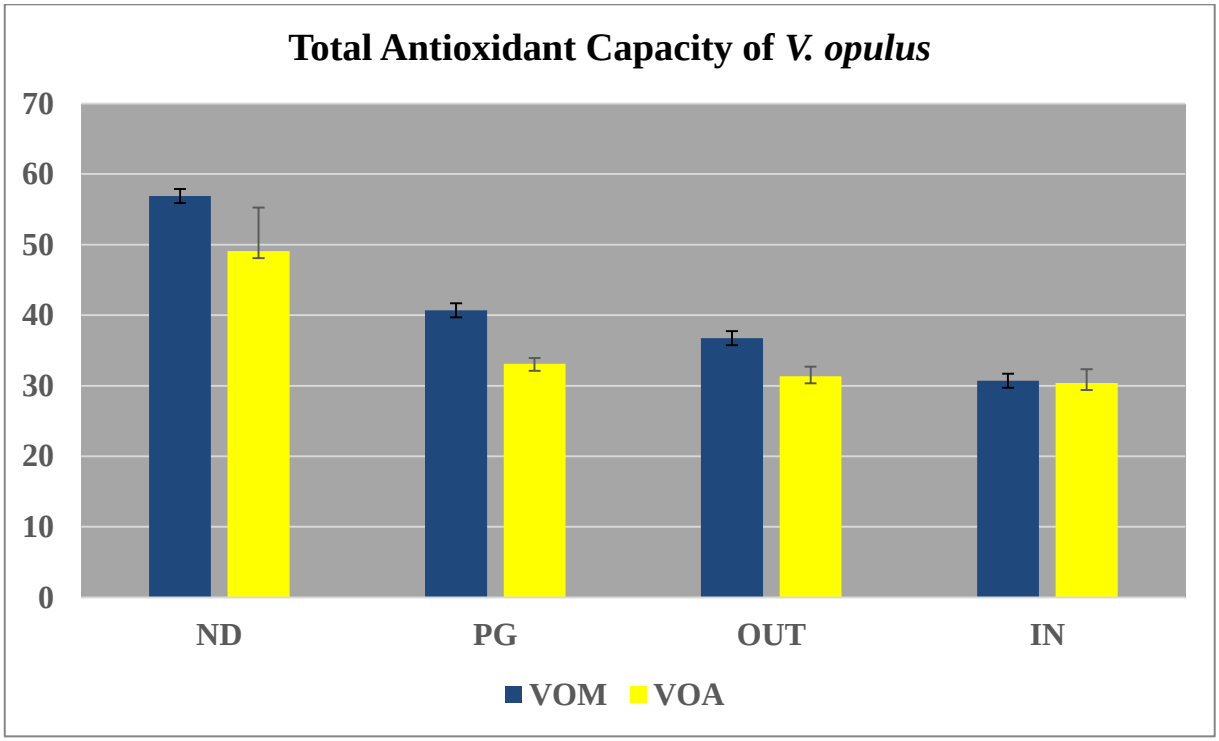

Figure 51. Total Antioxidant Activity of *Viburnum opulus* Fruit Extracts

4.11. Ferrous Ion Chelating Capacity

The metal chelating activity of the extracts was investigated by using four concentrations, *i.e.* 1, 2, 5 and 10 mg/mL. We found no activity of either extracts at the mentioned concentrations. EDTA, was used as a reference compound. Its EC50 value was measured as 8.7 ± 0.4µg/mL.

