

Short Communication

Quantitative phytochemical analysis and their antioxidant activity of *Cocculus hirsutus* (L.) Diels fruit

Rakkimuthu R¹, Kavithakrishna R¹, Suganyadevi P¹, Aravinthan K M^{1*}

*Corresponding author:

Aravinthan K M

¹Dr. Mahalingam Centre for Research and development, N.G.M College, Pollachi, India

Abstract

Anthocyanins, a large group of red-blue plant pigments, occur in flowers and fruits of higher plants. The fruits of *Cocculus hirsutus* (L.) Diels was extracted with acidified methanol and used for phytochemicals and antioxidant activity analysis. The total flavonoid, anthocyanin and phenol content were found to be 260±20 mg/g, 0.788±0.236 mg/g and 326.66±3.05 mg/g respectively. DPPH, ABTS and Nitric oxidescavenging activity exhibited an IC₅₀ value of 111.35±1.12, 80.90±0.39 and 79.84±1.48 respectively. The IC₅₀ value of reducing power assay, inhibition of lipid peroxidase in egg yolk and Metal chelating and was identified to be 97.03 ±0.88, 107.6±0.48 and 200.27±1.85µg/ml respectively. The positive control showed an IC₅₀ value of 39.78 ±0.07, 23.68±0.06, 63.62±1.22, 53.74±1.34, and 70.59±2.8 and 51.26±0.39 µg/ml respectively. The total antioxidant activity of the fruit anthocyanin exhibited highest absorbance of 0.382±0.005 for 100µg/ml concentration.

Keywords: *Cocculus hirsutus*.L. fruit, Flavonoid, Anthocyanin, Antioxidant assays.

Introduction

The *Cocculus hirsutus* L. (Menispermaceae) is a threatened climber with green flowers which bloom during February –March and fruits can be obtained during May- June. It is widely distributed in tropical and subtropical climates [1]. Tribals of Jhabua, Khargone and Dhar use the fruit of *C. hirsutus* to cure Jaundice. The roots and leaves of *C. hirsutus* have great medicinal value and are used both internally, as well as externally [2].

Anthocyanins are water soluble pigments belonging to the family of flavonoids. They are responsible for the purple, blue, red and orange colours of several fruits and flowers. Mostly six anthocyanidins are present in fruits and vegetables namely cyanidin, pelargonidin, malvidin, delphinidin, petunidin and peonidin. The anthocyanin content and compositions are different in the pigmented fruits depending on the varieties and origin [3]. These compounds possess beneficial health properties like antioxidant activity, free-radicals scavengers [4,5], protective effect against cardiovascular disease [6,7], anti-inflammatory and anticarcinogenesis properties [8,9].

Some fruits are good source of natural antioxidants including carotenoids, vitamins, phenols, flavonoids, dietary glutathione and endogenous metabolites and high level of antioxidant capacity against free radicals species, superoxide radicals, hydrogen peroxide, nitric oxide and hydroxyl radicals [10]. The benefits of these natural antioxidant activity of fruits induce reduction of diseases in humans [11], protection against tumor development [12]. There are no reports in literature on antioxidant activities of *C.*

hirsutus L. fruit. Thus the aim of the study was investigate the antioxidant potentials of the acidified methanol extracts obtained from *C. hirsutus* L. fruit and the results were compared with standard antioxidant compounds.

Materials and Methods

Sample collection

C. hirsutus L. fruits (Figure 1 a and b) were collected from in and around Coimbatore, Tamilnadu, India during the month of May 2011. After sampling, the fruits were immediately transported to laboratory, put in plastic bags, and stored at -20°C in a plastic container. The plant was identified and authenticated (1523) by Botanical Survey of India, TNAU campus, Coimbatore, Tamilnadu, India, as *Cocculus hirsutus* (L.) Diels.

