(non-polar) in separate test tubes kept at 55°C for 5 min contact period.

Each extract was centrifuged at 9000 rpm for 5 min, and the procedure was repeated for 2–3 times to get a clear supernatant which was stored in a refrigerator at 5–10°C until use.

Estimations of total phenolic content

This was done by adopting the method of Singleton *et al.* [4]. About 0.5 ml of extract was taken to which 0.5 ml of water and 2 ml of Folin–Ciocalteu reagent (1:5H_2O) was added and kept for 3 min. 10% (w/v) Na_2CO_3 was added to this mixture and kept for 30 min after which reading was taken at 725 nm in a UV-spectrophotometer. Gallic acid was used as the standard. The total phenolic content was expressed as µg gallic acid equivalents (GAEs).

Estimation of total flavonoids

The method followed was as described by Kessler *et al.* [5]. 0.5 ml of each extract (1 mg/ml) was taken in separate test tubes, to which 0.1 ml of 10% $AlCl_3$ and 0.1 ml of 1M potassium acetate were added followed by 2.8 ml of distilled water. The resultant mixture was shaken vigorously for thorough mixing and read at 420 nm in a UV-spectrophotometer. Quercetin was used as the standard, and the total flavonoid content was expressed in terms of quercetin equivalent (QE).

DPPH assay to estimate antioxidant activity

This procedure was done according to the method described by George *et al.* [6]. To 50 µl of each extract taken in a separate well of a microtiter plate, 100 µl of DPPH (in 0.1% methanol) reagent was added and kept for 30 min in a dark room and observed for color change. The extracts which changed to yellow were considered as strongly positive for antioxidant activity and proceeded for quantitative estimation as given below.

The above procedure was repeated with 100 µl extract, and reading was taken at 517 nm in a UV-spectrophotometer. Butylated hydroxytoluene (BHT) was used as a standard. The following formula was used to calculate the free radical scavenging activity.

DPPH radical

$$\text{scavenging activity } (\%) = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

Statistical analysis

Each test was performed in triplicates, and the results were expressed as mean±standard deviation.

RESULTS

In the present investigation, tubers collected from Thiruvannamalai showed the maximum phenolic (38.82±0.22 mg GAE/g) as well as total flavonoid (21.34±0.32 mg QE/g) content and coincided with the maximum antioxidant activity of 87.6%. Antioxidant activity was found to be lesser compared to the standard BHT (97.2%). The samples collected from Thiruvannamalai showed the maximum antioxidant activity (87.6%) with the ethanolic extract. This was followed by samples of Kallakuruchi (76.2%), Salem (73.7), Kanchipuram (68.3%), and Vandalur (62.1%) (Figs. 1-3). Regarding other solvents, also maximum values were obtained only for samples collected from this place.

DISCUSSION

Free radical production cannot be avoided as they are produced in almost all fundamental processes of aerobic organisms including plants. DPPH is a stable free radical compared to other antioxidants, and hence, it is considered as an important method to assess the potential of natural compounds to scavenge toxic oxygen molecules. The study clearly indicated that the various extracts of *C. forskohlii* act as a potent material to scavenge free radicals. The plant extract reduced DPPH to the hydrogen available by making unpaired electron to form pairs in the outer orbit, and hence, it is called antioxidant activity. This might be due to the high content of phenol and flavonoids as reported by many [7-9]. Among the different solvents used, extract done by ethanol showed the maximum antioxidant activity followed by acetone, aqueous (water), chloroform, and petroleum ether. This trend holds good for tubers collected from different places. The variations of antioxidant activity based on solvents might be due to their potential of extraction of these compounds from tuber biomass.

According to a study, petroleum ether extracts of *Mucuna pruriens* showed the maximum DPPH activity of 40.29% at 100 µg/ml, whereas rutin (standard) showed 69.83% [7]. They also found the different extents of DPPH scavenging activity for different solvents used, i.e., for ethyl acetate extract, 66.97%, and for methanol extract, it was 49.83%.

DPPH activity is concentration dependent (data not shown). In *Cressa cretica* 72.37% DPPH activity was observed at a concentration of

