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Research Article

In- Vitro Studies on the Antioxidant Activity and Pharmacological Properties of *Ciassampelos Pariera* L.

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Abstract: The current investigation stated that the antioxidant properties of *Cissampelos pareira* L. leaf extract of medicinal plants are a rich resource of ingredients that can be used in drug development for human life. The medicinal plant *Cissampelos pareira* with different solvents such as aqueous, acetone, ethanol, and methanol performed different concentrations like 100, 200, 300, 400, and 500µg/ml were carried out by three methods such as hydrogen peroxide scavenging (H_2O_2), DPPH and thiobarbituric acid assay. As per the antioxidant properties of *C. pareira* an aqueous extract of hydrogen peroxide scavenging (H_2O_2) showed the highest percentage of activity compared to the other methods of antioxidant. The minimum rate of antioxidant properties of DPPH in the *C. pareira* with 500 µg/ml in high activities than the low concentration. According to the acetone extracts of *C. pareira* leaf, the maximum percentage of inhibition in hydrogen peroxide scavenging (H_2O_2) whereas low percentages of inhibition by thiobarbituric acid activity at 500 µg/ml concentration, respectively. Although the extraction solvent ethanol reaction is a concern, the same trend of results of antioxidant properties of hydrogen peroxide scavenging (H_2O_2) assay showed excellent properties and minimum in thiobarbituric acid was found to be recorded with a maximum concentration of 500 µg/ml respectively. According to the methanolic extract of *C. pareira* leaf exhibited maximum antioxidant properties at 500µg/ml concentration and minimum percentage of activity at thiobarbituric acid, activity was found to be recorded respectively. Among the three methods, hydrogen peroxide scavenging (H_2O_2) showed excellent antioxidant activity compared with other methods and ascorbic acid as a standard. Antioxidant activities play a significant role in pharmacological functions because of bioactive ingredients. However, there are some health problems due to the deficiencies of macronutrients that establish an effective manner. This research article describes the current status of the world's herbal medicinal products and properties suggestions for facilitating *C. pareira* was better tomorrow in humanity.

Keywords: *Cissampelos pariera,* leaves extract, different solvent, antioxidant, ascorbic acid.

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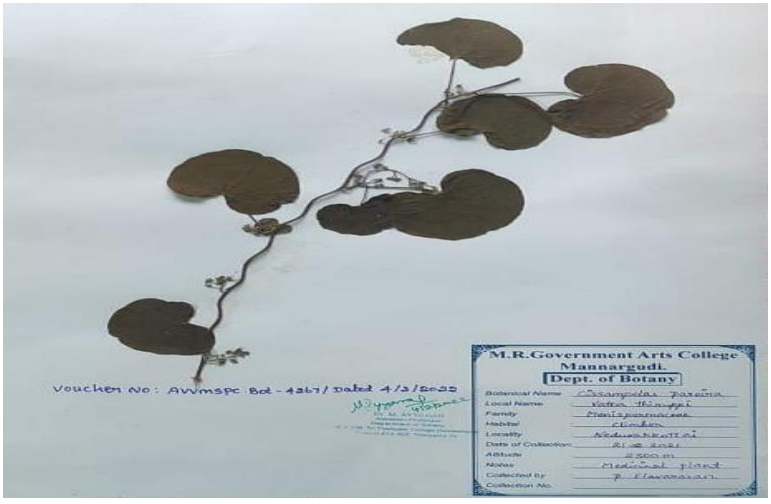
1. INTRODUCTION