Pigment Extraction

C. hirsutus (L.) Diels fruits were washed and dried. Then, 10gm fruits were extracted by crushing with 1% HCL in methanol (v/v). The extract was stirred with a glass rod for every 5 minutes to ensure a complete extraction. The extract was incubated in dark at room temperature for 4 h. The extract was centrifuged (5000 rpm/min) at 4°C for 20 minutes. The supernatant (Figure 1c) was separated and was evaporated under reduced pressure and the extract was used for further analysis.

Qualitative Test for Flavonoid [13]



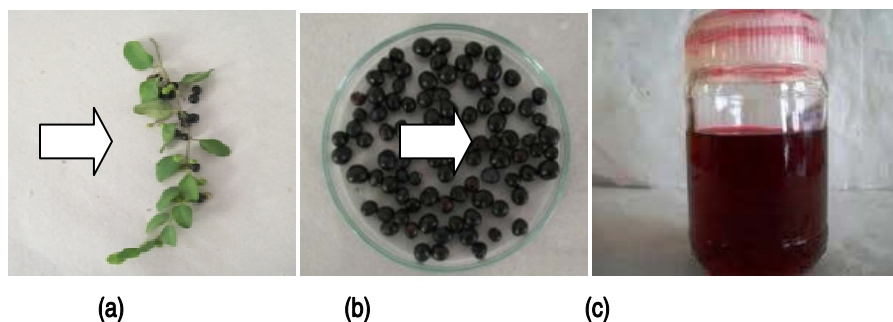


Figure 1. Images of *Cocculus hirsutus* fruits: (a) The fruits of *Cocculus hirsutus* L. (Menispermaceae) with leaves; (b) Collected fruits of *Cocculus hirsutus* L., (c) The pigment extracted from fruits of *Cocculus hirsutus* L. (Menispermaceae).

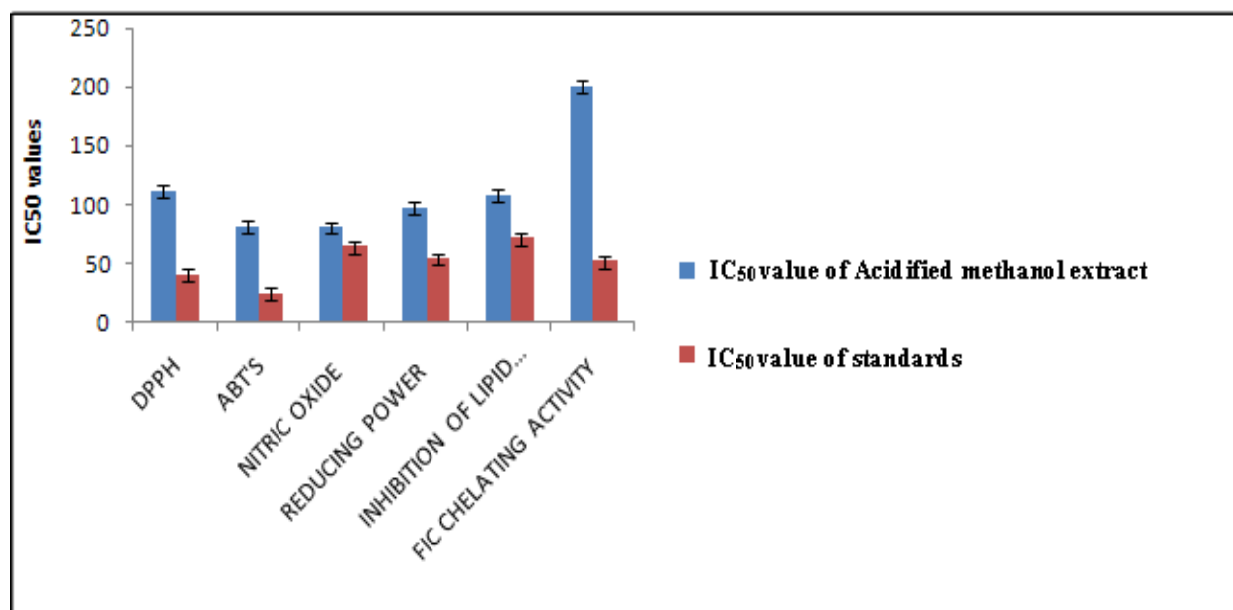


Figure 2. IC₅₀ value of radical scavenging assays of *Cocculus hirsutus* L. fruit (Menispermaceae).

