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# EVALUATION OF ANTIOXIDANT POTENTIAL OF *CIPADESSA BACCIFERA (ROTH) MIQ.*, A HERB USED BY TRIBALS OF WAYANAD

**P. N. Sajith Kumar*[1], Sr. Molly Mathew[2], T. K. Shahin Muhammed[3] and P. L. Rajagopal[4]**

[1,4]Department of Pharmacognosy and Phytochemistry, College of Pharmaceutical Sciences, Government Medical College, Kannur, Kerala, India.
[2]Former Professor and Principal, Malik Deenar College of Pharmacy, Kasaragod, Kerala, India.
[3]Department of Pharmaceutical Chemistry, College of Pharmaceutical Sciences, Government Medical College, Kannur, Kerala, India.

***Corresponding Author: P. N. Sajith Kumar**
Department of Pharmacognosy and Phytochemistry, College of Pharmaceutical Sciences, Government Medical College, Kannur, Kerala, India.

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## ABSTRACT
In the present study, investigation of antioxidant potential of the hydro-alcoholic extract of stem and leaves of *Cipadessa baccifera* (Meliaceae), was carried out. Antioxidant activity was assessed by performing DPPH free radical, hydrogen peroxide, hydroxyl, nitric oxide and superoxide free radical scavenging assays. The study demonstrates that *Cipadessa baccifera* is a good source of antioxidants which can be utilized for aiding treatments of liver disorders and several pathological conditions.

**KEYWORDS**: *Cipadessa baccifera*, CBHAE, DPPH, hydrogen peroxide radical, hydroxyl, nitric oxide, superoxide radical.

## INTRODUCTION
Abnormal levels of free radicals, due to insufficiency of the antioxidant defence system, may lead to disruption of cellular function, oxidative damages to membranes and enhance their susceptibility to lipid peroxidation.[1,2] There is an ample amount of evidence indicating the association between a diet rich in fruits and vegetables and a decreased risk of cardiovascular disease and diabetes mellitus.[3] It is generally assumed that the active principles contributing to these protective effects are nothing but primarily, the antioxidant phytochemicals. The search for new antioxidant principles is becoming, therefore, essential to improve the pharmacological treatment of liver disorders and several pathological conditions such as diabetes mellitus, rheumatic diseases, atherosclerosis and neurodegenerative conditions.[3,4] *Cipadessa baccifera* (Meliaceae), commonly known as *Pulippanchedi* is an important medicinal plant used in Kerala (India), especially by the tribals of Wayanad region and Kani tribals of Thirunelveli hills for snake bite, insect bite, scorpion bite and liver diseases.[5] Though widely used in the indigenous system of medicine, there is however no available data with relation to its antioxidant activity. The present study therefore aims to assess the antioxidant activity of hydro-alcoholic extract of *Cipadessa baccifera* using *in vitro* methods.

## Experimental
### DPPH radical scavenging assay
Different concentrations of the hydro-alcoholic extract of the stem and leaves of *Cipadessa baccifera* (CBHAE) such as 12.5µg/mL- 200µg/mL from stock solution were made up to a final volume of 20µl with DMSO, and 1.48ml DPPH (0.1mM) solution was incorporated. The reaction mixture incubated in dark room at room temperature for 20 minutes. Then the absorbance of the mixture was noted at 517nm. 3ml of DPPH was taken as control.[6,7,8]

### Hydrogen peroxide scavenging assay
A solution of H2O2 (40mM) was prepared in phosphate buffer (pH 7.4). Different concentration of CBHAE such as 125 - 2000µg/mL from a stock concentration of 10mg/mL was added to H2O2 solution (0.6 ml). OD was read at 230nm at 0 minutes and after 10 minutes of incubation. Optical density was noted. Ascorbic acid (10mg/mL) was used as standard.[9]