The term medicinal plant includes various types of plants used in herbalism. It is the uses of plants for medicinal purposes and the study of such benefits of medicinal purposes long before the prehistory period. The traditional system of medicine continues to be widely practised in many historical events. Population rise in a degraded supply of drugs, the prohibitive cost of treatments, side effects of several synthetic drugs, and the development of resistance of currently used drugs for infectious diseases have led to increased emphasis on the uses of plant materials as a source of medicines for a wide variety of human ailments. The importance of plants is known to us well. Therefore, the plants selected for medicinal use over thousands of years constitute the most obvious choice of examination. The current search for therapeutically effective new drugs such as anticancer, antimicrobial, and antihepatotoxic compounds[1]. These antioxidants protect against damage caused by free radicals played essential roles in developing many chronic diseases, including cardiovascular diseases, ageing, heart disease, anaemia, cancer, and inflammation[2]. Antioxidants are compounds that neutralize free radicals and reactive oxygen species (ROS) in the cell[3]. Antioxidants are compounds that neutralize free radicals and reactive oxygen species (ROS) in the cell[3]. Antioxidant activity in food and beverages has become one of the most exciting features in the science community. Natural antioxidants are generally derived from plant sources, and their activities vary depending on the plant's species, diversity, extracts and processing methods, and growing conditions. These biological properties explored in macro molecules claimed this potential application in drug development[4]. Furthermore, antioxidant peptides are given significant attention in the products due to their ability to remove ROS and force radicals to damage skin cells[5]. Since many relatively simple bioassays are readily available for assessment of antioxidant activities, many plant bioactive compounds and their extracts have been shown to act as antioxidants by the in vitro method. This activity will be expected to protect against all unwanted problems in the human body. Almost all organisms are protected, up to some extent, by free radical damage with the help of enzymes and various methods. Presently, much attention has been focused on the case of natural antioxidants to protect the human body, especially brain tissues, from the oxidation damage caused by free radicals. In the present investigation, different methods were performed for the antioxidant properties of *C. pareira*, and promising candidature for antioxidant activities showed extraordinary life in the behaviour of the human world.

2. MATERIALS AND METHODS

2.1 Collection of plant

The fresh, healthy plant materials of *C. pareira* L. var. hirsuta (Buch.-Ham. ex D.C.) Forman, plant collected from the Mannargudi regions of Thiruvarur Dt, Tamilnadu, India and Voucher no: Avvmspc. Bot-4267/Dated/04/02/2022.



2.2 Preparation of dried leaf

The leaves were thoroughly washed under running tap water, then with distilled water, and shade dried at room temperature, which removed the moisture completely. The dried leaves are then homogenized into a fine powder using a mixer grinder and stored in airtight containers for further study.

2.3 Solvent extraction preparation

Ten grams of the dried powder of leaves from *Cissampelos pareira* were taken separately in labelled, airtight bottles, and 50 ml of solvents such as acetone, ethanol, methanol and aqueous were individually added and prepared.

2.4 Scavenging of H_2O_2

Solution[6] of 0.2M potassium dihydrogen phosphate and 0.2M sodium hydroxide solutions were prepared per the Indian Pharmacopoeia 1996 standards. 50 ml potassium dihydrogen phosphate solution was placed in a 200 ml volumetric flask, and 39.1 ml of 0.2M sodium hydroxide solution was added.Finally, the volume was up to 200ml with distilled water to prepare phosphate buffer (pH-7.4). 50 ml phosphate buffer solution was added to an equal amount of hydrogen peroxide and generated free radicals. The solution was kept at room temperature for 5min to complete the reaction. Extracts (1 ml) in distilled water were added to 0.6 ml hydrogen peroxide solution. The absorbance was measured at 230 nm in a spectrophotometer against a blank solution containing phosphate buffer solution without hydrogen peroxide. The percentage of scavenging of H_2O_2 of the extract was measured. The ability to scavenge the H_2O_2 radical was calculated using the following equation:

$$H_2O_2 \text{ scavenging activity (\%)} = (A0 - A1)\ /A0 \times 100$$

Where A0 is the absorbance of the control and A1 is the absorbance in the presence of an extract sample. A standard of ascorbic acid was run using the same concentrations as that of extract. The antioxidant activity of the model was expressed as the sample's concentration (mg/ml) that inhibited the formation of H_2O_2 radicals by 50%.

2.5 *DPPH assay*

The antioxidant[7] activity of the *Cissampelos pareira* based on the scavenging movement of the stable 2, 2- diphenyl-2-picrylhydrazyl (DPPH) free radical was determined according to the method described with slight modification. The extraction solvents, such as aqueous and methanol extract, were prepared in 100, 200, 300, 400 and 500 µg/mL. Five ml of each solution was prepared, and the concentration was mixed with 0.5 mL of 1 ml DPPH solution. The experiment was done in triplicate. The test tubes were incubated for 30 min at room temperature, and the absorbance was measured at 517nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Vitamin C (0.1 mg/ml) was used as a standard, and the same concentrations were prepared as the test solutions. The difference in absorbance between the test and the control (DPPH in methanol) was calculated and expressed as % scavenging of DPPH radical.