Ferric Chloride Test

1 ml of pigment solution was added with a small amount of FeCl₃ solution, and the result was observed.

Alkalic Aluminum Chloride Test

1 ml of pigment solution was added with 5% alkalic aluminum chloride solution.

Qualitative Test for Anthocyanin

To the extract added 2M HCL and heated for 5 min at 100 C and the results were observed.

To the extract, 2M NaOH was added drop wise and the results were observed.

The anthocyanin stability was tested by treating 1mL of extract with 1mL each of varying pH solution in the range of 1.0 and 4.5 and the color change was noted.

Quantitative tests

Determination of Total flavonoid

Aluminum chloride colorimetric method was used for flavonoid determination [14]. The extract of the *C. hirsutus* L. fruit (0.5mL of 10g/100ml) in was mixed with 0.1ml of 10% aluminum chloride, 0.1ml of 1M potassium acetate and 2.8mL of distilled water, remained at room temperature for 30 minutes. Then the absorbance of the reaction mixture was measured at 415nm with double beam Genesis-5 UV/Visible spectrophotometer (India). The calibration curve was prepared by prepared by using quercetin as standard.

Determination of Total Anthocyanin

The total amount of anthocyanin content was determined by using pH differential method. A spectrophotometer was used for the spectral measurements at 210nm and 750nm [15]. The absorbance of the samples (A) was calculated as follows:

$$\text{Anthocyanin pigment content (mg/liter)} = (A \times MW \times DF \times 1000) / (X \times 1)$$

Where, A=[(Absorbance λ vis max 210 - Absorbance λ vis max-A750) pH1.0] / [(Absorbance λ vis max 210 -Absorbance λ vis-max-A750) pH 4.5], Molecular weight of anthocyanin (cyd-3-glu) =449, extraction coefficient () =29,600, DF=Diluted factor.

Determination of Total phenols

Total phenolic contents of The extract was determined by the Folin-Ciocalteu's method [16]. Briefly, aliquots of 0.1 g *C. hirsutus* L. fruits extract was dissolved in 1ml of deionised water. This solution (0.5 ml) was mixed with 2.8ml of deionized water, 2ml of 2% sodium carbonate (Na₂CO₃), and 0.1ml of 50% Folin-Ciocalteu's reagent. After incubation at room temperature for 30min, the absorbance of the reaction mixture was measured at 750nm against a deionized water blank using a spectrophotometer (GENESIS-5). Gallic acid was used as a standard to obtain a standard curve. The levels of total phenolic contents in *C. hirsutus* L. fruits were determined using the standards curve.

Antioxidant Assays

Sample preparation

C. hirsutus L. fruit extract 1.0mg was dissolved in 10 ml of methanol. From the above 20,40,60,80 and 100µg/ml concentration of sample was prepared and used for different assays. Ascorbic acid (or) BHT (or) EDTA was used as standard for comparing the activities.