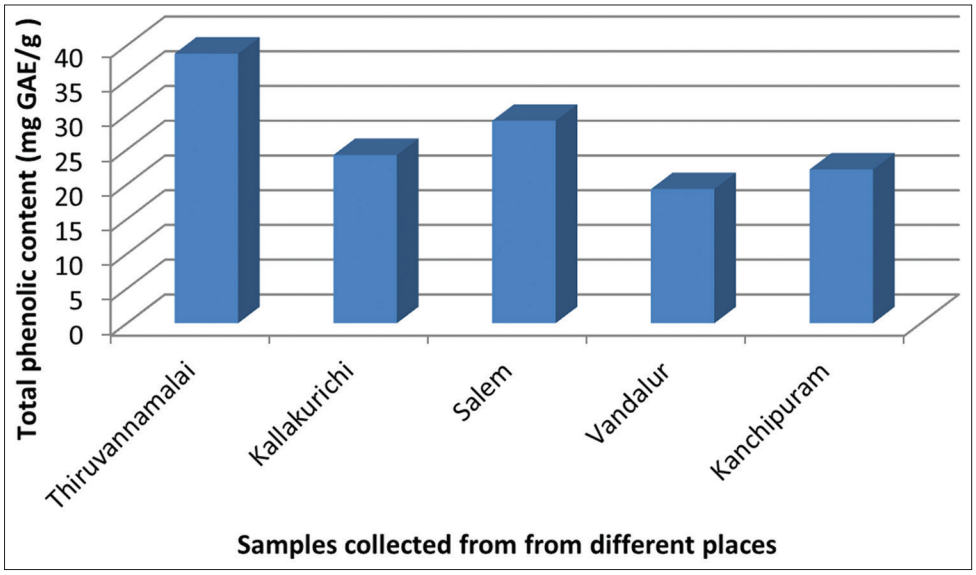

Fig. 1: Total phenolic content of *Coleus forskohlii* tubers collected from different places

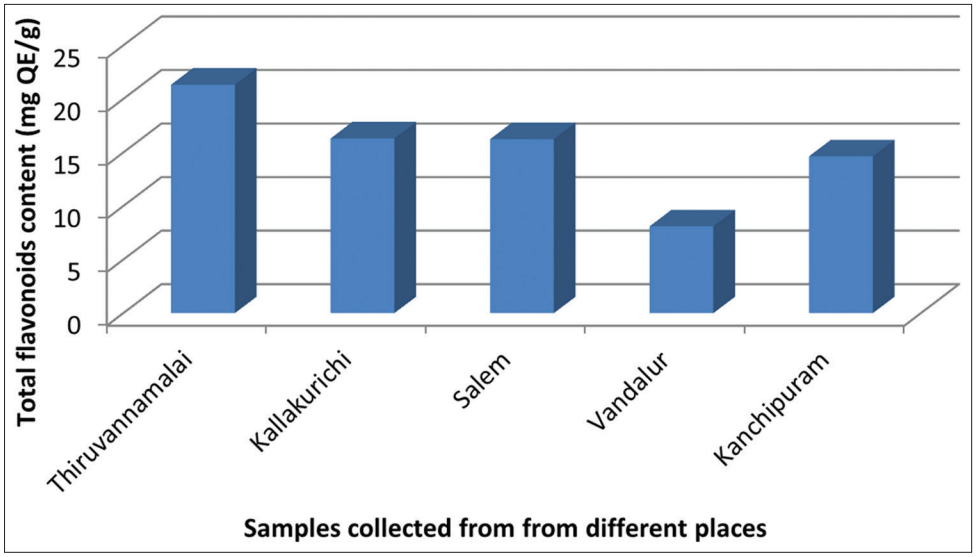

Fig. 2: Total flavonoids in *Coleus forskohlii* tubers collected from different places

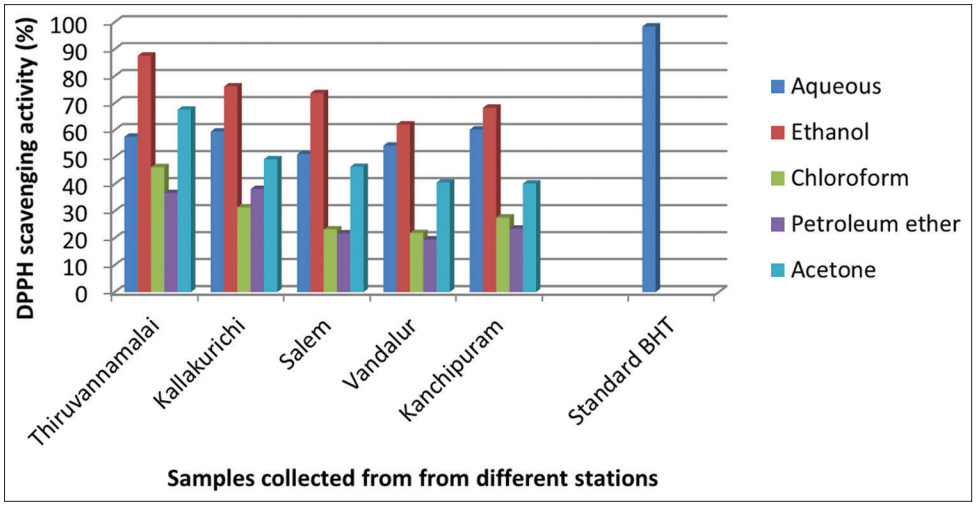

Fig. 3: Antioxidant activity of different solvent extracts of *Coleus forskohlii* tubers collected from different places

250 mg/ml [10]. In another study, a plant species *Salvia* used for the tea preparations, especially in Mediterranean countries, possessed 51.19–118.13 mg GAE/g of total phenols, 820.71–1000.65 µg RE/g of flavonoids, and 67.26–138.44 mg AAE of antioxidant capacity [11].