$$\text{\% Scavenged [DPPH]} = [(AC - AS)/AC] \times 100.$$

2.6 *Thiobarbituric acid (TBA)*

Preparation of TBA[8] Reagent. The standard solution of 4.0 mm TBA was prepared in glacial acetic acid. For this purpose, 57.66 mg of TBA was dissolved in 100 mL of glacial acetic acid. Samples of *C.pareira* plant leaf extract with 100% glacial acetic acid (A.A.) and 50% glacial acetic acid with water (A.W.). The extract of the leaf sample (1 mL) was mixed with 1 mL TBA reagent, and the above procedure was repeated five times (nn = 5). The TBARS was calculated using the formula as μM/g of the sample:

$$\text{TBARS } (\mu\mu\text{M/g}) = (\text{Ac} \times VV)\ /WW \quad , (1)$$

Where Ac is the amount determined from the calibration curve and WW is the weight of the sample taken, while VV is the volume in mL or dilution factor of the total leaf extract prepared.

3. STATISTICAL ANALYSIS

Experiments were carried out in triplicate, and the results are expressed as mean values with standard deviation.

4. RESULTS

The present investigation suggested the antioxidant activity of acetone, aqueous, ethanol and methanol in the leaf of *C. pareira.* The antioxidant activity was evaluated using the hydrogen peroxide (H_2O_2) radical scavenging activity assay, the DPPH radical scavenging activity assay, and the thiobarbituric acid (TBA) assay. Several methods were used for the analysis of the antioxidant compounds. The result obtained by H_2O_2 hydrogen peroxide radical scavenging activity recognized that the aqueous leaf extract showed maximum radical scavenging potential at 42µg/ml and the lowest value of 17µg/ml in the acetone extract. According to the results established in the investigation, it was found that all of the test plant extracts showed notable scavenging activities against the DPPH model in a concentration-dependent manner. The higher the concentration, the higher the scavenging potential (Figure 1-4). The leaf has a methanol extract value of 37µg/ml and the lowest value of 19 µg/ml in acetone. The TBA assay was carried out from the leaf's aqueous, acetone, ethanol, and methanol extracts. The value of TBA aqueous extracts of the leaf is 35µg/ml, with the lowest value of 20µg/ml in an aqueous extract.

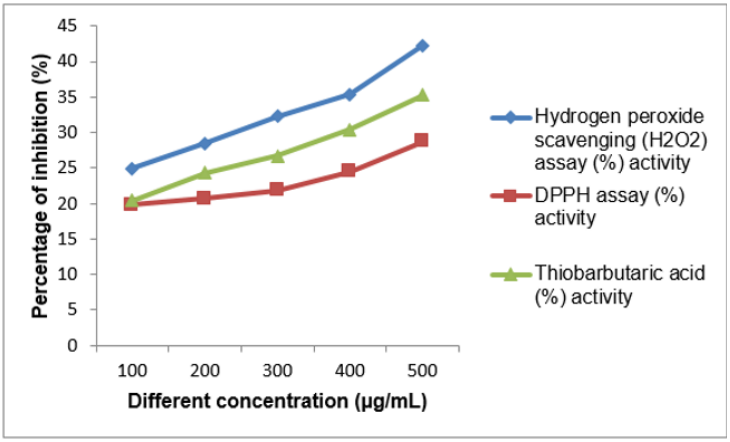


Fig 1: Analysis of Antioxidant activity of *Cissampelos pareira* L. leaf with aqueous extract by various methods

The different concentrations of *Cissampelos pariera* Leaf extracts contain other solvents and various methods of detecting antioxidant activity, such as the hydrogen peroxide scavenging assay, the DPPH assay and the thiobarbituric acid assay. An antioxidant activity of *C.pariera* leaves performed with an aqueous solvent in the hydrogen peroxide scavenging assay showed 100µg/ml in 24%, 200µg/ml in 28%, 300µg/ml in 32%, 400µg/ml in 35% and 500µg/ml in 42%. DPPH antioxidant activity showed 100µg/ml in 19%, 200µg/ml in 20%, 300µg/ml in 21%, 400µg/ml in 24% and 500µg/ml in 28% and the thiobarbituric acid assay in this assay also 100µg/ml in 20%, 200µg/ml in 24%, 300µg/ml in 26%, 400µg/ml in 30% and 500µg/ml in 35% were recorded respectively. The highest inhibition of antioxidant activity in an aqueous solution is the H_2O_2 assay. (Fig -1).

