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In Vitro Antioxidant and Free Radical Scavenging Activity of *Curcuma longa*, *Acorus calamus* and *Camellia sinensis*

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Abstract

The purpose of the current study was to determine the total phenolic and flavonoid content and total antioxidant activity of the *Curcuma longa*, *Acorus calamus*, and *Camellia sinensis* ethanolic extracts and their free radical scavenging activity. The study concluded that the *Curcuma longa*, *Acorus calamus*, and *Camellia sinensis* ethanolic extracts have a good source of phenolics, flavonoids, and antioxidant sources in turn which opens the high possibility of the extracts being used as food preservatives. The DPPH assay for scavenging free radicals showed that the IC_{50} value was above 123% of *Curcuma longa* ethanolic extract, 129.9% μg/ml of *Acorus calamus* ethanolic extract and 25% of *Camellia sinensis* ethanolic extracts shows very strong inhibition of the free radicals. Thus, comparing the DPPH assay for scavenging free radicals of *Curcuma longa*, *Acorus calamus* and *Camellia sinensis* ethanolic extracts with the positive control ascorbic acid, *Curcuma longa* and *Camellia sinensis* ethanolic extracts showed strong inhibition of the free radicals.

Keywords

Total Phenolic Content, Total Flavonoid Content, DPPH, *Curcuma longa*, *Acorus calamus* and *Camellia sinensis*

1. Introduction

The formation of free radicals (reactive oxygen species, ROS) in the biological system causes oxidative stress (OS), which plays a role in various diseases such as cancer, neurodegenerative diseases, heart disease, rheumatoid arthritis, and ag-

ing. Both extrinsic and endogenous antioxidants are radical scavengers that play a role in preventing the development of chronic age-related degenerative diseases. Therefore, dietary antioxidants reduce the risk of these illnesses. In addition to health benefits, antioxidants prevent or delay the oxidation of foods caused by the formation of free radicals while exposed to environmental factors such as light, air and temperature [1] [2] [3] [4] [5] [6].

Free radicals are unpaired molecules and are highly actives that can damage surrounding molecules. Free radicals that steal electrons from the human body can cause changes in the structure of DNA (deoxynucleic acid) and mutate cells. Exposure of the human body to free radicals is cumulative, and various illnesses occur when the human immune system becomes intolerant of the presence of free radical compounds. The opposite antioxidant stabilizes free radicals and inhibits the formation of free radicals [7].

Many antioxidants come from plants that contain one spice, turmeric. There are several types of turmeric, including black turmeric, yellow turmeric, white turmeric, and red turmeric. Turmeric is a plant with the potential as an antipyretic, antipyretic, antihepatotoxic, anti-inflammatory agent, bacteriostatic agent, hypocholesterolaemia, bile secretion promoter, and antispasmodic agent due to the active ingredient curcumin. Studies have shown that curcumin in turmeric contains powerful antioxidants. Turmeric also acts as a food preservative, as the content of curcumin and essential oils can inhibit the growth of bacteria [7] [8] [9] [10] [11].

Acorus calamus is a perennial monocotyledonous wetland plant of the Acorus family. This plant has been studied to contain many chemical constituents from rhizomes, leaves and roots. Bioactive compounds identified with *Acorus calamus* include eugenol, asarial defida, asarone, carameon, caramidiol, isocaramendiol, acorenin, aconin, acroagelmacron, acoramonin, isocoramine, siobrin, isociobnin, episubunit, resin, also tannins. The major bioactive compounds identified in the iris are saponins and flavonoids, α- and β-asarone caryophyllene, isosalone, methyl isoeugenol and safrole. Therefore, the use of medicinal herbs is expected to affect health not only for livestock but also for products [12] [13].

Adding tea powder to foods enhances antioxidant activity and significantly reduces peroxide production during product storage. Green tea powder is high in catechins, lutein, and vitamin K, and when taken daily, prevents cognitive decline in older women. The chemical composition of green tea powder includes chemical compositions that promote health benefits such as anti-cancer, anti-inflammatory, cardioprotective, anti-virus and regulate carbohydrate metabolism. Also, from a health perspective, adding tea powder to baked goods lowers the glycemic index. Green tea powder also contains a lot of vitamin C [14] [15] [16] [17]. Therefore, the purpose of the current study was to determine the total phenolic and flavonoid content and total antioxidant activity of the *Curcuma longa*, *Acorus calamus* and *Camellia sinensis* ethanolic extracts and their free radical scavenging activity.