DPPH radical scavenging activity

The free radical scavenging capacity of the evaporated fruit sample of *C. hirsutus* L. methanolic extract was determined using DPPH by Blois (1958)[17]. DPPH solution was prepared in 95% methanol. Different concentrations of *Cocculus hirsutus* L. fruit extracts was reacted with freshly prepared 3.0ml DPPH solution (0.1mM) and incubate room temperature. After 30 minutes, the absorbance was taken at 517nm using a spectrophotometer (Genesis-5 UV-Visible spectrophotometer). Ascorbic acid was used as a reference standard. The Control was devoid of the sample. Standard ascorbic acid was treated similar to the extracts. The percentage of inhibition was calculated by following formula.

$$\% \text{ of DPPH radical scavenging activity} = \text{Control} - \text{Absorbance of sample} / \text{control} \times 100$$

ABTS radical scavenging assay

ABTS (2, 2'-azinobis-3-ethylbenzothiazoline- 6-sulphonic acid) assay is based on the scavenging of light by ABTS radicals. An antioxidant with an ability to donate a hydrogen atom will quench the stable free radical, a process that is associated with a change in absorption. The relatively stable ABTS radical was green and it was quantified spectrophotometrically at 734nm. Fresh ABTS solution was prepared for each assay. Different concentration of *C. hirsutus* L. fruit extracts (20-100 µg/ml) were allowed to react with 1mL of the ABTS solution and the absorbance was taken at 734nm after 7minutes using a spectrophotometer [18]. The percentage of inhibition was calculated by following formula.

$$\% \text{ of ABTS radical scavenging activity} = \text{Control} - \text{Absorbance of sample} / \text{control} \times 100$$

Nitric oxide assay

The Nitric oxide scavenging capacity of the extract of *Cocculus hirsutus* L. was determined using nitric oxide generated by oxidation of sodium nitroprusside [19]. Sodium nitroprusside (10mM, 2ml) in phosphate buffer saline was incubated with the *C. hirsutus* L. fruit extract in different concentration (20– 100 µg/ml) at room temperature for 30 minutes. After 30 minutes, 0.5 ml of the solution was mixed with 1ml of Griess reagent. The absorbance was measured at 546nm. Ascorbic acid was used as a reference standard. The percentage of inhibition was calculated by following formula.

$$\% \text{ of inhibition} = (\text{Absorbance of sample} - \text{control} / \text{control}) - 1 \times 100$$

Reducing power assay

The reducing power of *C. hirsutus* L. fruit extract was determined according to the method previously described by Oyaizu (1986)[20]. Different concentrations of *Cocculus hirsutus* L. fruit extracts was mixed with phosphate buffer (2.5ml, 0.2M, pH 6.6) and potassium ferricyanide [K₃Fe(CN)₆] (2.5 ml, 1%). The mixture was incubated at 50 C for 20 min. Then, 2.5 ml of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5ml) was mixed with distilled water (2.5ml) and FeCl₃ (0.5 ml, 0.1%) and the absorbance was measured at 700 nm. Ascorbic acid was used as the standard. Phosphate buffer (pH 6.6) was used as blank. The percentage of increase in reducing Power is calculated by following formula.

$$\% \text{ increase in Reducing Power} = (\text{Absorbance of sample} - \text{control} / \text{control}) - 1 \times 100$$

Inhibition of lipid peroxidation

A modified thiobarbituric acid reactive species (TBRAS) assay [21] was used to measure the lipid peroxide formed, using egg yolk homogenate as lipid rich media [22]. Egg homogenate (0.5 ml, 10% in distilled water v/v) and different concentration of *C. hirsutus*

.L. fruit extracts (20-100 µg/ml) were allowed to react with 0.05 ml FeSo₄ (0.07M) and then incubated for 30 minutes to induce lipid peroxidation. Thereafter 1.5ml of 20% acetic acid (pH adjusted to 3.5 with NaOH) and 1.5ml of 0.8% TBA (w/v) was (prepared in 1.1% sodium dodecyl sulfate and 0.5ml of 20% TCA) added, vortexed and then heated in a boiling water bath for 60 minutes. After cooling 5.0 ml of 1-butanol was added to each tube and centrifuged at 3000rpm for 10 minutes. The absorbance of the organic upper layer was measured at 532 nm. BHT was used as a reference standard. The percentage of inhibition was calculated by following formula.