4.12. Comparison of all studied extracts

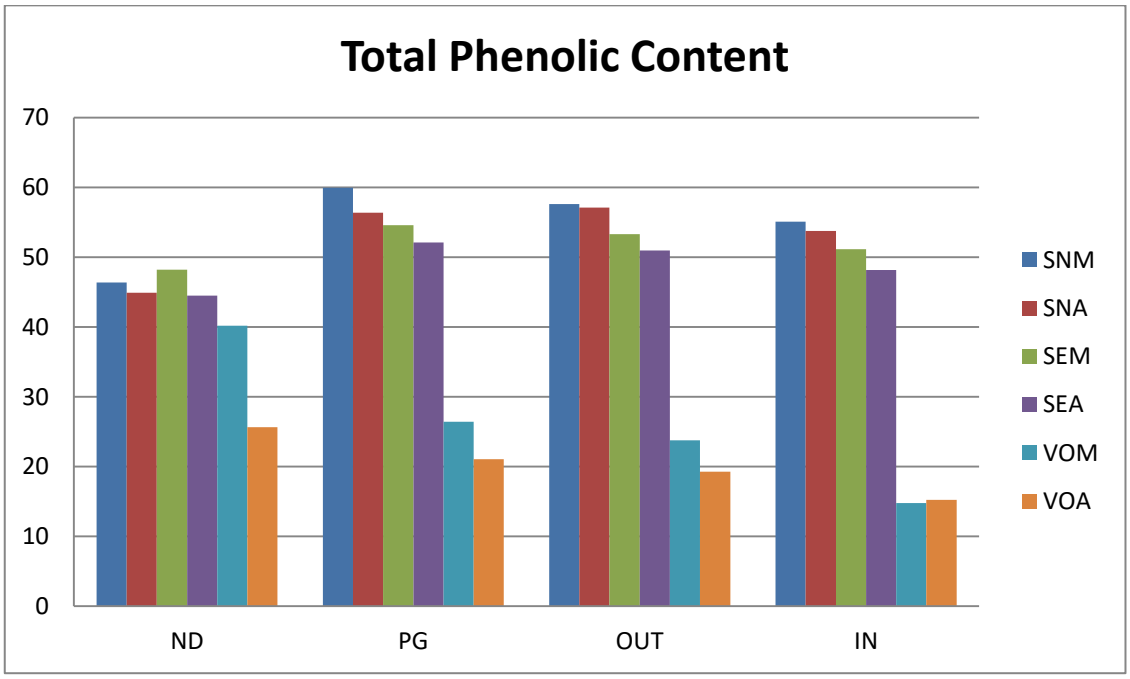

Figure 52. Comparison of Total Phenolic Content Assay Results

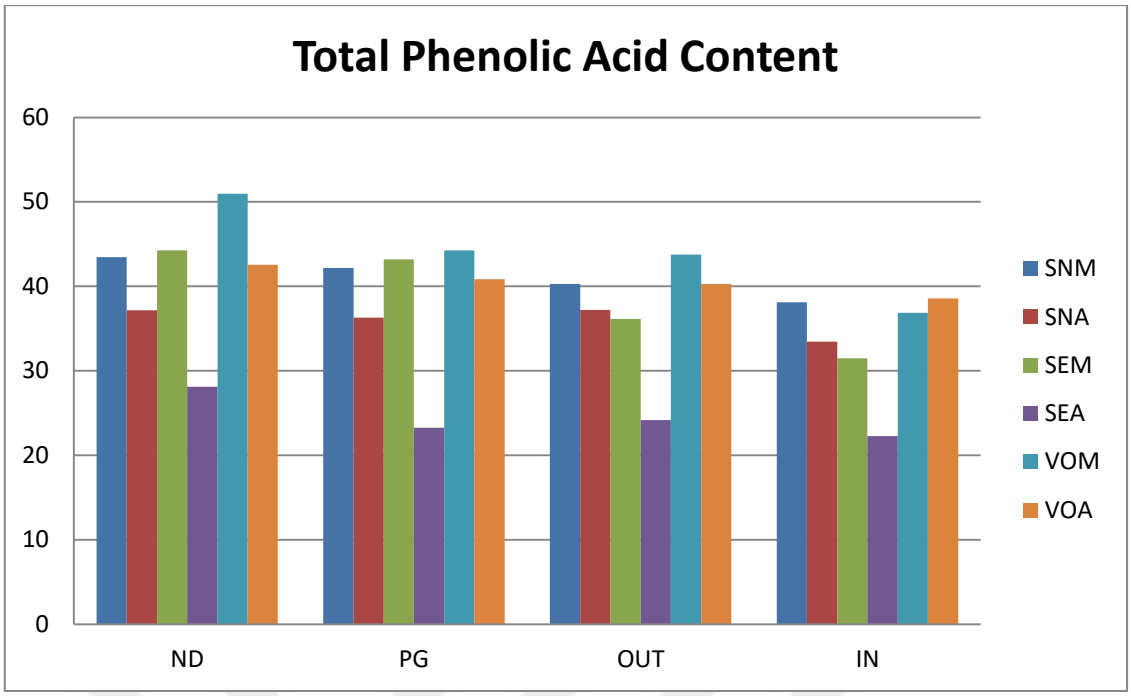

Figure 53. Comparison of Total Phenolic Acid Content Assay Results

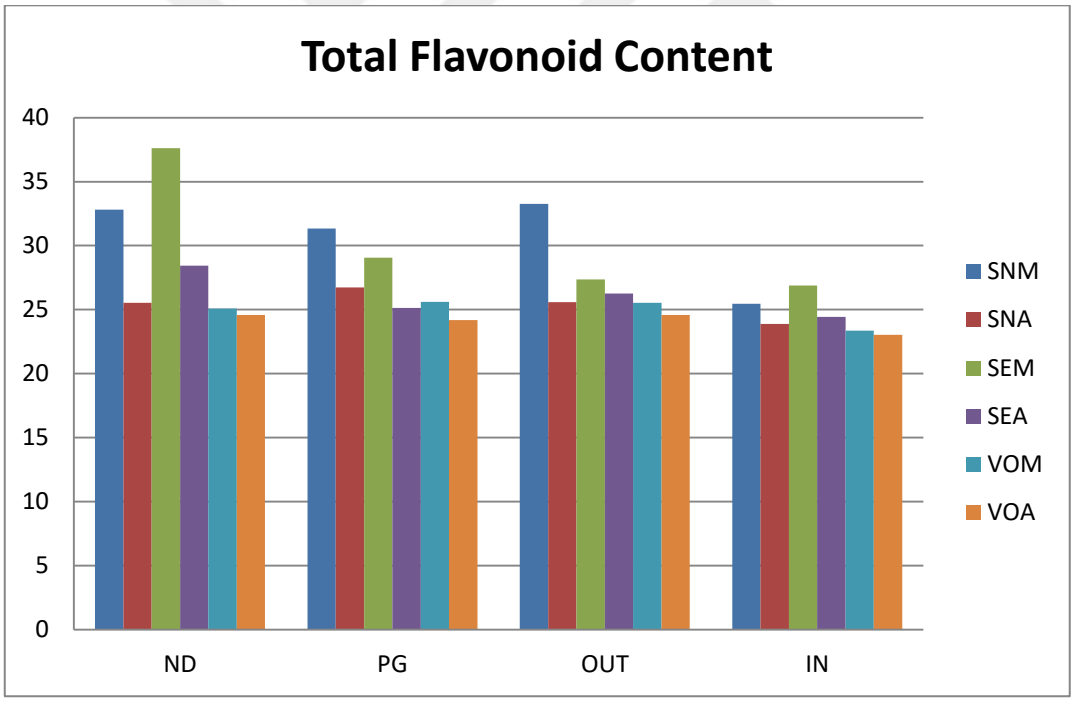

Figure 54. Comparison of Total Flavonoid Content Assay Results

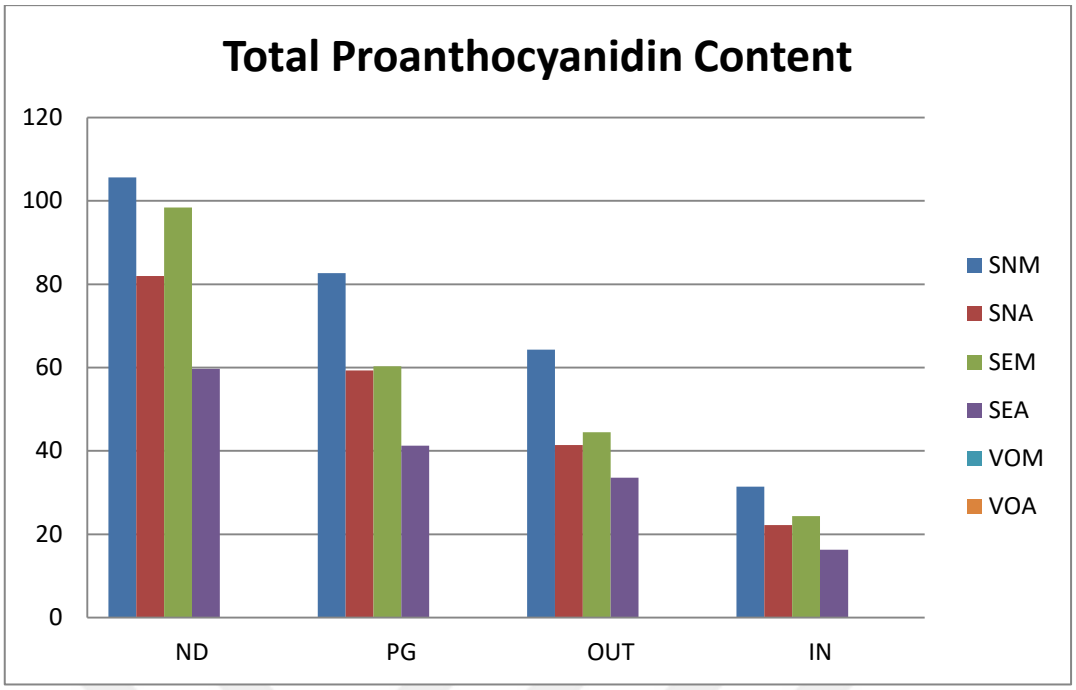

Figure 55. Comparison of Total Proanthocyanidin Content Assay Results

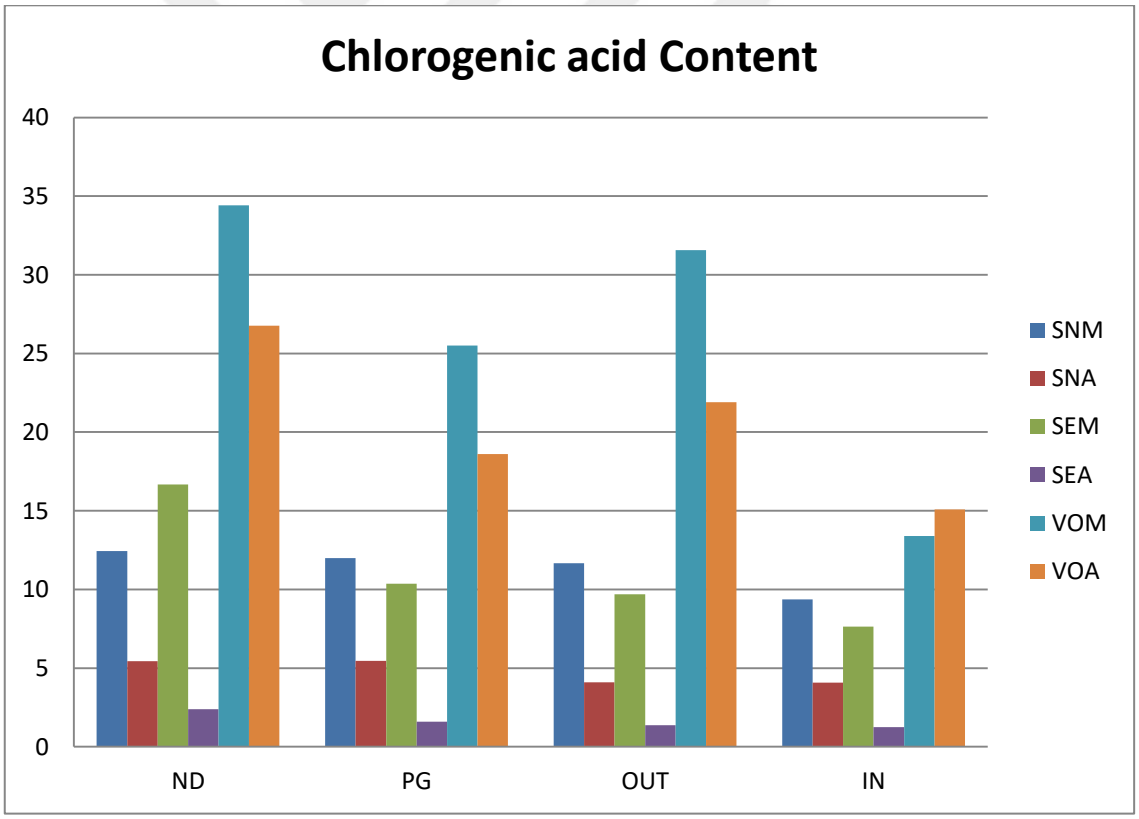

Figure 56. Comparison of Chlorogenic acid Content Assay Result

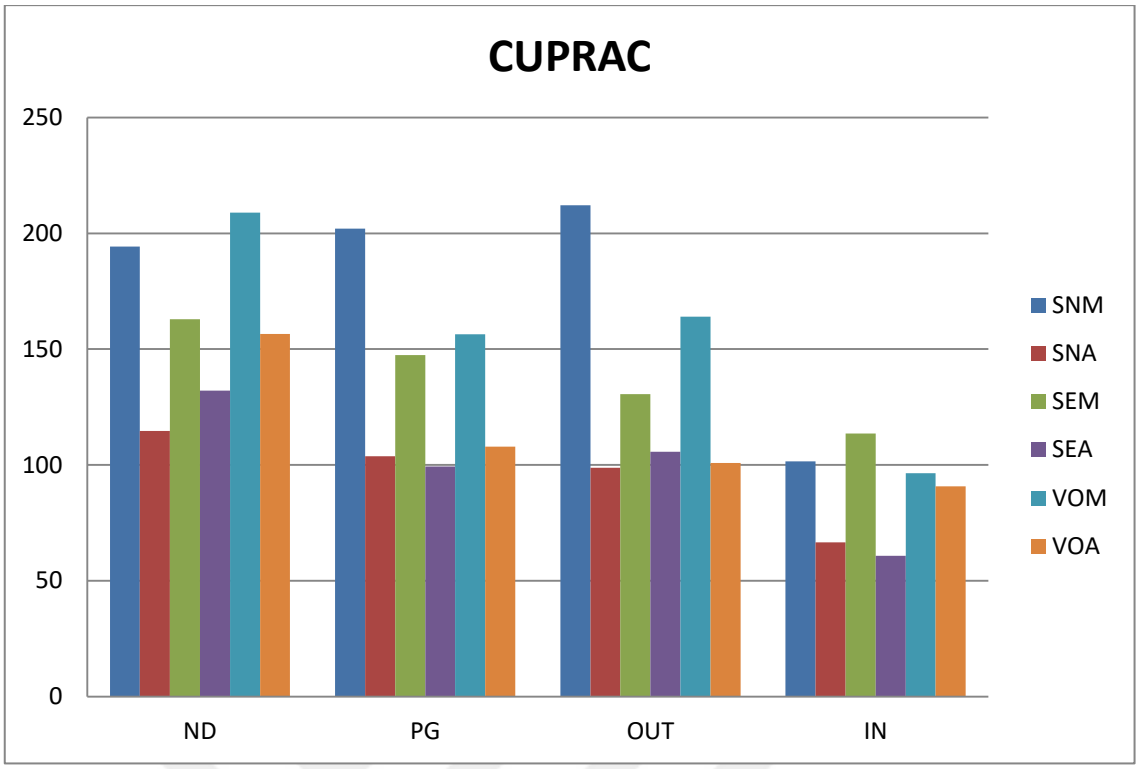

Figure 57. Comparison of CUPRAC Assay Results

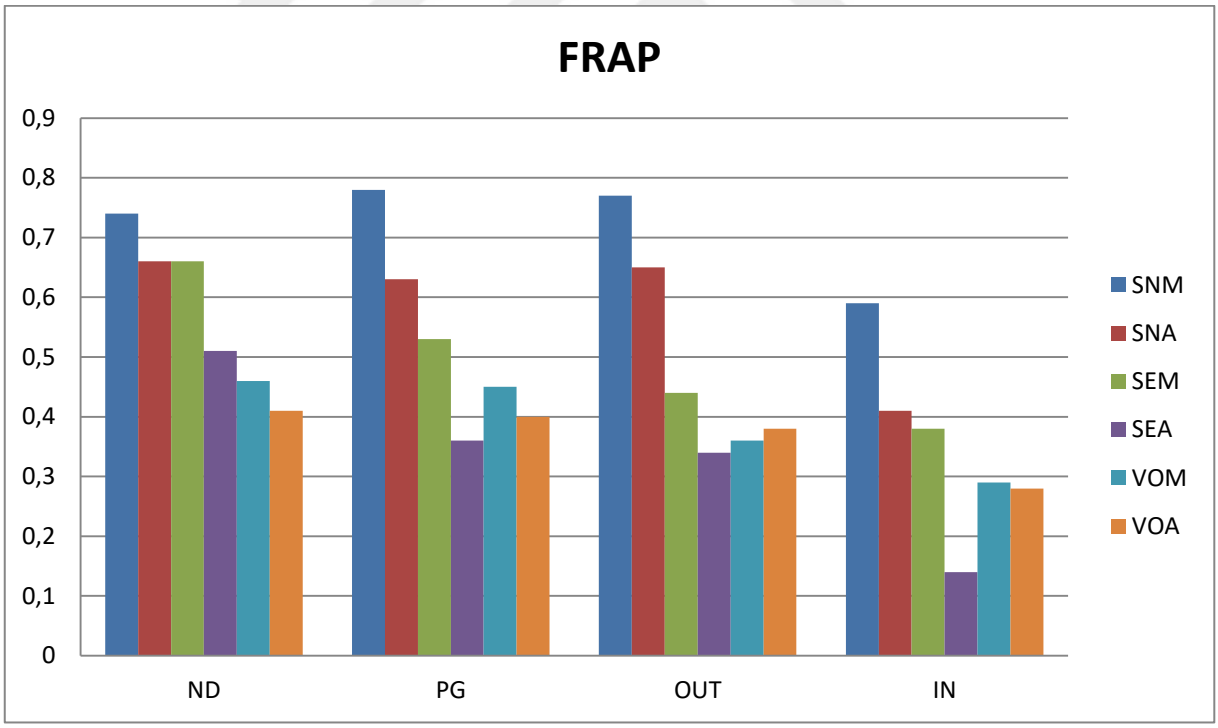