2. Materials and Methods

2.1. Raw Materials

The samples of Turmeric (*Curcuma longa*) were collected from the local market in Erode, Sweet Flag (*Acorus calamus*) and Tea (*Camellia sinensis*) was collected from the local field at Kotagiri, Nilgiris district.

2.2. Preparation of Crude Extract

Ethanolic extracts were prepared by adding 20 g of samples (**Figure 1**) to 80 mL of ethanol at 90˚C. The obtained mixture was filtered with a 200-mesh sieve, and the filtrate contained in a beaker was cooled to 10˚C in an ice water bath, then centrifugated with a centrifuge for 15 min to remove impurities. The upper layer was collected and concentrated in a vacuum at 60˚C and then dried powdered extract was stored in airtight bottles and refrigerated at 40˚C until use.

2.3. Determination of Total Phenolic Content

The total phenolic content of the sample was determined by using Folin-Ciocalteu reagent following a slightly modified method by Ainsworth and Gillespie [18]. Gallic acid was used as a reference standard for plotting calibration curve (25 to 500 μg/mL). A volume of 0.1 mL of the sample was mixed with 2 mL of the Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water) and neutralized with 4 mL of sodium carbonate solution (7.5%, w/v). The reaction mixture was incubated at room temperature for 30 min with intermittent shaking for color development. The absorbance of the resulting blue color was measured at 765 nm using double beam UV/VIS SPECTROPHOTOMETER LMSP-UV-1200-PC (LABMAN SCIENTIFICS, India). The total phenolic contents were determined from the linear equation of a standard curve prepared with gallic acid. The content of total phenolic compounds is expressed as mg/L gallic acid equivalent (GAE) of a sample.

2.4. Determination of Total Flavonoid Content

Total flavonoid content was measured spectrophotometrically by using Aluminium chloride colorimetric method [19]. 0.1 ml of the extract was mixed with 1.5 ml of ethanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M sodium acetate and 2.8 ml of distilled water. It was kept at room temperature for 30 min and absorbance of the reaction mixture was measured at 415 nm with a double beam UV spectrophotometer. The calibration curve was prepared by preparing

Figure 1. Powdered form of the samples *Curcuma longa*, *Acorus calamus* and *Camellia sinensis*.

Quercetin solutions at concentrations 6.25, 12.5, 25, 50 and 100 μg/ml in methanol. The total flavonoid content was expressed as mg/g Quercetin equivalents (QE).

2.5. Anti-Oxidant Study of *Curcuma longa, Acorus calamus and Camellia sinensis* Ethanolic Extracts Using DPPH Assay

DPPH radical scavenging activity of the extract was determined according to the method reported by Blois [20]. An aliquot of 0.5 ml of the sample solution in methanol was mixed with 2.5 ml of 0.5 mM methanolic solution of DPPH. The mixture was shaken vigorously and incubated for 30 min in the dark at room temperature. The absorbance was measured at 517 nm using UV spectrophotometer. Ascorbic acid was used as a positive control. DPPH free radical scavenging ability (%) was calculated by using the formula.

$$\% \text{ of inhibition} = \text{absorbance of control} - \text{absorbance of sample}/\text{absorbance of control} \times 100.$$

3. Results and Discussion

3.1. Total Phenolic and Flavonoids Content in *Curcuma longa, Acorus calamus* and *Camellia sinensis* Ethanolic Extracts

TPC and TFC of the extracts were calculated on the basis of a standard gallic acid curve and the results were expressed as mg/L gallic acid equivalent (GAE) of sample extractives. TPC of *Curcuma longa*, *Acorus calamus* and *Camellia sinensis* ethanolic extracts were found to be 15.53% ± 0.53%, 12.22% ± 0.68% and 17.20 ± 0.70 mg of GAE/g of *Curcuma longa*, *Acorus calamus* and *Camellia sinensis* ethanolic extracts, respectively (**Figure 2**). From the data, it was shown that the phenolic content of *Camellia sinensis* as well as *Curcuma longa* was higher than *Acorus calamus* extract. Hence these extracts might serve as a good source for phenolics as well as antioxidants. The TFC of *Curcuma longa*, *Acorus calamus* and *Camellia sinensis* ethanolic extracts were 13.67% ± 1.63%, 12.07% ± 0.23% and 13.92% ± 0.76% mg/g Quercetin equivalents (QE), respectively (**Figure 3**). The data indicates that *Camellia sinensis*, as well as *Curcuma longa*, contained higher amounts of flavonoids than that of *Acorus calamus* extract, the results demonstrated that these extracts are a significant source of flavonoids.