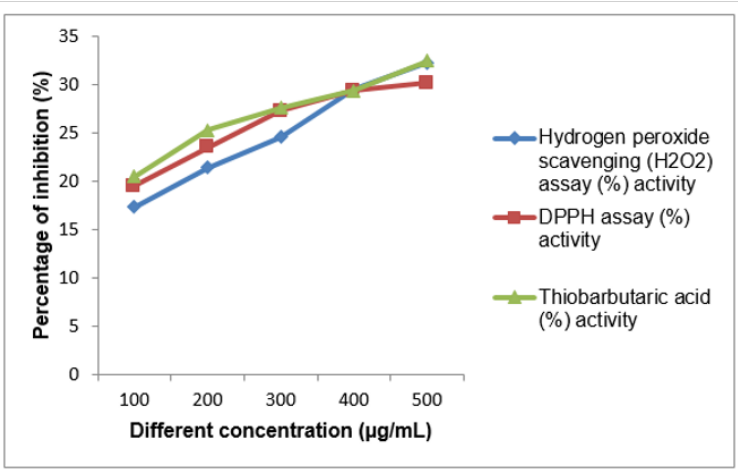


Fig 2: Analysis of the antioxidant activity of *Cissampelos pareira* L. leaf with acetone extract by various methods

In an acetone extract of *C.pariera* leaves, a hydrogen peroxide scavenging assay showed 100µg/ml in 17%, 200µg/ml in 21%, 300µg/ml in 24%, 400µg/ml in 29% and 500µg/ml in 32%. On the other hand, DPPH antioxidant activity showed 100µg/ml in 19%, 200µg/ml in 23%, 300µg/ml in 27%, 400µg/ml in 29% and 500µg/ml in 30%, and thiobarbituric acid assay performed showed 100µg/ml in 20%, 200µg/ml in 25%, 300µg/ml in 27%, 400µg/ml in 29%. As compared with the other assays, thiobarbituric acid shows the highest inhibition of antioxidant activity in acetone solvent (Fig -2).

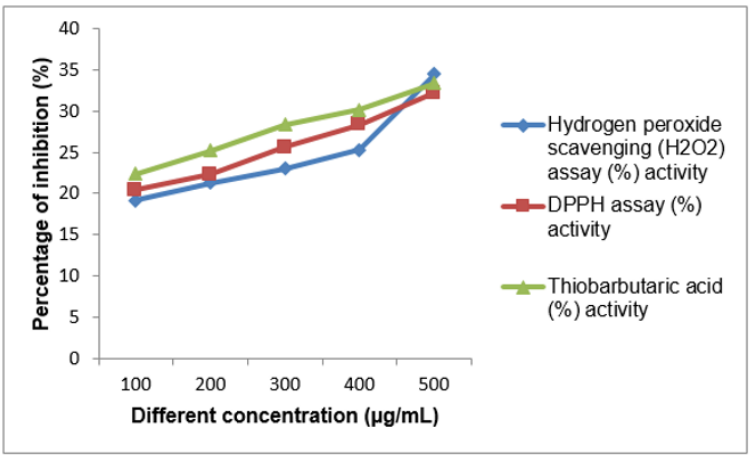


Fig 3: Analysis of the antioxidant activity of *C. pareira* L. leaf with ethanol extract by various methods

An ethanol extract of the leaves of *C.pariera* was performed in a hydrogen peroxide scavenging assay, which showed 100µg/ml in 19%, 200µg/ml in 21%, 300µg/ml in 23%, 400µg/ml in 25% and 500µg/ml in 34%. DPPH antioxidant activity showed 100µg/ml in 20%, 200µg/ml in 22%, 300µg/ml in 24%, 400µg/ml in 28% and 500µg/ml in 32%. The thiobarbituric acid assay performed showed 100µg/ml in 22%, 200µg/ml in 25%, 300µg/ml in 28%, 400µg/ml in 30% and 500µg/ml in 33%, respectively. As with the other assays, H_2O_2 exhibits the highest inhibition of antioxidant activity in acetone solvent (Fig -3).

