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A study on preliminary phytochemical screening and antioxidant activity of *Capparis divaricata* L

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Abstract
Plants are being played an important role by their medicinal values in food and pharmaceutical industries for human welfare like disease prevention and treatment. The currents study characterizes the preliminary Phytochemical and antioxidant activity of *Capparis divaricata* by using colorimetric, and spectrophotometric assays. Results revealed that the methanolic leaf extracts of *C. divaricata* (Caperbush) displayed the highest content of total phenols (26.6 mg gallic acid per gram of plant dry weight), flavanoid (52.6 mgRE/g extracts), and tannins (16mg GAE/g DW). The highest antioxidant capacity was measured by DPPH, hydrogen radical scavenging activity, and total antioxidants (26.29µg/ml, 161.26gDMSO/g extract, 17.61 mg GAE/g DR respectively) methods were also obtained in *C.divaricata* leaf extracts. Such potent antioxidant activity of *C.divaricata* leaves can be attributed to the presence of different types of phenolic compounds and the high content in tannins, phenolic, and flavonoids were found to be dominant. Consequently, despite the well-known antioxidant properties of these plant species, our study suggests *C.divaricata* leaf can be used more valuable plant source of natural bioactive molecules for developing novel functional food-pharma ingredients for the betterment of human society.

Keywords: *Capparis divaricata*, antioxidant activity, phytochemical, caperbush

Introduction
India is known to be a rich repository of Medicinal Plants. Plants have been used even from prehistoric times for therapeutic purposes. The ancient cultures-figures like Unani Hakims, Indian Vaids, European and Mediterranean leaves used Medicinal Plants based on customary practice. As herbs are natural drugs they are free from side effects, eco-friendly and cost-effective, and easily available. They save us from the blind dependence on synthetic drugs naturally society is returning to the natural herbs with faith in their security and safety. But the optimum commercial production of herbal drugs is still limited. Population rise, inadequate supply of herbal drugs, the prohibitive cost of treatments, side effects of several synthetic drugs and develop resistance to current drugs for infectious diseases, etc., have contributed to the greater emphasis on the use of plant materials as sources of medicine.

Capparis divaricata is a native plant of the Mediterranean region and is commonly known as Caperbush C. divaricata belongs to the Capparidaceae family and it is a type genus of the family consisting of more than 80 species. Different parts of the plant exhibit pharmacological properties like Antirheumatic, Expectorant, Tonic, Antipyretic and Antispasmodic analgesics (Tlili *et al*, 2011; Patel and Sharma, 2015; Mollica *et al*, 2017) [15, 11, 9]. Phytochemical screening of *C.divaricata* reveals that it consists of various functional Phyto-compounds such as glycosides, saponins, flavonoids, alkaloids, phenols, and tannins (Khandare *et al*, 2012; Hirare and Kondawar, 2016) [7, 5].

The bark extract of *C.divaricata* pounded with leaves of *Erythrina variegate* ginger, garlic, and turmeric mixed with goat's milk is given orally for the treatment of trypanosomiasis (Umberto, 2012). Free radicals are responsible for the damage to lipids, proteins, and nucleic acids in cells. Natural antioxidants have gained importance in the Pharmaceutical and food industry to limit the use of synthetic antioxidants; they have side effects (Namiki, 1990). The biological property of Phenolic compounds is commonly impulsed by metabolism (Hollman, 2001) [6]. It was found that colonic microflora extensively changes most dietary phenols into different molecules (Olthof *et al*, 2003) [10], modifying their chemical structures is not so easily predictable ways (Eddouks *et al*, 2005) [1]. Such changes are very important as they indicate changes in biological properties like antioxidant activity, which are known to be structure-dependent (Flora, 2009) [3]. Phenolic compounds can serve as antioxidants through different mechanisms, like terminating free radicals, reducing oxygen concentration, transforming primary products of oxidation into non-oxidant molecules, or acting as metal chelators (Shahidi and Naczk, 2004). The present investigation aimed to estimate the antioxidant property of methanolic extracts of *Capparis divaricata*.

Materials and Methods
Plant Material
Fresh leaves, fruit, seed, and bark of *C.divaricata* were collected during March 2019 from the Lembalakkudi, Pudukkottai District, Tamil Nadu, India (Latitude: 10.244033; Longitude: 78.747514). The plant samples were brought to the laboratory immediately. The plants were washed thoroughly with tap water to remove dust and exogenous particles. The samples were shade-dried at room temperature until a constant weight was obtained and ground in an electric blender. The powdered samples were stored in the refrigerator for further use.