3.2. Anti-Oxidant Study of Ascorbic Acid Using DPPH Assay

From **Table 1** % inhibition of free radicals by ascorbic acid at various concentrations (5, 10, 20, 40 and 50 μg/ml) can be determined. Ascorbic acid is taken as the positive control for the DPPH assay of scavenging free radicals. From the IC_{50} graph (**Figure 4**), the value 41.21% ± 1.56% μg/ml for IC_{50} is obtained. Ascorbic acid at various concentrations (5, 10, 20, 40 and 50 μg/ml) showed an inhibition percentage of 3.35%, 8.64%, 13.52%, 43.45% and 82.54% respectively. Two graphs were plotted between the concentration of the sample and log values for the concentration of the sample and % inhibition as shown in **Figure 4**. The

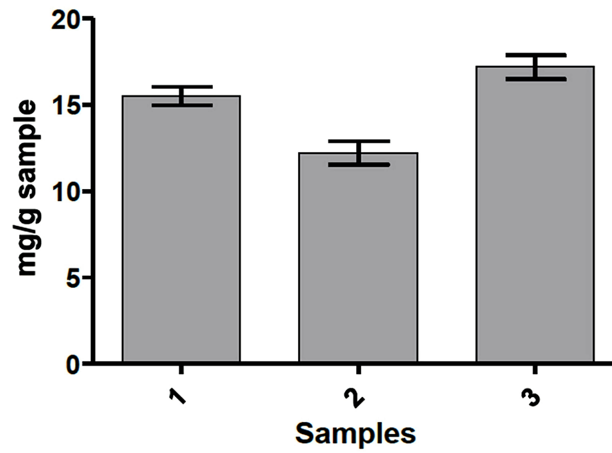

Figure 2. TPC of *Curcuma longa*, *Acorus calamus* and *Camellia sinensis* ethanolic extracts. *Here samples 1, 2 and 3 refers to *Curcuma longa*, *Acorus calamus* and *Camellia sinensis* ethanolic extracts.

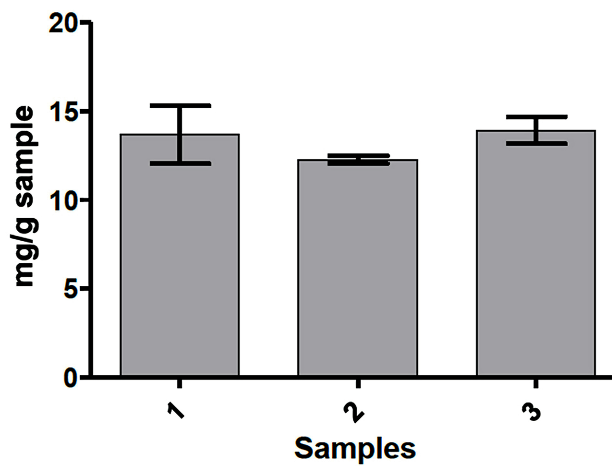

Figure 3. TFC of *Curcuma longa*, *Acorus calamus* and *Camellia sinensis* ethanolic extracts. *Here samples 1, 2 and 3 refers to *Curcuma longa*, *Acorus calamus* and *Camellia sinensis* ethanolic extracts.