Figure 58. Comparison of FRAP Assay Results

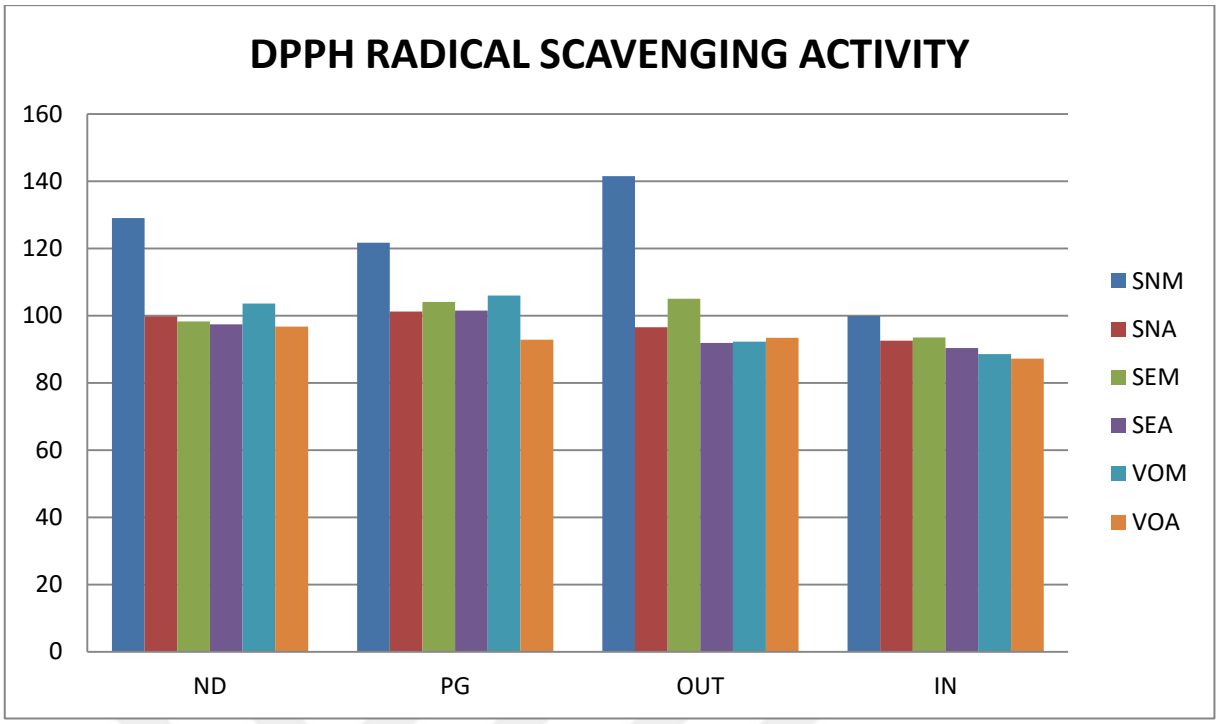

Figure 59. Comparison of DPPH Radical Scavenging Assay Results

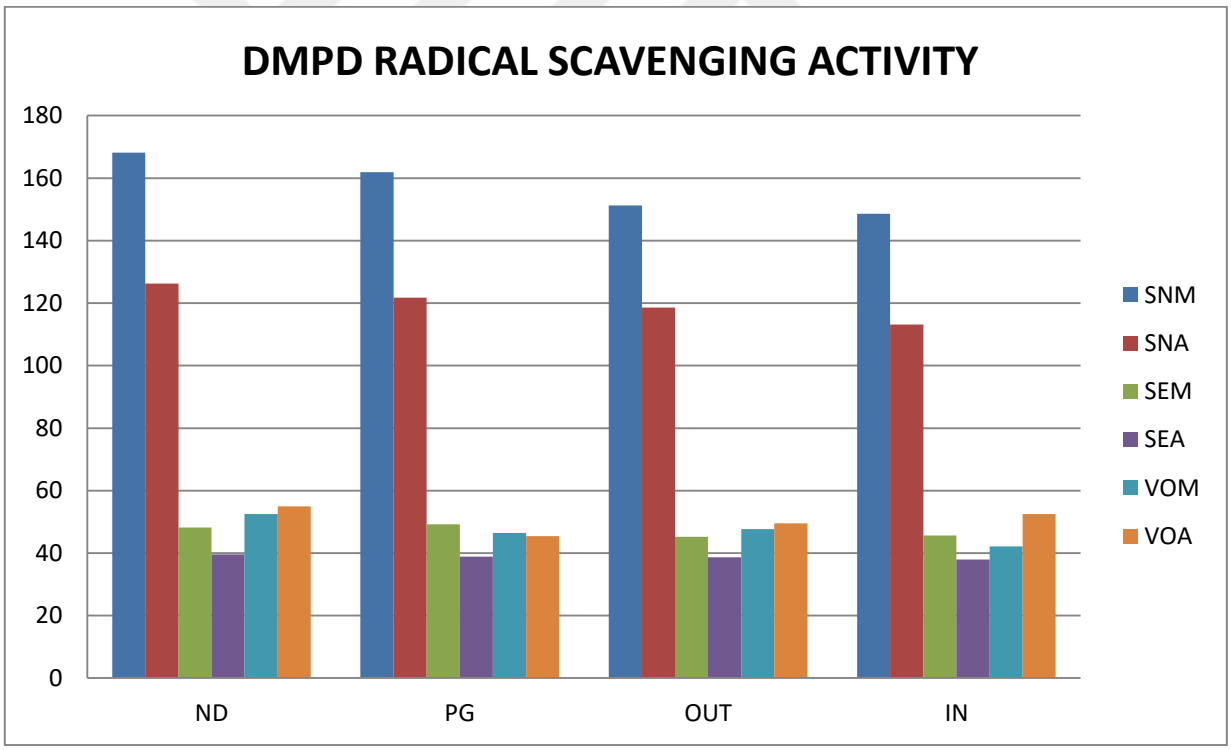

Figure 60. Comparison of DMPD Radical Scavenging Assay Results

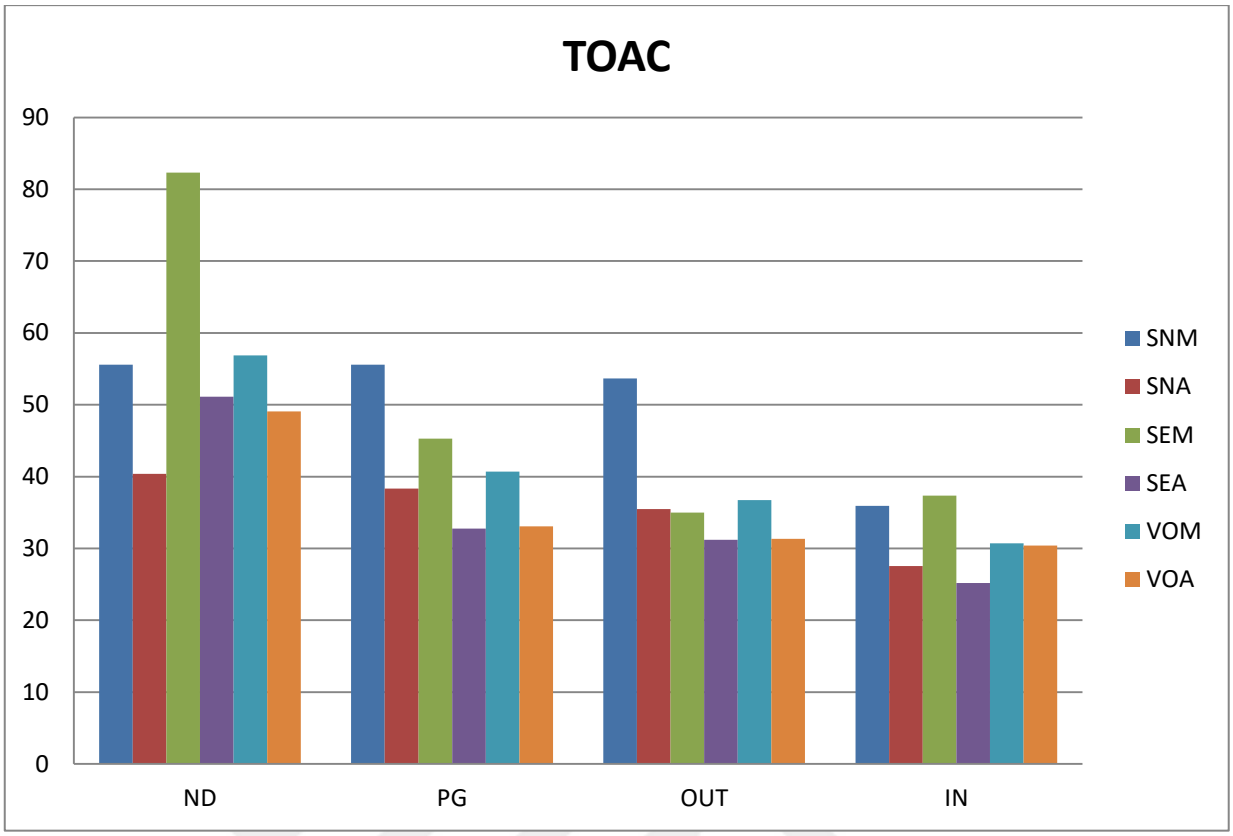

Figure 61. Comparison of TOAC Assay Results

5. DISCUSSION

Current medicinal paradigm considers plants as a primary option for prophylaxis and treatment due to their better coherence with patients and low rate of side effect frequency comparing to conventional drugs. Oxidative stress is currently regarded as one of the primary factors for numerous chronicle diseases (e.g. Alzheimer's, cardiovascular diseases, diabetes) (141). These diseases do not have a specific cure, thus after diagnosis, patients require constant treatment. In this context, antioxidants have a key role for their ability for protection from oxidative stress. Prevention from such diseases is more convenient for both promotion of public health and lowering the cost of social security systems. Phenolic compounds are known for their potent antioxidant bioactivity; therefore fruits rich in phenolic ingredients are considered to be beneficial for homeostasis. It has been considered that daily consumption of 1 g phenolic compounds through fresh fruits and vegetables might be favourable for preventing from carcinogenesis and mutagenesis (141).