Preparation of Extract
The various parts of *C.divaricata* were powdered and extracted for 72 h in methanol at room temperature. The

extraction was repeated twice and filtered using a glass funnel and Whatman No.1 filter paper. In such extraction, the solvent was evaporated using a rotary evaporator. Finally, the *C.divaricata* extracts were cooled in desiccators for 30 min. The extracts from different parts of *C.divaricata* were kept at - 80°C before further analysis.

Chemicals and Reagents

Ascorbic acid, Boric acid, 1-1-diphenyl - 2 - picryl - hydroxyl (DPPH) were brought from Sigma. Aldrich, Methanol, Chloroform, Tween 200, trichloroacetic acid, linoleum acid, and Ammonium from Fisher Scientific. Folin-Ciocalteu'sphenol reagent and Sodium Carbonate from Merck, Mumbai, India. All the Chemicals reagents and buffer solutions have purchased the brand of analytical grade.

Total Phenol Content

The total phenol content of *C.divaricata* extracts was determined by the modified Folin-Ciocalteuassay (Ismail *et al*, 2010). The methanolic extracts were individually mixed with 10ml of methanol. Then 0.1 ml of Folin-Ciocalteureagent and 2.5 ml of 7.5% Sodium Carbonate. The reaction mixer's vortexes were for 15 sec. and they were allowed to stand for 30 min. at 40oC for incubation. Then absorbance was measured at 760mm by using a spectrophotometer (Perkin Elmer Lamda 25 UV-Vis spectrophotometer). Gallic acid was used as a standard and the total phenol content of *C.divaricata* extracts was expressed in milligram of Gallic-acid equivalents (mg GAE/g extract).

Total Flavonoid Content

Total flavonoid content was estimated by the Aluminum Calorimetric Method and rutin used as standard (Quettier - Deleu *et al*, 2000). The various extracts of C. divaricata were individually dissolved in DMSO. 150 ml of extract was mixed with an equal amount of 2% AlCl3. After the incubation time (10 min.) the absorbance of the supernatant was measured at 435nm by using a UV-Vis spectrophotometer (Perkin Elmer Lamda 25 UV-Vis spectrophotometer). Three replicates were made for each test sample. The total flavonoid content was expressed as rutin equivalents in microgram per gram extract (mg RE/g extract).

Total Tannin Content

The total tannin content was determined using the Folic Ciocalteu method described by Mejri *et al* (2014). The extracts of *C.divaricata* with absorbent material were homogenized and stored at 4oC for 2h to form a tannin – pulp complex. The pH of the reaction solution was adjusted to 3 and it was centrifuged. The values obtained were subtracted from the total phenolic compound levels and the content of total tannins was expressed as mg of Gallic acid equivalents per gram of dry residue (mg GAE/g DR). The total tannin content was assessed by scheming the Gallic acid calibration curve (from 1 to 1500 µg/ml) and articulated as milligrams of garlic acid equivalents (GAE) per gram of dried extract.

DPPH Scavenging Activity

The DPPH Scavenging Activity of the methanol extracts of *C.divaricata* was determined by the method described by Tlili *et al*, (2013) [15]. 0.5ml of different concentrations of methanolic extracts were added to 3.0ml of a 6mm methanolic solution of DPPH. The mixtures were stirred vigorously and allowed to stand in the dark at room temperature for 30min. Finally, the absorbance was measured by using a UV-Vis spectrophotometer (Perkin Elmer Lamda 25 UV-Vis spectrophotometer). The percentage of inhibition (1%) of free radical DPPH was determined using the formula.

$$1\% = (A \text{ blank} - A \text{ sample}) / A \text{ blank}) \times 1000$$

DPPH Scavenging activity is expressed as an IC50 value, which is the concentration of sample required for scavenging 50% of the free radicals present in the test solution. Butylated hydroxytoluene (BHT) was used as a control standard.

Hydroxyl Radical Scavenging Activity

The Hydroxyl radical scavenging activity of the *C.divaricata* methanolic extracts was determined by using electron spin resonance, spectrometer JEOL-JES-PA100 to Kg, Japu. The Hydroxyl radical was generated by using the Fenton reaction, with 5, 5 – dimethyl N-Oxide Pyrroline (DMPO) as the trapping agent. The reaction contained 40 ml of DMPO (400mM), 375 ml of FeSo4 (0.4mm), 112.5 ml of EOTA (0.1mm), 60 ml of extract or blank and 150 ml of H2O2 (2.0mm). The electron was conducted 1min after preparing each reaction mixture at room temperature. The ESR measurement was conducted using seeping field, 336.45 + 5mT; microwave powders, 8mm, mod width 0.1 MT, sweep time, 2 min, time constant, 0.15 and amplitude, 160 Dimethyl Sulphoxide (DMSO) was used as standard and the hydroxyl radical scavenging activity of *C.divaricata* extracts were studied and the activity was expressed in gram DMSO equivalent per gram of sample (g DMSO/g sample).