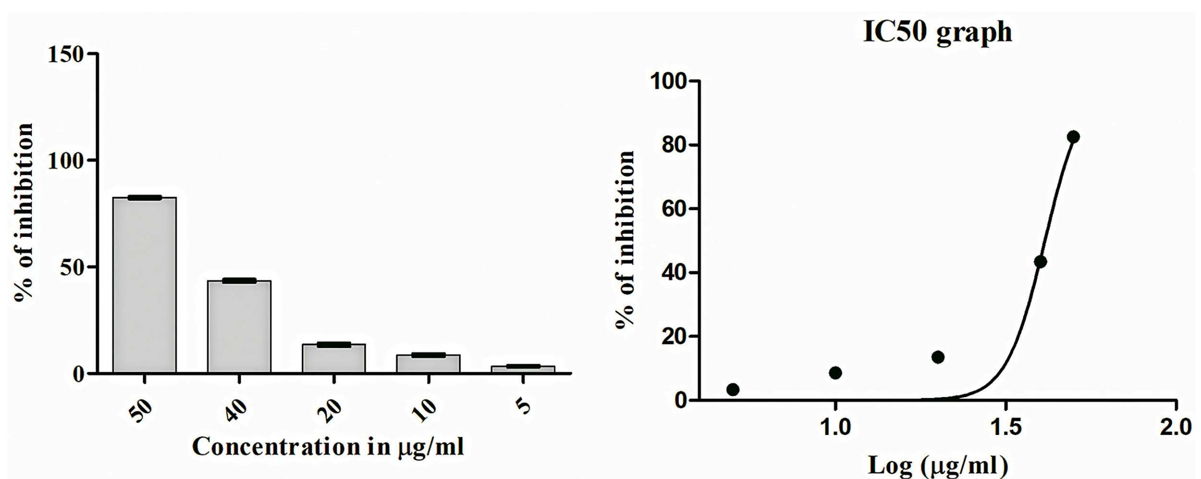

Figure 4. % of inhibition of free radicals and IC_{50} graph of Ascorbic acid using DPPH assay.

IC_{50} value above 42% of ascorbic acid shows very strong inhibition of the free radicals.

3.3. Anti-Oxidant Study of *Curcuma longa* Ethanolic Extracts Using DPPH Assay

From Table 2 % inhibition of free radicals by *Curcuma longa* ethanolic extracts at various concentrations (25, 50, 100, 250 and 500 µg/ml) can be determined. *Curcuma longa* ethanolic extracts at various concentrations showed less inhibition of free radicals than the positive control ascorbic acid taken for the DPPH assay of scavenging free radicals. From the IC_{50} graph (Figure 4) the value 123.20% ± 0.06% µg/ml for IC_{50} is obtained. *Curcuma longa* ethanolic extracts at various concentrations (500, 250, 100, 50 and 25 µg/ml) showed an inhibition percentage of 83.89%, 73.54%, 38.92%, 24.55% and 18.69% respectively. Two graphs were plotted between the concentration of the sample and log values for the concentration of the sample and % inhibition as shown in Figure 5. The IC_{50} value above 123% of *Curcuma longa* ethanolic extract shows very strong inhibition of the free radicals [21].

3.4. Anti-Oxidant Study of *Acorus calamus* Ethanolic Extracts Using DPPH Assay

From Table 3 % inhibition of free radicals by *Acorus calamus* ethanolic extracts at various concentrations (25, 50, 100, 250 and 500 µg/ml) can be determined. *Acorus calamus* ethanolic extracts at various concentrations showed less inhibition of free radicals than the positive control ascorbic acid taken for the DPPH assay of scavenging free radicals. From the IC_{50} graph (Figure 6) the value 129.9%

Table 1. % of inhibition of free radicals by ascorbic acid using DPPH assay.

Tested concentration (µg/ml)	% of Inhibition	Standard Deviation	Standard Error
5	3.35	0.71	0.41
10	8.64	1.23	0.71
20	13.52	1.41	0.82
40	43.45	1.32	0.77
50	82.54	0.98	0.57
Control	0.00	0.00	0.00

Table 2. % of inhibition of free radicals by *Curcuma longa* using DPPH assay.

Tested concentration (µg/ml)	% of Inhibition	Standard Deviation	Standard Error
500	83.89	0.10	0.06
250	73.54	0.18	0.10
100	38.92	0.44	0.26
50	24.55	0.13	0.07
25	18.69	0.18	0.10
Control	0.00	0.00	0.00

Table 3. % of inhibition of free radicals by *Acorus calamus* using DPPH assay.

Tested concentration (μg/ml)	% of Inhibition	Standard Deviation	Standard Error
500	32.51	0.27	0.16
250	19.64	0.27	0.16
100	9.41	0.27	0.16
50	7.76	0.18	0.10
25	6.06	0.27	0.16
Control	0.00	0.00	0.00