A vast number of studies have shown that there are irrefutable correlations between the amount of phenolic compounds in plant extracts and their antioxidant potentials (142). In this context, several studies have reported the antioxidant potentials of *S. nigra*, *S. ebulus* and *V. opulus* fruits (143-145). Among them, none had taken into consideration the possible influence of GI tract on chemical properties and antioxidant capacity of their extracts. As mentioned earlier, bioactive compounds responsible for the antioxidant activity are supposed to reach target tissues in sufficient concentrations to exhibit their properties. Hence, it is crucial to determine the stability and bioavailability of phenolic compounds for higher rate of accuracy for bioactivity. For this reason, in order to estimate the genuine activity profile of these plant extracts, the aforementioned human digestion simulation model was applied in this study. Both the methanolic and aqueous extracts of selected fruits from Adoxaceae were investigated for their phenolic profiles and antioxidant potentials after subjected to human digestion procedures.

Phenolic profile of *S. nigra* fruits and flowers were investigated in various previous studies. Total phenolic content of *S. nigra* fruits which were collected from Turkey, were measured in a previous study (146) (Table 65). Variances in the values were originated in different genotypes. Also, different genotypes of *S. nigra* fruits from

USA were investigated in another study and results were found significantly similar with that the genotypes from Turkey (147) (Table 65). Ochiman et al. (148) and Wu et al. (149) also calculated TPC of *S. nigra* fruits and results were given at Table 65. In our study, TPC of both extracts were analogous. TPC of SNM was measured 46.37 ± 1.28 and SNA was 44.91 ± 2.15 GAE/g dry extract. Digestion process affected the extracts correspondingly. Following the gastric simulation, total phenolic content rate and bioavailability was amplified and total bioavailability was resulted 118.76 % and 119.66 %, respectively (Table 29). Flavonoid profile of *S. nigra* was studied several times in previous studies as well. Total flavonoid content of *S. nigra* fruits from Romania was calculated (90) and in another study, fruit extracts of elderberry were investigated and results revealed the total amounts of flavonols (71) (Table 65). Same study investigated flavonol content of elder flowers. Results revealed that flowers had higher amount of flavonols. In our study, total flavonoid content of both extracts and their variance after GI digestion simulation were investigated (Table 35). Results showed that methanolic extract contains significantly higher amount of flavonoids. Nevertheless, human digestion simulation affected methanolic extract in higher in negative manner and in serum-available concentrations of both extracts were observed highly equivalent. Total phenolic acids and proanthocyanidins of *S. nigra* fruits were investigated in a previous studies (148, 149) and results given in Table 65. Correspondingly with previous data, our study revealed that fruits of *S. nigra* had substantial amount of phenolic acids and proanthocyanidins (Tables 32, 38). Methanolic extracts had higher amounts in all conducted phenolic profile assays. Total proanthocyanidin content was affected more intense than any other phenolic compounds and showed lowest bioavailability.

Kaack et al. (150) investigated different genotypes of *S. nigra* fruits for determination of suitable genotype for industrial utilization. Results that rutin and chlorogenic acid are abundant metabolites. Two other studies confirmed previous findings; different cultivars from USA were investigated for its rutin and chlorogenic acid contents (147) and also rutin and chlorogenic acid content of fruits collected from Turkey was measured (151). All of these studies revealed that rutin and chlorogenic acid are most abundant phenolic metabolites of *S. nigra* fruits (Table 66). The results of our study revealed rutin and chlorogenic acid content of both extracts which were investigated via HPTLC and our results exhibited that rutin and chlorogenic acid content of methanolic extracts were significantly higher than aqueous extracts (Table

40). After digestion process, bioavailability of rutin and chlorogenic acid were higher than 70%, except in rutin amount of aqueous extract of *S. nigra.*

As mentioned before, previous studies conducted on the phenolic profile and antioxidant activity of *S. ebulus* fruits disregarded the effects of human digestion. In previous studies Meriç et al. (151) measured total phenolic acid content of methanolic extracts of *S. ebulus* fruits, Jimenez et al. (88) measured TPC of ripe fruits while Cvetanovic et al. (89) measured TPC of the supercritical water extract of fruits. In addition, Yaldiz et al. (152) reported the phenolic and total flavonoid contents of fruits and Mikulic-Petkovsek et al. (123) investigated the total hydroxycinnamic acid content of *S. ebulus* fruits. All of the results from previous studies that mentioned above were given in Table 65. In the present study, with a different manner from those previous reports, two extracts of *S. ebulus* fruits were investigated for their phenolic profiles after being subjected to human digestion simulation. Results demonstrated in Table 30 revealed that TPC was directly influenced by the simulated digestion process. This minor yet significant increase might be originated from the liberation of phenolic compounds from macromolecules such as fibers and peptides, after digestion procedure (153). This phenomenon was only observed in total phenolic content assay, while all other assays showed downtrend in the serum available phase. Both increment and decrement in phenolics were reported in various previous studies. Chen et al., (154) reported diverse alterations regarding the concentrations of phenolic compounds in 33 studied samples, while 25 of them exhibited degradation, on the contrary 8 of them showed ascent. Besides, our research group reported converse bioavailability results in different studies (155,156). Results from previous studies obviously indicated that variations arise from exclusive properties of different samples. A significant decrease in serum-available phase was determined in total phenolic acid content comparing to non-digested phase. Bioavailability of total flavonoids was found to be coherent with phenolic acids. Unlike other assays, this is the first time total proanthocyanidin assay was conducted in *S. ebulus* fruit extracts to our knowledge. Total content of proanthocyanidins exhibited reduction in every step of digestion. Total proanthocyanidins showed lowest bioavailability and stability among all phenolic assays. Since proanthocyanidins consist of oligomers, it is possible to conclude that new molecules might be formed after extracts confronted physical and chemical environment of GI tract. Also results from a previous study demonstrated that

Table 64: Results of Previous Studies on the Phenolic Profiles of the Aforementioned Fruit Samples