Total Antioxidant Activity

Total antioxidant activity of the extracts of *C.divaricata* was determined by using the method of Prieto (1999). 0.1ml of the extract was mixed with 1 ml of reagent solution (0.06 M Sulphuric acid, 28mm sodium phosphate, and 4mm Ammonium molybdate). The reaction solution was incubated at 95oC for 90 min. in a water bath. Absorbance was read at 695 mm by using UV-Vis spectrophotometer (Perkin Elmer Lamda 25 UV-Vis spectrophotometer). Total antioxidant activity is expressed as milligrams of Gallic acid equivalents per gram of dry residue (mg GAE/g-DR).

Statistical Analysis

Data were presented as mean + standard deviation from triplicate determination. Analysis of variance (ANOVA) accompanied with LSD and Tukey tests (SPSS for Windows, Version 15) were performed to identify the significant difference among samples ($p<0.05$).

Results and Discussion

Preliminary Phytochemical Analysis

Methanolic extracts of leaf, seed, fruit, and bark of C.divaricata were analyzed for total phenolic, total flavonoid content, and total tannins. The total phenolic content of *C.divaricata* extracts was observed using a linear Gallic acid standard curve (z=8.2313xx + 0.078; r2 = 0.9971). The total phenol content was carried from 3.31 to

26.61 mg GAE/g DW) and the maximum content of total phenol content was observed in leaves of C. divaricata (26.61 mg GAE/g DW) whereas the lowest content was observed in fruit (3.31 mg GAE/g DW) (Table 1). The total phenol content of *C.divaricata* extracts is arranged in acceding order: fruit < seed < bark < leaf. This result also agreed with *Cucumis melo* (Ismail *et al*, 2010) Coriandrum sativum (Wong and Kitts, 2006). It suggests that the plant leaf might be a rich phenolic compound in many higher plants. Several earlier reports revealed that the phenolic content is associated with their antioxidant potential, due to their redox potential, which insists them to act as the reducing agent, hydrogen donors, and single oxygen quench (Cheng *et al*, 2001).

According to Yanishlieva Maslarova, (2001) the flavonoid is the most common and widely distributed group of Phenolic compounds and is a very effective antioxidant. In the current study, the total flavonoid content of methanolic extracts of *C.divaricata* was measured by using the Aluminium Colorimetric method. Rutin was used as standard (y=0.0097x + 0.0127, r2 = 0.9995) and the total flavonoid content of C. divaricata extract was expressed in microgram of rutin equivalents per gram of extract (Mg Re/g extract). The total flavonoid content of *C.divaricata* was varied from 2.43 to 52.61 Mg RE/g extract (Table 1).The minimum flavonoid content of 52.61 Mg Re/g (Table.1) extract was observed in the extracts of leaf and the minimum content of the flavonoid was observed in the extracts of seed (2.43Mg RE/g extract). Total flavonoid content of C.divaricata is arrange in the following order: seed < bark < fruit < leaf ($p<0.05$). The total flavonoid content of *C.divaricata* extracts is in clear correspondence to the total phenolic content. These results are coincidental with the *Cucumis melo* (Ismail *et al*, 2010).

The total tannin contents of the extracts of *C.divaricata* is represented in table.1. The Total tannin content of *C.divaricata* extracts was ranged from 2.12 to 16.2 mg GAE/g DW. Among the tested extracts the highest amount of the total tannin content was estimated in leaf extract (62.2 mg GAE/g DW) and the lowest yield was observed in leaf extract (2.12 mg GAE/g DW). The yield of the methanolic extracts of *C.divaricata* is presented as follows: bark < fruit < seed < leaf. It clearly showed that the number of Polyphenols varied largely from part to part. These findings also agreed with previous reports indicating that secondary metabolite content could be varied from parts to parts of various medicinal plants (Rengasamy, Ragupathi Raja Kannan *et al*, 2010; Avello *et al*, 2013; Chetoui *et al*, 2013).

Table 1: Contents of total phenol, total flavonoid, and Total tannins of different parts of the *C. divaricate*

Sl.No.	Parts of *C.divaricata*	Total phenols (unit) (mg GAE/g extract)	Total flavonoids (unit) (mg RE/g extract)	Total Tannins (mg GAE/g DW)
1.	Leaf	26.61 ± 2.61^{b}	52.61 ± 0.31^{d}	16.2 ± 0.17^{a}
2.	Seed	6.42 ± 0.4^{e}	2.43 ± 0.21^{e}	8.62 ± 0.26^{b}
3.	Fruit	3.31 ± 0.26^{b}	10.41 ± 0.19^{c}	5.61 ± 0.16^{c}
4.	Bark	8.2 ± 0.19^{a}	6.23 ± 0.12^{b}	2.12 ± 0.21^{d}

Values are different. The Superscript letters indicate Significant differences at $p<0.05$ (Tukey tests).