$$\% \text{ of inhibition} = (\text{Absorbance of sample} - \text{control} / \text{control}) \times 100$$

Metal chelating activity assay

The chelating activity of the extracts for ferrous ions Fe²⁺ was measured according to the method of Dinis *et al.*, (1994)[23]. Different concentration of *C. hirsutus* L. fruit extracts (20-100 µg/ml) were combined with 1.6 mL of deionized water and 0.05 mL of FeCl₂ (2 mM). After 30 s, 0.1 mL ferrozine (5 mM) was added. Ferrozine reacted with the divalent iron to form stable magenta complex species that was soluble in water. After 10 min at room temperature, the absorbance of the Fe²⁺- Ferrozine complex was measured at 562 nm. The chelating activity of the extract for Fe²⁺ was calculated as Chelating rate (%) = $(A_0 - A_1) / A_0 \times 100$, Where A₀ was the absorbance of the control (blank, without extract) and A₁ was the absorbance in the presence of the extract. EDTA was used for standard.

Total antioxidant capacity by phosphomolybdenum assay

The spectrophotometric measurement of Total antioxidant capacity (TAC) is based on the reduction of Mo (VI) to Mo (V) by antioxidant compound and the formation of green phosphate / Mo (v) complex at acidic pH [24]. Different concentration of *C. hirsutus* L. fruit extracts (20-100 µg/ml) were combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 90 min. Then the absorbance of the solution was measured at 695 nm. Methanol (0.3 ml) in the place of extract is used as the blank. Ascorbic acid was used as a reference standard.

Statistical Analysis

In all cases analyses were performed in triplicate and data were averaged over the three measurements. The standard deviation (SD) was also calculated. The IC₅₀ value is calculated by using MS-EXCEL software.

Results and Discussion

Qualitative Test for Flavonoid

In the presence of FeCl₃, the extract showed brown color which confirmed the presence of flavonoid [25]. In the presence of AlCl₃ dark color was observed in the extract (reference).

Qualitative Test for Anthocyanin

Table 1 shows the confirmatory test from *C. hirsutus* .L. fruit extract. Flowers, fruits and vegetables have health benefits and are good sources of phenolics, flavonoids, anthocyanins and carotenoids [26]. Giusti and Wrolstad (2003)[27] reported that the anthocyanins are stable at low pH.

Quantitative Analysis

Determination of total flavonoid

The *C. hirsutus* .L. fruit extract showed more significant total flavonoid content of 260±20 mg/ g of fresh weight (Table-2).The flavonoid content in sweet cherries of different genotype cultivars samples contains 0.42 ± 0.13 to 5.63 ± 0.05 mg/g [28].

Table-1 Confirmation test for Anthocyanin

S.No	Confirmation Test	Anthocyanins
1.	Extract+Heat +2M HCL for 5min at 100°C	Colour stable
2.	Extract+2M NaoH drop wise	Changes blue to green and slowly fades
3.	Stability at Variable pH. Extract+ pH 1 Extract+ pH 4.5	Appears red colour Colour disappeared

Determination of total anthocyanin

The *C. hirsutus* .L. fruit extract showed more significant total anthocyanin content of 0.788±0.236 of fresh weight (Table-2).Total anthocyanin content in sweet cherries of different cultivars samples contains 29 to 62 mg/100 g of fresh weight [29]. These values of total anthocyanin content are in general lower, compared to some earlier reports in the literature, but show good agreement with some recent investigations relying on acidic extraction [30].

Table 2: Quantitative determination of important phytoconstituents of *Cocculus hirsutus* fruit

S.NO	Phytoconstituents	Concentration in mg/g Acidified Methanol extract
1	Flavonoids	260±20
2	Anthocyanin	0.788±0.236
3	Phenolic compounds	326.66±3.05

Determination of total phenols

In the present study the *C. hirsutus* .L. fruit extract contains 326.66±3.05 mg / g of fresh weight (Table-2). Total phenolic content in sweet cherries of different cultivars samples contains 97 to 197 mg / 100 g of fresh weight [29]. The total phenolic content in strawberry, raspberry and red plum was containing 78.3±12, 228 ±6 and 133±12 respectively [31].