Sample	Assay	Results	Ref
S. nigra fruit (fresh)	Total phenolic content	371-472 mg GAE/100 g FW	146
S. nigra fruit (fresh)	Total phenolic content	354-582 mg GAE/100 g FW	147
S. nigra fruits (fresh)	Total phenolic content	513.6 mg GAE /100 g FW	148
S. nigra fruit (fresh)	Total phenolic content	19.5 mg GAE/g FW	149
S. nigra fruit MeOH ext.	Total flavonoid content	38.26 mg/100g FW	90
S. nigra fruit EOH ext.	Total flavonols	20.1836 g/100 g DE	71
S. nigra fruit (fresh)	Total phenolic acids	20 mg /100 g FW	148
S. nigra fruits (fresh)	Total proanthocyanidins	23.3 mg /100 g FW	149
S. ebulus fruit MeOH ext.	Total phenolic content	24.28 ± 1.39 mg GAE /g FW	151
S. ebulus fruit MeOH ext.	Total phenolic content	7.56 ± 0.08 mg GAE/g FW	88
S. ebulus fruit supercritical H_2O ext.	Total phenolic content	72.94 ± 0.91 mg CAE /g DE	89
S. ebulus fruit $CHCl_3$ ext.	Total phenolic content	10.128% of dry extract	152
S. ebulus fruit supercritical H_2O ext.	Total flavonoid content	27.40 ± 0.46 mg RE/g dry extract	89
S. ebulus fruit $CHCl_3$ ext.	Total flavonoid content	6.084% of dry extract	152
S. ebulus fruit MeOH ext.	Total hydroxycinnamic acid content	916.1 mg/kg FW	123
V. opulus fruit juice	Total phenolic content	5.47 ± 0.24 - 10.61 ± 0.42 mg GAE/g DE	104
V. opulus fruit MeOH ext.	Total phenolic content	67.73 mg GAE/g	106
V. opulus fruit EtOH/H_2O ext.	Total phenolic content	80 ± 0.15 - 8.31 ± 0.21 mg GAE/g	159
V. opulus fruit acetone ext.	Total phenolic content	621 ± 15 to 987 ± 32 mg GAE/100 g FW	160
V. opulus fruit acetone ext.	Total flavonoid content	202 ± 12 to 318 ± 16 mg rutin equivalent/ 100 g FW	160
V. opulus fruit EtOH/H_2O ext.	Total flavonoid content	3.14 ± 0.17 - 4.89 ± 0.17 mg RE/g	159

proanthocyanidins content of chokeberry decreased significantly after digested simulation and bioavailability of proanthocyanidins was expressively low (157). Methanolic extract of *S. ebulus* exhibited higher content of phenolics in each assay. Even though aqueous extract had relatively higher bioavailability, this was still insufficient for reaching the phenolic content levels of methanolic extract.

There are several studies in which chlorogenic acid was found to be the major phenolic component of *S. ebulus* extracts. In fact, it was reported that *S. ebulus* fruits were characterized with the highest level of chlorogenic acid among other elderberry species (123). Dulf et al. (158) measured the chlorogenic acid content of fresh fruits and in another study supercritical extract of *S. ebulus* fruits were investigated (89). Results were given in Table 66. For this reason, chlorogenic acid contents of all simulation phases obtained from both extracts were measured with HPTLC in order to support data for further assessment of bioavailability of bioactive compounds. Data in Table 41 demonstrated that chlorogenic acid content was significantly higher in non-digested SEM as well as other phenolic compounds especially total phenolic content. Reduction in chlorogenic acid content was continuous in all phases and total bioavailability was measured 45.77%. Chlorogenic acid content of non-digested SEA was expressively lower. Consistent with the TPAC assay, bioavailability was higher in SEA extract, however total amount of chlorogenic acid was greater in methanolic extract.

Phenolic profile of *V. opulus* fruits were investigated earlier in several studies. However, these studies overlooked the influence of gastrointestinal system on extracts and the bioavailability of biologically active major metabolite. According to a study conducted on six different genotypes of *V. opulus* fruit parts of Lithuania, total phenolic contents were calculated (105) (Table 65). Total phenolic content of methanolic extract of *V. opulus* fruits collected from Kayseri was calculated by Eken et al. (106) and also, Rop et al. (159) measured total phenolic content and total flavonoid content of different *V. opulus* cultivars from different years. Ersoy et al., (160) investigated both total phenolic and flavonoid content of ten different genotypes of *V. opulus* fruits collected from Sivas province, Turkey (Table 65). Total phenolic acid content assay has not been conducted on *V. opulus* fruits before, according to our knowledge. Although various researchers studied the phenolic profile of *V. opulus*, phenolic bioavailability had not

been taken into consideration in any of them. In this study, bioavailability of the phenolics and their effects on antioxidant capacity were revealed. Results demonstrated that all IN fractions possessed the lowest phenolic profile. Total phenolic and phenolic acid contents of both extracts decreased significantly at IN fraction, while the only exception is total flavonoid content, which showed no significant modification. Alterations in phenolic acid and total phenolic amount were more substantial than total flavonoid content; since *V. opulus* fruits are meager in flavonoids wherefore its biological activities might depend majorly on its total phenolics and phenolic acids. These results imply that phenolic structures in the *V. opulus* extracts have low stability in the GI tract. Reduction of the phenolic compounds seems to be reasoned by some physical properties of the GI tract like significant pH changes, body temperature. Especially, alkaline medium of the small intestines is a major parameter of descent of phenolic compounds (161). Another element that may cause reduction of the phenolics is excessive enzyme activity in the intestines. Enzymes may accelerate the hydrolization of the compounds after gastric digestion phase. Previous reports revealed opposite outcomes such as some studies showed escalation in the amount of phenolic contents in contrast other studies demonstrate descent (156,161-163). It might be postulated that these variations in the results of various studies originate from unique characteristic of the studied sample.

Chlorogenic acid is known as the major secondary metabolite of *V. opulus* fruits (103). Perova et al. (164) measured the amount of chlorogenic acid in eleven different samples collected from Russia meanwhile, Velioglu et al. (165) investigated phenolic composition of *V. opulus* fruits and showed that chlorogenic acid is far more dominant metabolite than other phenolic compounds (Table 66). However, the bioavailability of chlorogenic acid of *V. opulus* had not been investigated before. Therefore, in this study the amount of chlorogenic acid in both extracts and all phases of simulated human digestion for ascertainment was measured by HPTLC. Descending trend in chlorogenic acid amount was consistent with total phenolic acid and total phenolics assays. Decrease in chlorogenic acid level subsequent to digestion was reported previously by other studies (166,167). Besides, methanolic extract seemed to possess the highest phenolic profile in all assays (total phenolics, total phenolic acids, total flavonoids and HPTLC analysis). For all assays, bioavailability index rate was calculated higher in aqueous extract. After the digestion procedure, bioavailable amount of these compounds was

measured as the same. Therefore, it can be claimed that both extracts had a similar bioavailability trend. Simulated human digestion method used in this study excluded gastric absorption of polyphenolic compounds from extracts. A previous study reveals that chlorogenic acid is rapidly absorbed from stomach of rats in its intact form (168). Another report indicates that chlorogenic acid is highly bioavailable in humans (169). Based on these information, it can be speculated that post-gastric substantiality of chlorogenic acid enhances the biological activity of methanolic and water extracts of *V. opulus* since its biological activity is due to its major metabolite and chlorogenic acid, dominant metabolite major metabolite of *V. opulus,* measured higher amount in post-gastric phase than serum-available phase.

Total phenolic acid content assay was conducted for all extracts. Both extracts of the two *Sambucus* species showed significantly higher bioavailability when compared to *Viburnum opulus.* Before digestion procedure all of the extracts had highly similar total phenolic content except aqueous extract of *V. opulus.* However, after digestion simulation total phenolic content of both *Sambucus* extracts were escalated unlike *Viburnum* extracts, which were decreased significantly. In total phenolic acid content assay, methanolic extracts showed higher profile for all three species, while *Viburnum* species distinguished meaningfully before digestion. After simulation process all six extracts showed decline trend and low bioavailability. Aqueous extracts exhibited higher bioavailability thus; in IN fraction all extracts had equivalent phenolic acid profile except aqueous extract of *S. ebulus*, which had obviously lower than other extracts in serum available phase. Total flavonoid content assay revealed that methanolic extract of *Sambucus ebulus* fruits contained highest flavonoid content among all extracts. Even though methanolic extracts had higher bioavailability for all fruits, in serum available phase all six extracts had highly similar flavonoid concentrations, therefore from the viewpoint of flavonoids, all extracts may be used as counterparts. Total proanthocyanidin content assay was performed for all extracts, yet it had given negative results for *V. opulus.* These results indicate that total proanthocyanidin content of *V. opulus* extracts were lower than the limit of quantification (LOQ). On the other hand, *S. nigra* contained apparently higher proanthocyanidins than *S. ebulus* extracts. In addition, methanolic extracts had substantially higher proanthocyanidin content than aqueous extracts. Results also exhibited that, proanthocyanidins were affected the most from human digestion and