Antioxidant Activity Analysis

The DPPH assay is carried out based on the reduction of DPPH, a stable free radical. DPPH assay has been employed extensively to measure the antioxidant ability of samples in reducing DPPH by donating hydrogen ions to form neutral DPPH. (Tlili *et al*, 2015; Noipa *et al*, 2011). The IC50 values of all tested samples by DPPH assay ranged from 7.31 to 26.29 µg/ml. The DPPH scavenging activity is arranged as follows: Ascorbic acid > Gallic acid > leaf > fruit > seed > bark ($p<0.05$). In the present investigation, the DPPH assay of C. divaricata extracts showed similar to the total content of tannins, flavonoids, phenols. The presence of Phyto-constituents, such as tannins, phenols, and flavonoids in medicinal plants may be responsible for its high antioxidant activity in preventing several diseases by free-radical scavenging activity (Athi – Perumal Samy *et al*, 2010; Mestry *et al*, 2017; Fellah *et al*, 2020) [8, 2].

Hydroxyl radicals are the major active oxygen species causing lipid peroxidation and enormous biological damage. The hydroxyl radical scavenging activity of *C.divaricata* extracts ranged from 39.31 to 161.26 g DMSOE/g extract. Among the four tested samples, the leaf extract showed the highest hydroxyl radical scavenging activity (161.20 g DMSOE/g extract), while the bark extract exhibited the least hydroxyl-radical scavenging activity (39.31 g DMSOE/g extract) ($p<0.05$). The hydroxyl scavenging activity of C. divaricata extract is presented in the following descending order: bark < seed < fruit < leaf ($p < 0.05$). The correlation test shows that the hydroxyl radical scavenging activity of *C.divaricata* extract is correlated with its total phenolic and total flavonoid contents, supporting the previous statement on the contribution of phenolic compounds in the antiradical activity of C. divaricata extracts. Hydroxyl radical scavengers were produced by the reaction of hydrogen peroxide (H2O2) and the ferrous that would react with 2-deoxyribose. The reaction was stopped by adding a Thiobarbituric Acid Thiobarbituric Acid that would give a red colour if the malonaldehyde was formed as the result of the reaction between the radical and 2-deoxyribose (Rahman *et al*, 2015) [14]. The Hydroxyl radical scavenging capacity of an extract is directly proportional to its antioxidant activity which is depicted by the low intensity of the red color (Gulcin *et al*, 2005) [4].

The total antioxidant activity of *C.divaricata* was estimated and found to be much higher in fruit (17.61 mg GAE/g DR) than in the other samples tested. The bark of *C.divaricata* extract had the lowest total antioxidant potential (8.36 mg GAE/g DR). Their reducing power shows that the antioxidant compounds are electron donors, they reduce the oxidized intermediate compounds in the lipids peroxidation process, and they act as primary and secondary antioxidants (Yen and Chen, 1995). The positive control of ascorbic acid showed a significantly higher antioxidant activity than the sample. These results are correlated by Rengasamy *et al*, 2010; Kumaresan *et al*, 2007 in their study on the *Enhalusa coroides* and *Phyllanthus* species respectively.

Table 2: The antioxidant activity (DPPH, Hydrogen Scccavening activity, and total antioxidant) of different parts of the C.divaricata

Sl.No.	Parts of *C.divaricata*	DPPH (µg/ml)	Total Antioxidant (mg/GAE/g extract)	Hydrogen Radical Scavening Activity (g DMSO/g Extract)
1.	Leaf	26.29± 1.21[b]	17.61± 1.12[d]	161.26± 3.57[a]
2.	Seed	10.12± 1.12[e]	15.46± 1.41[e]	59.22± 1.26[b]
3.	Fruit	19.2± 1.26[b]	12.31± 2.14[c]	92.12± 1.24[c]
4.	Bark	7.31± 2.14[a]	8.32± 1.12[b]	39.31± 1.82[d]

Values are different. The Superscript letters indicate Significant differences at $p<0.05$ (Tukey tests).

Conclusions

High antioxidant activity is observed in the leaf, fruit, seed, and bark extracts of *C.divaricata* as compared to other tested extracts. These extracts can be considered as a new potential source of natural antioxidants for food and nutraceutical products.

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