### Hydroxyl radical scavenging activity
Different concentration of CBHAE such as 125-2000µg/mL from a stock concentration of 10mg/mL were mixed with 500µl reaction mixture ((2 deoxy 2 ribose (2.8mM), FeCl3 (100µm), EDTA (100µm), H2O2 (1.0mM), ascorbic acid (100µm) in KH2PO4 - KOH buffer (20 mM pH 7.4) was made up to a final volume of 1 ml. After incubation for one hour period at 37°C,

added 1ml of 2.8% TCA, then 1ml 1% aqueous TBA was also added, and the mixture was incubated at 90°C for 15 minutes to develop the colour. After cooling the absorbance was noted at 532nm against an appropriate blank solution.[8]

**Nitric oxide scavenging activity**

Sodium nitroprusside (5mmolL-1) in phosphate-buffered saline pH 7.4, was mixed with different concentration of CBHAE such as 125µg/mL -2000µg/mL from a stock concentration of 10mg/mL and incubated at 25°C for 30minutes. After 30minutes, 1.5mL of the incubated solution was removed and diluted with 1.5mL of Griess reagent. The absorbance was measured at 546nm, and the percentage scavenging activity was measured with reference to the standard.[10,11]

**Superoxide free radical scavenging activity**

Different concentration of CBHAE such as 125 - 2000µg/ml from a stock solution of 10mg/ml, 0.05ml of Riboflavin solution (0.12mM), 0.2 ml of EDTA solution [0.1M], and 0.1 ml NBT (Nitro-blue tetrazolium) solution [1.5mM] were properly mixed in test tube and reaction mixture was diluted up to 2.64ml with phosphate buffer [0.067M]. The absorbance of the solution was noted at 560nm after illumination for 5 minutes incubation in fluorescent light and also measured after illumination for 30 min. at 560 nm on UV visible spectrophotometer.[12,13]

**RESULTS AND DISCUSSION**

1, 1-diphenyl-2-picryl hydrazyl is a type of stable free radical with pink colour which changes to yellow when scavenged. The DPPH assay utilizes this character to demonstrate free radical scavenging activity. Antioxidants readily react with DPPH and reduce it to DPPH-H and as an outcome the absorbance decreases.[14,15] The extent of discoloration denotes the scavenging potential of the antioxidant compounds or extracts with regard to hydrogen donating ability. The effect of the hydro-alcoholic extract of stem and leaves of *Cipadessa baccifera* (CBHAE) on scavenging of DPPH free radical are depicted in table 1 and figure 1 which is comparable with standard antioxidant compound ascorbic acid. From the figure 1 it can be concluded that the scavenging activity of CBHAE on DPPH radicals were outstanding. The IC50 value of CBHAE was also found to be significant (table 6). The hydro alcoholic extract of *Cipadessa baccifera* demonstrated a maximum DPPH scavenging activity of 88.68% at 200 µg/ml whereas for the standard compound ascorbic acid was turned out to be 95.07% at 200 µg/ml. The IC50 of the CBHAE and ascorbic acid were calculated to be 45.03 µg/ml and 17.63µg/ml respectively.

Due to its strong oxidizing properties, hydrogen peroxide is regarded as a potent oxidizer of biomolecules. It readily crosses physiological membranes and cause slow oxidization of number of compounds. Table 2 and figure 2 demonstrates hydrogen peroxide scavenging property of the CBHAE and Antioxidant reference standard. CBHAE exhibited a moderate dose-dependent scavenging of hydrogen peroxide radical. The highest scavenging effect of CBHAE and ascorbic acid was exerted at a concentration level of 2000 µg/ml with a percentage inhibition of 54.29 and 95.97 respectively. The scavenging ability of CBHAE was comparable to the reference antioxidant compound. The $IC_{50}$ values were recorded to be 1541.01 µg/ml and 259.32 µg/ml respectively (table 6), for the test extract and standard ascorbic acid.