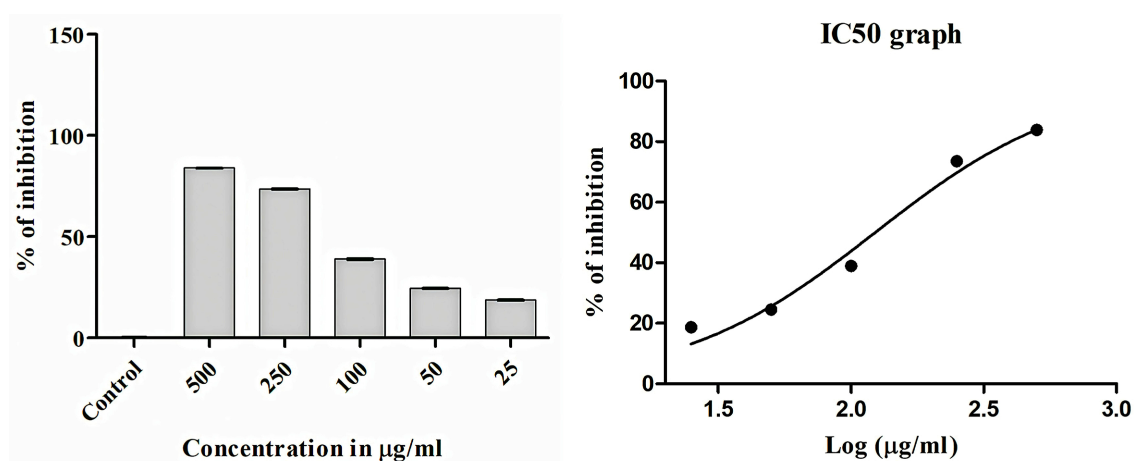

Figure 5. % of inhibition of free radicals and IC_{50} graph of *Curcuma longa* ethanolic extracts using DPPH assay.

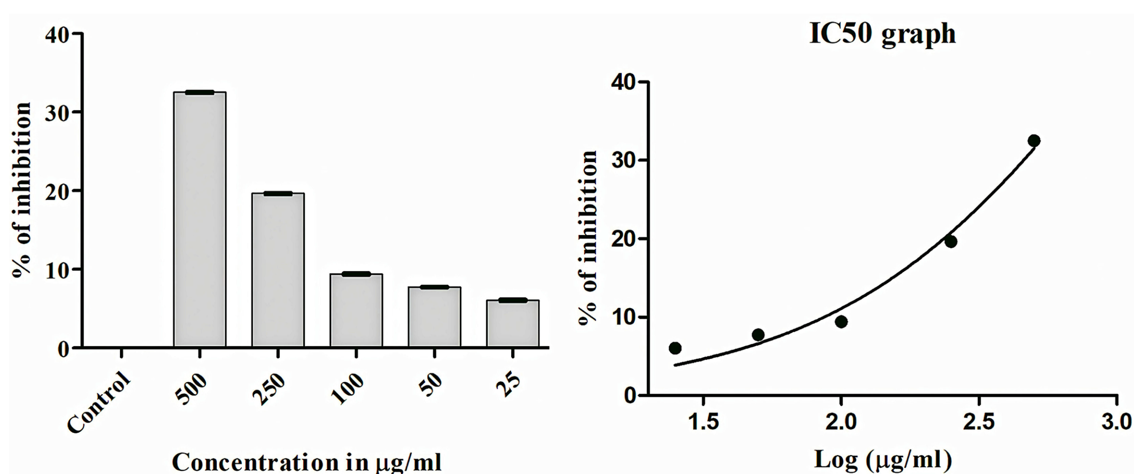

Figure 6. % of inhibition of free radicals and IC_{50} graph of *Acorus calamus* ethanolic extracts using DPPH assay.

± 21.47% μg/ml for IC_{50} is obtained. *Acorus calamus* ethanolic extracts at various concentrations (500, 250, 100, 50 and 25 μg/ml) showed an inhibition percentage of 32.51%, 19.64%, 9.41%, 7.76% and 6.06% respectively. Two graphs were plotted between the concentration of the sample and log values for the concentration of the sample and % inhibition as shown in **Figure 6**. The IC_{50} value above 129.9% μg/ml of *Acorus calamus* ethanolic extract should be used to

have a very strong inhibition of the free radicals [22].