showed the lowest bioavailability among other phenolic groups which were studied. In all steps of human digestion, total amount of proanthocyanidins reduced in high quantity and resulted with extremely low bioavailability. This indicated that proanthocyanidins are instable in human GI tract. This result is similar with previous various studies. Numerous studies demonstrated that proanthocyanidins were instable in human GI system (170). As mentioned before, chlorogenic acid content of all extracts was measured by HPTLC and additionally, rutin content of *S. nigra* extracts were calculated. *V. opulus* fruits had overwhelmingly higher amounts of chlorogenic acid for both extracts when compared to other species. Furthermore, methanolic extracts had significantly higher amounts of chlorogenic acid for all species. This outcome is analogues with numerous previous studies that propounded higher solubility of chlorogenic acid in methanol (171). HPTLC analysis of chlorogenic acid content showed corresponding outcomes with total phenolic content assay as expected. Aqueous extract of *S. ebulus* had expressively lower amounts of chlorogenic acid, however methanolic extract of *S. ebulus* had higher amount than *S. nigra* methanolic extract. These results were also corresponding with total phenolic acid content assay results. All extracts were affected from human digestion in chlorogenic acid content, all extracts exhibited moderate chlorogenic acid bioavailability. These results were parallel with a previous study investigated phenolic bioavailability of *S. nigra* after human digestion simulation (69). Rutin was the only flavonoid derivate which was able to be measured in the current study. *S. nigra* extracts had considerable amounts of rutin. Other studied extracts may also contained rutin yet it is possible that rutin amounts of them were lower than LOD of HPTLC analysis. Mikulic-Petkovsek et al. (123) was previously showed that rutin amount of *S. ebulus* was significantly lower than *S. nigra* which is supporting the results of our study.

Antioxidant effect of the plant parts originates from diversified mechanisms. To achieve an improved perspective, couple of metal reducing, free radical scavenging activity assays and additionally a total antioxidant capacity assay were performed for all extracts, in this study. Several *in vitro* activity assays were conducted on *S. nigra* fruits earlier. A couple of free radical scavenging assays were practiced in present study. DPPH radical scavenging activities of elderberries were investigated previously. In previous reports, various preparations from *S. nigra* fruits were investigated (172) and *S. nigra* determined as the second best activity among all studied fruit juices after

chokeberry fruit juice (173). In another study wines prepared from different resources were investigated and Elderberry wine was the highest among all wines (174). Results of DPPH assays were given in (Table 67). DMPD activity of Elderberry fruits were investigated for the first time in our study to our knowledge. Even though radical scavenging activities were investigated before, these studies neglected the effects of GI tract and bioavailability of phenolic compounds. Our results exhibited that methanolic extracts had higher bioactivity for both assays. After steps of digestion, bioactivities of the studied samples were significantly lowered corresponding to phenolic profile assays (Table 52).

Table 65: Results of the Previous Studies on the Chlorogenic acid and Rutin Contents of the said fruit Samples

Sample	Assay	Results	Ref
Rutin contents			
S. nigra fruit ACN ext.	Rutin	1.94-6.31 mg /g DW	150
S. nigra fruit MeOH ext.	Rutin	42.6-95.6 mg /100g FW	147
S. nigra fruit MeOH ext.	Rutin	21.95 mg/100 g FW	151
Chlorogenic acid contents			
S. nigra fruit ACN ext.	Chlorogenic acid	0.53-1.22 mg/g DW	150
S. nigra fruit MeOH ext.	Chlorogenic acid	26.4-35.9 mg /100g FW	147
S. nigra fruit MeOH ext.	Chlorogenic acid	14.69 mg/100 g FW	151
S. ebulus fruit MeOH ext.	Chlorogenic acid	46.8 mg/100 g FW	123
S. ebulus fruit (fresh)	Chlorogenic acid	24.32 ± 1.20 mg/100 g DW	158
S. ebulus fruit supercritical H_2O ext.	Chlorogenic acid	36.83 mg/L	89
V. opulus fruit H_2O ext.	Chlorogenic acid	250-580 mg / 100 mg FW	164
V. opulus fruit juice	Chlorogenic acid	54% of all phenolics	165

Free radical scavenging activity was performed with two different assays for *S. ebulus* fruits as well. DPPH radical scavenging activity assay was previously conducted several times on *S. ebulus* fruits. Jimenez et al. (88) and Cvetanovic et al. (89) measured the DPPH scavenging activity of *S. ebulus* fruits (Table 67). These studies did not

reveal any data about potential bioavailability and its impact on antioxidant activity. In the present study, no significant bioactivity changes were observed between non-digested and serum-available fractions of SEM by DPPH radical scavenging assay, while minor yet significant decrease in serum-available phase was determined for SEA. In contrast, SEA displayed no significant change in IN phase but had slightly lower DMPD scavenging activity. The statistically significant changes observed in activity were considerably minor. These results were more correlated with total phenolic content assay than others. It might be hypothesized that phenolic compounds sustain their average total concentrations after simulated human digestion and this condition leads analogue free radical scavenging activity after digestion.

As mentioned earlier free radical scavenging activity assays are prevalently used for the definition of antioxidant properties of plant parts (128). In this study, DPPH and DMPD radical scavenging activity assays were also conducted for *V. opulus* fruits. DPPH radical scavenging activities of different cultivars of *V. opulus* fruits were previously reported by Rop et al. (159) (Table 67). DMPD scavenging activity of *V. opulus* fruits was not studied before, according to our knowledge. In DPPH assay, both extracts showed slight but significant changes when compared to non-digested and serum available fractions. As observed in the metal reducing and total antioxidant assays, methanolic extract showed higher activity in DPPH assay, and both extracts had similar activity at IN fraction. In DMPD assay, contrary to other assays both extracts had similar scavenging activity in all phases of digestion. When compared to metal reducing, total antioxidant and total phenolic content results, free radical scavenging activity either remained the same or decreasing slightly after the digestion simulation (Table 51, 52).

In DPPH radical scavenging activity methanolic extract of *S. nigra* exhibited highest bioactivity before digestion. Other five extracts showed analogous bioactivities. All extracts were affected by human digestion simulation low or mildly. After the simulation procedure all extracts showed similar trends and results for DPPH radical scavenging bioactivity and there are no significant differences between aqueous and methanolic extracts. In DMPD radical scavenging activity, unlike DPPH radical scavenging activity, *S. nigra* extracts exhibited overwhelmingly higher bioactivity in comprasion with other fruits. *S. ebulus* and *V. opulus* extracts had comparable

bioactivities both not-digested and digested phases. They showed similar trends and digestion procedure affected them mildly. It is evident that *S nigra* fruit extracts were found to be superior DMPD radical scavenger than other species. For both radical scavenging assays, effect on human digestion procedure is mild or low, on the contrary to metal reducing assays. This trend is parallel to total flavonoid content assay. This study takes account of exclusively phenolic compounds, for that reason possible effects of non-phenolic compounds are incomputable. In a similar manner, Pavan et al. (174) reported similarity in antioxidant activity of digested papaya against undigested phase. That study hypothesized that non-phenolic compounds possibly affect antioxidant activity of plant sample, thus decline in phenolic amount is compensated on *in vitro* bioactivity. Variance in the free radical scavenging activity was also similar to total flavonoid content. It is also possible that flavonoids are the major phenolic compounds of the extracts which are responsible for the free radical scavenging activity rather than other phenolics. These results may indicate that flavonoids have higher priority in radical scavenging activity for extracts of Adoxaceae fruits growing in Turkey.