The principle behind hydroxyl radical scavenging assay in accordance with the qualification of the degradation product of 2 deoxy ribose by condensation with TBA. Hydroxyl radical was generated by the $Fe^{3+}$ - ascorbate-EDTA $–H_2O_2$ system (The Fenton reaction).[14,15] Gallic acid (10mg/mL DMSO) was used as reference. The hydro alcoholic extract of *Cipadessa baccifera* (CBHAE) exhibited a maximum hydroxyl radical scavenging activity of 57.4% at 2000 µg/ml whereas the standard compound gallic acid exhibited maximum activity of 93.93% at 2000 µg/ml. The results were depicted on table 3 and figure 3. The IC50 of the CBHAE and the standard were calculated to be 1392.7 µg/ml and 217.296µg/ml accordingly (table 6).

Sodium nitro prusside in water-based solution at physiological pH instantaneously yield nitric oxide which engage with oxygen to generate nitrite ions that can be assessed using Griess reagent.[14] Scavengers of nitric oxide emulate with oxygen, leading to minimal production of nitrite ions. Nitrite radical scavenging assay was performed on CBHAE selecting a concentration range of of 125 to 2000 µg/mL. Percentage scavenging of free radical was charted against various concentration of the extract as pictured in Figure 4. The data were also depicted in table 4. CBHAE exhibited antioxidant activity by contending with oxygen for the scavenging of nitrite radical. The antioxidant activity improved sharply with a rise in extract concentration. CBHAE had a highest scavenging activity at 2000 µg/mL which was comparable with reference antioxidant standard, Gallic acid. The IC50 value of CBHAE and gallic acid was counted as 1453.1µg/ml and 243.622µg/ml respectively (table 6).

Super oxide is biologically significant as it can produce singlet oxygen and hydroxyl radical as well. Excessive formation of super oxide anion radical forward to redox imbalance and associated with harmful physiological effects. Super oxide anion are produced in riboflavin-NADH system through the oxidation of NADH and assayed on the basis of the reduction of NBT lead to the formation of blue coloured formazan product.[14,15] The scavenging of superoxide anion was investigated at various concentrations of hydro alcoholic extract of *Cipadessa baccifera* (125, 250, 500, 1000 and 2000 µg/ml). The data were represented in table 5 and figure

5. The scavenging of superoxide free radical rose sharply with the graded concentration of CBHAE. The maximum scavenging activity of CBHAE and ascorbic acid at highest concentration was turned out to be 67.57% and 93.22% accordingly. The IC50 value of CBHAE and ascorbic acid was noted as 1017.2µg/ml and 214.62µg/ml respectively (table 6).

**Table 1**

| Sl.No. | Conc. µg/ml | CBHAE extract | | Ascorbic acid | |
|---|---|---|---|---|---|
| | | Abs | % Sca. | Abs | % Sca. |
| 1 | control | 0.5087 | 0.00 | 0.4972 | 0.00 |
| 2 | 12.5 | 0.4084 | 19.72 | 0.2849 | 42.70 |
| 3 | 25 | 0.2995 | 41.12 | 0.1966 | 60.46 |
| 4 | 50 | 0.1489 | 70.73 | 0.1584 | 68.14 |
| 5 | 100 | 0.0984 | 80.66 | 0.0952 | 80.85 |
| 6 | 200 | 0.0576 | 88.68 | 0.0245 | 95.07 |

**Table 2**

| Sl.No. | Conc. µg/ml | CBHAE extract | | Ascorbic acid | |
|---|---|---|---|---|---|
| | | Abs | % Sca. | Abs | % Sca. |
| 1 | control | 0.0350 | 0.00 | 0.0347 | 0.00 |
| 2 | 125 | 0.0321 | 8.29 | 0.0267 | 23.05 |
| 3 | 250 | 0.0285 | 18.57 | 0.0180 | 48.13 |
| 4 | 500 | 0.0227 | 35.14 | 0.0132 | 61.96 |
| 5 | 1000 | 0.0184 | 47.43 | 0.0075 | 78.39 |
| 6 | 2000 | 0.0160 | 54.29 | 0.0014 | 95.97 |