Antioxidant analysis

DPPH radical scavenging activity (Spectrophotometer)

DPPH is a stable free radical, The antioxidant activity of plant extract was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH [32]. The absorbance decreases as a result of a colour change from purple to yellow due to the power of hydrogen donating ability [17]. The *C. hirsutus* .L. fruit extract scavenged DPPH radical at 28.63±0.44 % and the corresponding concentration was 100 µg/ml. The IC₅₀ value was 144.51±1.11 µg/ml and was not significant compare to standard ascorbic acid with IC₅₀ value of 39.78±0.07µg/ml. (Figure-2). *Chrysophyllum cainito*, *Gaultheria shallon*, *Malpighia glabra*, *Myrciaria cauliflora* and *Sambucus caerulea* fruits were highly red-purple in colour and contain anthocyanin compounds. The IC₅₀ values of the H₂O: MeOH fraction of these fruits extracts

were as follows 7.9±0.3 µg/ml, 5.9±0.3µg/ml, 13.9±1.3µg/ml, 6.2 ±0.7 µg/ml and 16.9±0.6 µg/ml respectively [33]. *Syzygium cumini* fruit skin contains anthocyanin compounds and the IC₅₀ values of the water extract was 168 µg/ml [34]. So *C. hirsutus* .L. fruit extract has more significant antioxidant activity than *Syzygium cumini* fruit skin extract and less antioxidant activity than *Chrysophyllum cainito*, *Gaultheria shallon*, *Malpighia glabra*, *Myrciaria cauliflora* *Sambucus caerulea* and 70% less activity than standard ascorbic acid.

ABTS radical scavenging activity

Effective ABTS radical scavenging process was exhibited by the fruit extracts of *C. hirsutus* .L. Acidified Methanol extract showed 49.1±0.35% inhibition at 100µg/ml concentration (Table-4) with IC₅₀ value of 80.90±0.39 µg/ml. The ascorbic acid had an IC₅₀ value of 23.68±0.06 µg/ml. (Figure-2). The fruit of the two jabuticaba varieties (Paulista and sabara) presented significant antioxidant activities of 1.20±0.11mmol L⁻¹ g⁻¹, 1.21±0.06 mmol L⁻¹ g⁻¹ respectively [35]. The *C. hirsutus* L. fruit extract was 57% less antioxidant activity than synthetic antioxidant of ascorbic acid.

Nitric oxide radical scavenging activity

The available nitric oxide radical is linked with various carcinomas and inflammatory conditions [36]. The nitric oxide generated from sodium nitroprusside reacts with oxygen to form nitrite. The extract directly competes with oxygen to react with nitric oxide and thereby inhibits nitrite formation. The *C. hirsutus* .L. fruit extract showed scavenging action against nitric oxide (NO) induced release of free radicals and the value was 69.79±1.8% at 100µg/ml concentration (Table-5) with 79.84±1.48µg/ml IC₅₀ value. The IC₅₀ value for Ascorbic acid 63.62±1.22 µg/ml (Figure-2). The scavenging of nitric oxide by Berry's anthocyanin extract increased in a dose-dependent manner 10-80µg/ml of extract contain 20-60% of inhibition [37]. The fruit extract was 16% less antioxidant activity than synthetic antioxidant of ascorbic acid.