A couple of metal reducing activity assays were conducted on both extracts of *S. nigra* fruits for this study. In previous studies, FRAP assay was conducted on elderberries several times. Nonetheless this is the first time CUPRAC assay was operated on elderberries to our knowledge. FRAP activities of wild genotypes collected from Turkey was investigated in an earlier studies (146, 175) (Table 67). All the previous studies still neglected the effects of GI digestion. Likewise, phenolic profile assays and free radical scavenging bioactivities, metal reducing activities of methanolic extracts were significantly higher in methanolic extracts. In serum-available phase, all extracts had lost their bioactivity significantly. CUPRAC of SNA was affected mostly among all other extracts in metal reducing assays. In this study, TOAC assay was conducted on elderberry fruits for the first time to our knowledge. Similar to all phenolic and bioactivity assays, TOAC activity was highly affected from GI digestion simulation. Before and after digestion, methanolic extracts had significantly superior total antioxidant capacity (Table 55)

In this study, both metal reducing assay and total antioxidant capacity assay exhibited similarities for *S. ebulus* extracts, *inter se.* Unlike free radical scavenging activity assays, metal reducing and total antioxidant activity assays demonstrated

decline in all phases. In CUPRAC assay, SEM and SEA extracts showed notable decline. Correspondingly, in FRAP assay extracts showed major decline in ferric reducing activity, as well. Likewise, TOAC assay had similar variance in bioactivity with metal reducing activity assays. Gradient in results of metal reducing activity assays and TOAC were consistent with amount of phenolic acid, proanthocyanidin and flavonoids. Also chlorogenic acid content demonstrated similar alteration. Results indicated that possibly these classes of phenolic compounds in extracts had a more major role in metal reducing and total antioxidant capacity contrasting to free radical scavenging activity.

Table 66: Results of the Previous Antioxidant Tests on the said Fruit Samples

Sample	Assay	Results	Ref
S. nigra fruit juice	DPPH radical scavenging activity	62.14 µmol TE/ml	172
S. nigra fruit wine	DPPH radical scavenging activity	9.95 mM TE/L	173
S. ebulus fruit MeOH ext.	DPPH radical scavenging activity	07 ± 0.08 mg TE/g FW	88
S. ebulus fruit supercritical H_2O extract	DPPH radical scavenging activity	0.069 ± 0.004 mg/ml IC_{50}	89
V. opulus fruit EtOH/ H_2O ext.	DPPH radical scavenging activity	9.79 ± 0.14 g AAE/kg fresh mass	159
S. nigra fruit (fresh)	FRAP	5.04 - 6.37 mmol TE/100g FW	146
S. nigra fruit wine	FRAP	135.83 mmol TE/kg	175
V. opulus fruit juice	FRAP	55.77 ± 1.77 - 109.76 ± 1.37 Fe^{2+} µmol/g	104
V. opulus fruit MeOH ext.	TOAC	315.50 ± 8.2 mg/g AAE	108

Metal reducing activity was also studied for *V. opulus* extracts with two different assays. CUPRAC assay was studied for the first time on *V. opulus* fruits to our knowledge. In a previous report about ferric reducing antioxidant power of *V. opulus*, results were given (104) (Table 67). However, that study ignored the effect of gastric tract on the bioactivity. Results of CUPRAC and FRAP assays were demonstrated in (Table 45, 48). Both extracts showed coherent gradient with their phenolic profile and major metabolite in metal reducing activity. Sagdic et al. (108) measured total antioxidant capacity of methanolic extracts of some parts of the *V. opulus* fruit via phosphomolybdenum complex method before, still disregarding the effects of digestion (Table 67). Likewise, total antioxidant activity was reduced at IN fraction just as total

phenolics and metal reducing capacity. Previous reports affirmed similar conclusions on fruit samples. Bouayed et al. (176) reported reduction of total phenolics at IN fraction conduced to decline in ferric reducing activity. Our research group reported reduction in cupric ion reducing capacity and total antioxidant capacity corresponds with alleviation on phenolic content in fruit wines (164).

Metal reducing potentials of all extracts were investigated via two different methods; Cupric reducing antioxidant capacity (CUPRAC) and Ferric reducing antioxidant power (FRAP). In CUPRAC assay, methanolic extract of *V. opulus* showed higher copper reducing capacity. Likewise, in phenolic assays, methanolic extracts exhibited higher activity before digestion for all three species. After digestion procedure, bioactivity recessed for all extracts significantly. In serum available phase, methanolic extract of *S. nigra* had higher bioactivity by a narrow margin. On the contrary, aqueous extract of *S. nigra* had lowest activity, of all extracts. Similarly in FRAP assay methanolic extract of *S. nigra* had highest ferric reducing power among all extracts. *V. opulus* extracts had lowest activity, nonetheless, they were affected fewest from digestion simulation among other extracts. After digestion simulation, *S. nigra* extracts had still highest bioactivity in serum available phase. Before digestion *S. ebulus* extracts had moderate bioactivity however aqueous extract of *S. ebulus* highly affected from gastrointestinal conditions subsequently had lower bioactivity in IN phase. It is apparent that human digestion affected metal reducing bioactivities of all extracts for both assays. There was an observable correlation between phenolic contents and metal reducing bioactivities. It may be hypothesized that metal reducing activities are due to their total phenolic contents. In a previous review, it is evidently presented that amounts of phenolic contents are decidedly correlated with metal reducing bioactivities (177). Our results homologates with numerous previous findings. In total antioxidant capacity assay methanolic extract of *S. ebulus* showed significantly higher bioactivity in none-digested phase. However, after digestion procedure methanolic extracts of *S. ebulus* fruits affected farther than other extracts and in IN fraction all extracts, including aqueous extracts, exhibited more or less similar bioactivities therefor it may be stated that extracts may be used as counterparts.

6. CONCLUSION

In this study, methanolic and aqueous extracts of three Adoxaceae fruits growing in Turkey were investigated for their phenolic profile and *in vitro* antioxidant activity. *Sambucus nigra*, *Sambucus ebulus* and *Viburnum opulus* fruits were selected for their economic importance and broad utilization in Turkish folk medicine. A number of previous studies have investigated and reported their phenolic profiles and antioxidant capacities via several methods. However, none of them has taken into consideration the influence of human gastrointestinal system on these extracts. This is the first comparative study which investigated the effects of in *vitro* human gastric simulation on some Adoxaceae fruits growing in Turkey. Every extract divided into four fractions after gastric simulation; extract before digestion (BFR), after gastric phase (PG), colon-available phase (OUT) and serum-available phase (IN) were prepared for all extracts. Total phenolic, total phenolic acid, total flavonoid and total proanthocyanidin contents were assayed of twenty four prepared fractions for determining the precise variations on phenolic profile of extracts. Moreover, DPPH and DMPD radical scavenging activities, FRAP, CUPRAC and TOAC assays were conducted on all fractions for determination of the incidence of phenolic alteration before and after the gastrointestinal simulation on antioxidant capacity. Further advanced survey of *in vitro* pharmacokinetic properties of extracts, HPTLC analysis was applied for known active phenolic metabolites in the extracts; chlorogenic acid and rutin. Nonetheless, present study was the first to reveal the influence of human digestion simulation on some Adoxaceae fruits growing in Turkey.

Results indicate that, simulated GI digestion had significant effect on both phenolic profile and antioxidant capacity of extracts. In general, methanolic extracts have superior properties as antioxidants. All extracts affected from GI digestion simulation more or less which is discussed comprehensively in discussion section. In conclusion, even though GI digestion had negative effect on antioxidant capacity of extracts, they still had significant bioactivity. Fruits from Adoxaceae species growing in Turkey are valuable sources for antioxidants and implementing diet with them as food additives or nutraceuticals may promote long term health.

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8. CURRICULUM VITAE

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Barak, T. H., Celep, E., İnan, Y., & Yesilada, E. (2019). Influence of in vitro human digestion on the bioavailability of phenolic content and antioxidant activity of Viburnum opulus L.(European cranberry) fruit extracts. *Industrial Crops and Products*, *131*, 62-69.

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Certificates

Certificate of Animal Use in Experimental Research