**Table 3**

| Sl.No. | Conc. µg/ml | CBHAE extract | | Gallic acid | |
|---|---|---|---|---|---|
| | | Abs | % Sca. | Abs | % Sca. |
| 1 | control | 0.0847 | 0.00 | 0.0860 | 0.00 |
| 2 | 125 | 0.0724 | 14.52 | 0.0598 | 30.47 |
| 3 | 250 | 0.0684 | 19.24 | 0.0421 | 51.05 |
| 4 | 500 | 0.0601 | 29.04 | 0.0227 | 73.60 |
| 5 | 1000 | 0.0484 | 42.86 | 0.0147 | 82.91 |
| 6 | 2000 | 0.0329 | 61.16 | 0.0048 | 94.42 |

**Table 4**

| Sl.No. | Conc. µg/ml | CBHAE extract | | Gallic acid | |
|---|---|---|---|---|---|
| | | Abs | % Sca. | Abs | % Sca. |
| 1 | control | 0.3582 | 0.00 | 0.3462 | 0.00 |
| 2 | 125 | 0.3012 | 15.91 | 0.2462 | 28.89 |
| 3 | 250 | 0.2647 | 26.10 | 0.1472 | 57.48 |
| 4 | 500 | 0.2117 | 40.90 | 0.0855 | 75.30 |
| 5 | 1000 | 0.1840 | 48.63 | 0.0482 | 86.08 |
| 6 | 2000 | 0.1526 | 57.40 | 0.0210 | 93.93 |

**Table 5**

| S.No. | Conc. µg/ml | CBHAE extract | | Ascorbic acid | |
|---|---|---|---|---|---|
| | | Abs | % Sca. | Abs | % Sca. |
| 1 | control | 0.1252 | 0.00 | 0.1268 | 0.00 |
| 2 | 125 | 0.0987 | 21.17 | 0.0872 | 31.23 |
| 3 | 250 | 0.0890 | 28.91 | 0.0540 | 57.41 |
| 4 | 500 | 0.0681 | 45.61 | 0.0351 | 72.32 |
| 5 | 1000 | 0.0521 | 58.39 | 0.0207 | 83.68 |
| 6 | 2000 | 0.0406 | 67.57 | 0.0086 | 93.22 |

**Table 6**

| Sl.No | Free radical | IC50 value (µg/mL) | | |
|---|---|---|---|---|
| | | CBHAE (test Extract) | Ascorbic acid (standard) | Gallic acid (standard) |
| 1 | DDPH radical | 45.0376 | 17.638 | Not employed |
| 2 | Hydrogen peroxide radical | 1541.01 | 259.32 | Not employed |
| 3 | Hydroxyl radical | 1392.7 | Not employed | 217.296 |
| 4 | Nitric oxide radical | 1453.1 | Not employed | 243.622 |
| 5 | Superoxide radical | 1017.2 | 214.62 | Not employed |