Table-3 *In vitro* free radical scavenging effect of *Cocculus hirsutus* fruit on DPPH

Percentage Scavenging (mean±SD) of Triplicates						
concentration	20µg/ml	40µg/ml	60µg/ml	80µg/ml	100µg/ml	IC ₅₀ µg/ml
Acid-Methanol	15.01±0.14	21.78±0.14	28.51±0.29	33.01±0.24	43.63 ±0.44	111.35 ±1.12
concentration	10µg/ml	20µg/ml	30µg/ml	40µg/ml	50µg/ml	
Ascorbic acid	16.18±0.31	27.68±0.20	36.99±0.29	49.31±0.26	62.32 ±0.41	39.78 ±0.07

Table-4 *In vitro* free radical scavenging effect of *Cocculus hirsutus* fruit on ABTs

Percentage Scavenging (mean±SD) of Triplicates						
concentration	20µg/ml	40µg/ml	60µg/ml	80µg/ml	100µg/ml	IC ₅₀ µg/ml
Acid-Methanol	34.74±0.08	37.54±0.14	45.41±0.21	47.16±0.28	49.10±0.35	80.90±0.39
concentration	10µg/ml	20µg/ml	30µg/ml	40µg/ml	50µg/ml	
Ascorbic acid	65.67±0.57	74.68±0.64	76.48±0.28	78.60±0.37	80.54±0.43	23.68±0.06

Table-5 *In vitro* free radical scavenging effect of *Cocculus hirsutus* fruit on nitric oxide assay

Percentage Scavenging (mean±SD) of Triplicates						
concentration	20µg/ml	40µg/ml	60µg/ml	80µg/ml	100µg/ml	IC ₅₀ µg/ml
Acid-Methanol	11.46±1.81	22.91±1.8	29.16±1.81	48.95±3.61	69.79±1.8	79.84±1.48
concentration	10µg/ml	20µg/ml	30µg/ml	40µg/ml	50µg/ml	
Ascorbic acid	44.95±0.62	38.72±0.97	31.09±0.65	26.83±0.98	21.91±0.98	63.62±1.22

Reducing power assay

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [38]. The reduction power of acidified methanol extract was summarized in table-6. The *C. hirsutus* .L fruit extract showed potent ferric reducing power of 52.17±0.88 % at 100µg/ml concentration. The data showed that reducing power of the extracts increased with increased concentration of extracts. The reducing power of red sorghum bran anthocyanins was 0.984(absorbance) at 100 µg/mL concentration [40]. However, the activity was found to be less when compared to the standard. IC₅₀ value of *C. hirsutus* fruit extract was 97.03±0.88 µg/ml and 53.74 ± 1.34µg/ml for standard ascorbic acid (Figure-2). The fruit extract was 44% less antioxidant activity than synthetic antioxidant of ascorbic acid.

Inhibition of lipid peroxidation using egg yalok

A modified thiobarbituric acid reactive species was used to measure the lipid peroxide formed, using egg-yolk homogenates as lipid rich media. Malondialdehyde (MDA), a secondary end product of the oxidation of polyunsaturated fatty acids, reacts with two molecules of TBA yielding a pinkish red chromogen with an absorbance maximum at 532 nm. The *C. hirsutus* .L fruit showed a maximum of 37.72 ±0.36% at 100mg concentration where as the standard had 34.05±1.41% at an similar concentration (Table-7). The IC₅₀ value for fruit extract was 200.27±1.85 µg/ml and 51.26 ± 0.39µg/ml for standard BHT (Figure-2). The fruit extract was 37% less antioxidant activity than synthetic antioxidant of BHT.

Metal chelating activity assay

The *Cocculus hirsutus* fruit extract showed chelating capacity of 15.07±1.08 % at 100µg/ml concentration (Table-8). However, the chelating capacity was found to be less when compared to the standard. IC₅₀ value for acidified methanol extract was 200.27±1.85 µg/ml and 51.26 ± 0.39µg/ml for standard EDTA (Figure-2). The fruit extract was very less antioxidant activity than synthetic antioxidant of EDTA. The sorghum anthocyanins exhibited the highest activity of 85.2% at 100µg/mL whereas 48.3% inhibition was noted at 1µg/mL, respectively. The acidified methanol extract of the sorghum anthocyanins had the highest chelating capacity than BHT and ascorbic acid[39].