3.5. Anti-Oxidant Study of *Camellia sinensis* Ethanolic Extracts Using DPPH Assay

From Table 4 % inhibition of free radicals by *Camellia sinensis* ethanolic extracts at various concentrations (25, 50, 100, 250 and 500 μg/ml) can be determined. *Camellia sinensis* ethanolic extracts at various concentrations showed better inhibition of free radicals than the positive control ascorbic acid taken for the DPPH assay of scavenging free radicals. From the IC_{50} graph (Figure 4) the value 24.74% ± 0.1% μg/ml for IC_{50} is obtained. *Camellia sinensis* ethanolic extracts at various concentrations (500, 250, 100, 50 and 25 μg/ml) showed an inhibition percentage of 92%, 87.95%, 85.19%, 67.90% and 48.56% respectively. Two graphs were plotted between the concentration of the sample and log values for the concentration of the sample and % inhibition as shown in Figure 7. The IC_{50} value above 25% of *Camellia sinensis* ethanolic extracts shows very strong inhibition of the free radicals [23].

Table 4. % of inhibition of free radicals by *Camellia sinensis* using DPPH assay.

Tested concentration (μg/ml)	% of Inhibition	Standard Deviation	Standard Error
500	92.00	0.44	0.26
250	87.95	0.62	0.36
100	85.19	0.35	0.20
50	67.90	0.53	0.31
25	48.56	0.54	0.31
Control	0.00	0.00	0.00

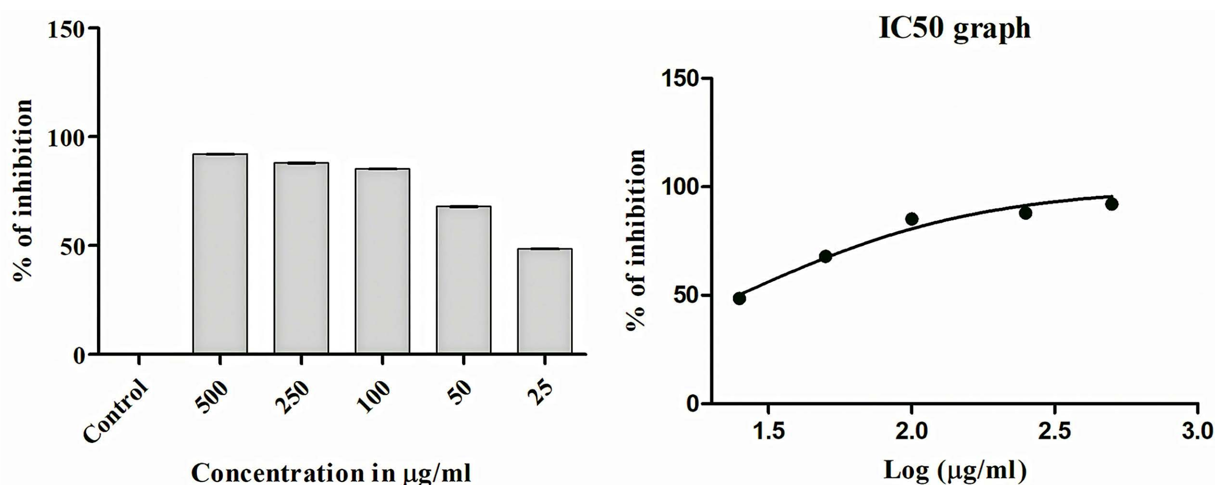

Figure 7. % of inhibition of free radicals and IC_{50} graph of *Camellia sinensis* ethanolic extracts using DPPH assay.

4. Conclusion

The study concluded that the *Curcuma longa*, *Acorus calamus* and *Camellia si-*

nensis ethanolic extracts have a good source of phenolics, flavonoids and antioxidant sources in turn which opens the high possibility of the extracts being used as food preservatives. It was shown that the phenolic content of *Camellia sinensis* as well as *Curcuma longa* was higher than *Acorus calamus* extract. Hence these extracts might serve as a good source of phenolics as well as antioxidants. It also indicates that *Camellia sinensis*, as well as *Curcuma longa*, contained higher amounts of flavonoids than that of *Acorus calamus* extract; the results demonstrated that these extracts are a significant source of flavonoids. The DPPH assay for scavenging free radicals showed that the IC_{50} value was above 123% of *Curcuma longa* ethanolic extract, 129.9% µg/ml of *Acorus calamus* ethanolic extract and 25% of *Camellia sinensis* ethanolic extracts shows very strong inhibition of the free radicals. Thus, comparing the DPPH assay for scavenging free radicals of *Curcuma longa*, *Acorus calamus* and *Camellia sinensis* ethanolic extracts with the positive control ascorbic acid, *Curcuma longa* and *Camellia sinensis* ethanolic extracts showed strong inhibition of the free radicals.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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