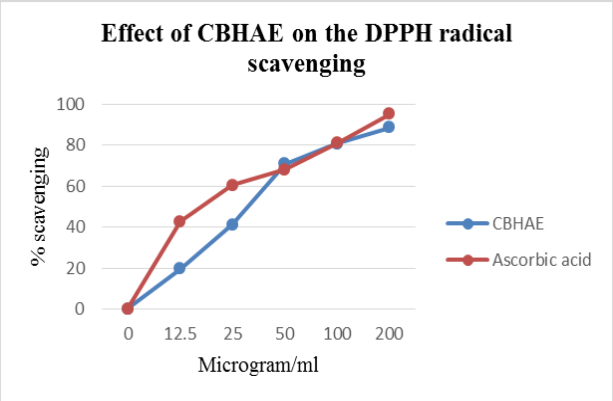


**Figure 1**

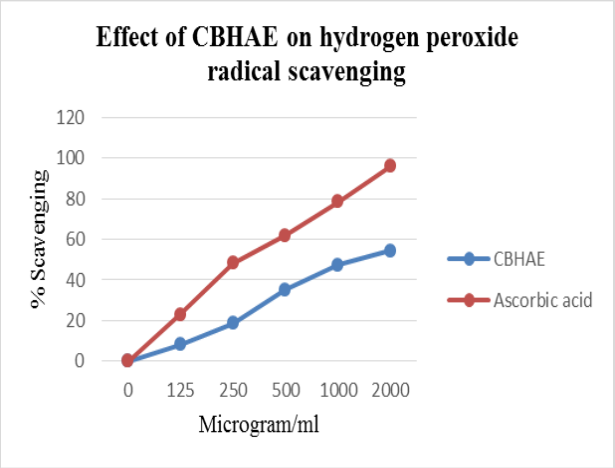


**Figure 2**

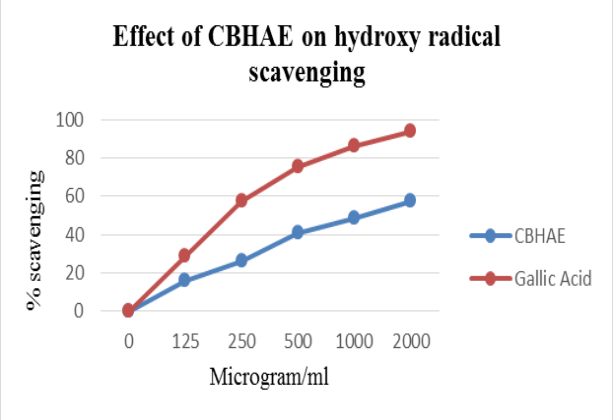


**Figure 3**

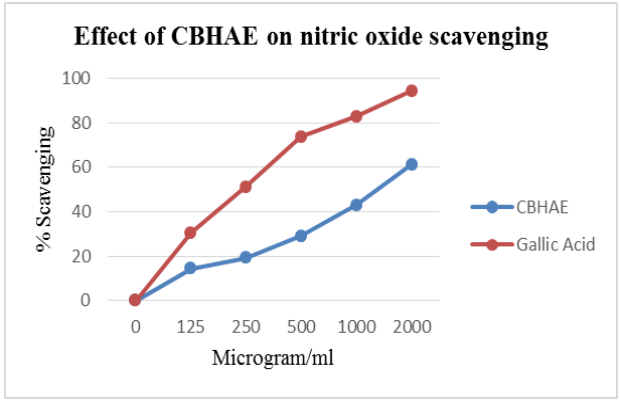


**Figure 4**

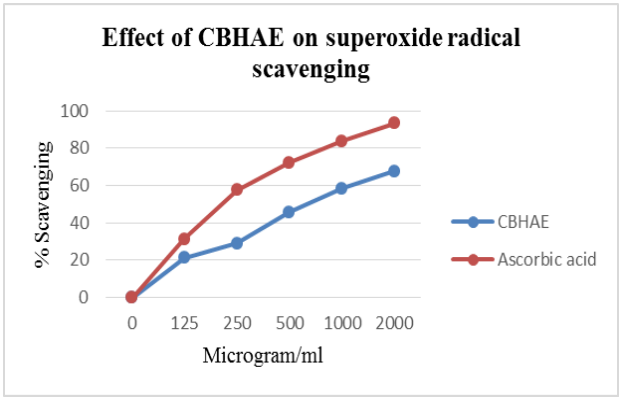


**Figure 5**

## CONCLUSION

From the results acquired in the present study, it has come to the conclusion that the hydro alcoholic extract of the stem and leaves of *Cipadessa baccifera* may contain considerable amounts of poly phenolic compounds, which in turn demonstrate marked antioxidant and free radical scavenging properties. The experiments performed with *in vitro* free radical scavenging assays denote that CBHAE is a reliable source of antioxidant phytoconstituents, which may be advantageous in the prevention of various oxidative stress related disease conditions. Therefore, further research to be conducted to identify and isolate the antioxidant phytoconstituents exist in the plant extract.

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