Total antioxidant capacity by phosphomolybdenum assay

Increase in absorbance indicates increase in total antioxidant capacity. The *Cocculus hirsutus* fruit extract exhibited significant activity and it s absorbance increased with increase in concentration. And the values are in comparison with Ascorbic acid. The fruit anthocyanin exhibited highest absorbance of 0.382±0.005for 100µg/ml concentration (Table-9). The fruit of the two jaboticaba varieties (Paulista and sabara) presented high antioxidant activities of 113.53±18.8 mg/g,127.56±3.23 mg/g respectively. These values were lower than standard rutin (290.89±15.7 gm/g) [35].

Table-6 *In vitro* free radical scavenging effect of *Cocculus hirsutus* fruit on Reducing power assay

Percentage Scavenging (mean±SD) of Triplicates						
concentration	20µg/ml	40µg/ml	60µg/ml	80µg/ml	100µg/ml	IC ₅₀ µg/ml
Acid-Methanol	11.24±0.65	25.17±1.06	30.23±0.64	38.53±1.35	52.17 ±0.88	97.03 ±0.88
concentration	10µg/ml	20µg/ml	30µg/ml	40µg/ml	50µg/ml	
Ascorbic acid	6.3±2.06	14.41±0.78	25.67±1.35	36.93±2.06	50.45±2.07	53.74±1.34

Table-7 *In vitro* free radical scavenging effect of *Cocculus hirsutus* fruit on Inhibition of lipid peroxidation in Egg yolk

Percentage Scavenging (mean±SD) of Triplicates						
concentration	20µg/ml	40µg/ml	60µg/ml	80µg/ml	100µg/ml	IC ₅₀ µg/ml
Acid-Methanol	21.82±0.55	24.45±0.45	35.37±0.46	36.62±0.48	37.72±0.36	107.6±0.48
concentration	10µg/ml	20µg/ml	30µg/ml	40µg/ml	50µg/ml	
BHT	6.8±0.34	11.74±1.06	23.82±0.88	29.55±0.95	34.05±1.41	70.59±2.8

Table-8 *In vitro* free radical scavenging effect of *Cocculus hirsutus* fruit on FIC chelating activity

Percentage Scavenging (mean±SD) of Triplicates						
concentration	20µg/ml	40µg/ml	60µg/ml	80µg/ml	100µg/ml	IC ₅₀ µg/ml
Acid-Methanol	23.26±0.28	22.09±0.28	20.35±0.28	17.83±0.31	15.07±1.08	200.27±1.85
concentration	10µg/ml	20µg/ml	30µg/ml	40µg/ml	50µg/ml	
EDTA	18.78±0.28	23.35±0.74	32.02±0.74	39.04±0.56	43.78±0.28	51.26±0.39

Table-9 Total antioxidant capacity on phosphomolybdenum assay

concentration	20µg/ml	40µg/ml	60µg/ml	80µg/ml	100µg/ml
Acid-Methanol	0.150±0.004	0.220±0.01	0.278±0.004	0.318±0.004	0.382±0.005
concentration	10µg/ml	20µg/ml	30µg/ml	40µg/ml	50µg/ml
Ascorbic acid	0.308±0.010	0.443±0.008	0.617±0.005	0.781±0.003	0.94±0.047

Conclusion

The *Cocculus hirsutus* L. fruits could be evaluated as major source of anthocyanin, Flavonoid and polyphenols. The antioxidant activity of the fruit extract showed potential activity when compared to the synthetic antioxidants. So this fruit can be used as a natural source

of anthocyanin and antioxidants. Future research should include, identification of constituents in the acidified methanol fractions, and study of the anticancer effects of this extracts and purified components *in vitro*, using cell proliferation and cell cycle analysis, as well as biochemical receptor assays.

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