Plants may contain varieties of phenolic compounds with varying antioxidant potential [12] which might be true in the present study also. As plant phenolics are the prime antioxidants or free radical terminators, their quantity must be estimated [2]. High content of phenolics was observed in *O. sanctum* (48.93±0.24 mg/g), DPPH activity was more in the methanolic extracts of all the five medicinal plants tested and the antioxidant values varied from 3.43±0.2 and 48.93±0.24, and strongest DPPH activity was noted in *Desmodium gangeticum* and *Amaranthus caudatus* [13]. The antioxidant activity of methanolic extracts of four Indian medicinal plants revealed the maximum of 77.0% DPPH activity for *Hemidesmus indicus* (stem) and the minimum of 20.06% for *Holarrhena antidysenterica* (bark) [14]. The ethanolic extracts of *Alpinia* speciosa and *Alpinia calcarata* rhizome showed antioxidant and free radical scavenging activity [15].

In a study done on the methanolic extract of different parts of the plant *Lantana camara,* total phenolic content was in the order of leaves>flower>root>fruits so also the DPPH reduction activity [16]. The present study along with other studies given above insisted that the total phenol might be involved in quenching of free radicals, leading to higher antioxidant activity. This might be due to their hydrogen-donating property of their hydroxyl groups. Work on DPPH activity on *Salvia amplexicaulis* found that the methanol and ethanol extracts showed the highest DPPH reduction activity as in the present investigation. The methanol and ethanol extracts of *S. amplexicaulis* showed the highest DPPH reduction activity [17,18] as in the present investigation. Interestingly in *S. amplexicaulis,* the ethanol extract of leaves showed maximum DPPH activity, whereas, in the whole plant, methanol extract showed the maximum DPPH reduction activity. However, in the present study, only tuber samples were used, as forskolin is an important compound in this plant, and it is present predominantly in tubers.

Different species of *Salvia* plant was attempted earlier [19], whereas in the present study, *C. forskohlii* available in different areas of Tamil Nadu was studied. The results showed the importance of the area of plantation related to pharmacologically important crops. *Ludwigia octovalvis* and *Vitis thunbergii* exhibited very strong scavenging of DPPH radicals when 26 species of different plants were tested and IC_{50} values were 4.6 and 24.1 µg/ml, respectively [20]. *Ginkgo biloba,* a popular medicinal herb leaf extract, studied for its antioxidant activity showed an IC_{50} value of 930 µg/ml [21].

The antioxidant activity of three methanolic rhizome extracts *Hedychium* spp. was studied and 60.86% of free radical scavenging activity as the highest was noted with *Hedychium rubrum* [22], whereas 71.21% of antioxidant capacity was observed with *C. forskohlii* [9]. Compared to these plants, the potency exhibited by tubers of *C. forskohlii* in the present study seemed to be more. The antioxidant property of *C. forskohlii* revealed the possibility of formulating functional foods from this plant extracts as high content of flavonoids and phenolics might have a preventive role in many diseases including heart diseases and cancer [23]. Red wine [24,25], carotenoid-containing fruits and vegetables [26], and their role in saving lives from heart diseases insist the food value of plants with antioxidative potential [27].

Flavonoids and DPPH activity had been reported in different plants by different researchers, for example, in leaf extracts of *Stevia rebaudiana* Bert. [28], *Clusia fluminensis Planch*. and *Triana* (*Clusiaceae Lindl*.) [29], and *Sida cordata* [30].

Oxidative stress generating free radicals seemed to be controlled by natural flavonoids in foods consumed by human beings as well as animals. The total flavonoids in methanolic extracts of three *Hedychium* spp. in *H. spicatum, H. coronarium,* and *H. rubrum* are to be, respectively, 4.22, 2.47, and 21.25 µg/100 g in dried rhizomes. Accordingly, 42.74%, 32.42%, and 60.86% of antioxidant activity in DPPH assay were obtained [22]. Similar results have been observed in various *Hedychium* spp. in different regions [31-33] and in *Polyalthia longifolia* [34]. As more than 6000 flavonoids in different plant species were reported, it became a practical issue, to estimate total flavonoids as an index of their antioxidant potential [35]. The oxygen with a double bond in the basic structure of flavonoids seemed to be responsible for their scavenging activity. The results of the present study are in agreement with various other researches who have done in different kind of samples opined that antioxidant activity might be due to phenols as well as flavonoids [29,30].

CONCLUSION

The study clearly indicated that the antioxidative activity potential of different extracts of *C. forskohlii* might be due to phenols as well as flavonoids and these properties can be used in drug formulations as well as in exploiting this tuber for other purposes such as cosmetics and preparation of health tonics.

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AUTHORS' CONTRIBUTION

SS: Collection of plant materials, acquisition, analyzed, interpretation of data, and wrote the manuscript. VU: In a consolidation of results. BVP: Reviewing and corrected the manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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