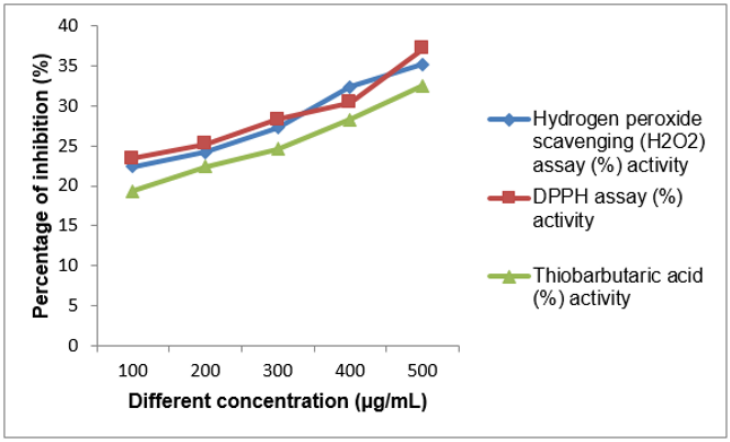


Fig 4: Analysis of the antioxidant activity of *Cissampelos pareira* L. leaf with methanol extract by various methods

Methanol solvent of leaf extract *C.pariera* was expressed following antioxidant activity such as hydrogen peroxide scavenging assay, which showed 100µg/ml in 22%, 200µg/ml in 24%, 300µg/ml in 27%, 400µg/ml in 32% and 500µg/ml in 35%. DPPH antioxidant activity showed 100µg/ml at 23%, 200µg/ml at 25%, 300µg/ml at 28%, 400µg/ml at 30%, and 500µg/ml at 37% and thiobarbituric acid assay performed showed 100µg/ml in 19%, 200µg/ml in 22%, 300µg/ml in 24%,400µg/ml in 28% and 500µg/ml in 32% were recorded respectively. Compared with the other assays, the highest inhibition of antioxidant activity in acetone solvent is DPPH (Fig -4).

5. DISCUSSION

The methanolic extracts of *C. pareira* leaf exhibited the highest free radical scavenging activity[9]. The antioxidant assays were carried out from the methanolic extracts of the leaf, stem, root and callus[10]. The value of DPPH (32.5±0.62µg/ml) and ABTS (55.17 µg/ml) radical activity was found to be maximum in the methanolic extract of the stem compared to other extracts. The extracts exhibited concentration-dependent hydrogen peroxide scavenging activities. The scavenging efficiency increased: CPW < CPA < CPH < CPE. CPE was most efficient with the lowest IC_{50} value, 75.7±1.63 µg/ml.[11] Therefore, *C. pareira* extracts quench hydrogen peroxide, which can be due to the existence of phenolic groups that exhibited proton donating ability, thereby neutralizing it into water. The CPE scavenged as much as 80.7% of DPPH radicals at 50 µg/mL concentration.[12] The antioxidant activities of H_2O_2, DPPH, and TBA assays leaf of *C. pareira* were maximum and performed aqueous extract compared to other extracts.Screened the alkaloid fraction of *Cissampelos pareira* roots for antioxidant activity.[13] The strong antioxidant activity expressed ability to DPPH, Superoxide ion and lipid peroxidation in rat liver. The total phenol antioxidant assay was more effective than the DPPH and H_2O_2 antioxidant with hexane, ethyl acetate and ethanol leaf extract of *C.pariera.* The DPPH assay was found high in *C.pariera* leaves, and the antioxidant activity of leaves was poor compared with standard ascorbic acid.[14]The polyphenol extract of *Cissampelos pariera* expressed high antioxidant activity in ABTS followed by lipid peroxidation inhibition and superoxide scavenging. The flavonoid and phenol content was responsible for the antioxidant activity.[15] The alkaloid fraction of *C. pareira* roots demonstrated potent antioxidant activity by scavenging superoxide ion, the stable free radical DPPH, and inhibiting lipid peroxidation in rat liver homogenate induced by iron/ ADP/ ascorbate complex.It gets reduced in the presence of antioxidants in the sample, which is considered a measure of their antioxidant activity. The results demonstrate the in vitro antioxidant activity of ethyl acetate, ethanol and hexane leaf extract of *Cissampelos pareira*. The result revealed good antioxidant activity and was comparable with that of standard ascorbic acid. The higher concentration of the ethyl acetate extract exhibits more antioxidant activity compared to lower concentrations. The data suggests that the extract contains compounds effectively utilized as a wide spectrum of antioxidant agents.The extracts demonstrated hydrogen peroxide (H_2O_2) radical scavenging activity in a dose-dependent manner. The highest scavenging activity was noticed in the ethyl acetate extract at 100 µg/ml (82.1 ± 1.76%). Low activity was noted in hexane extract at 100 µg/ml (43.6 ± 1.23%). While other concentrations showed moderate scavenging activity. The standard ascorbic acid showed maximum activity at 100 µg/ml concentration (94.7 ± 1.18%). Similar dose-dependent scavenging was observed for standard ascorbic acid. The IC_{50} values of ethyl acetate extract (46.19%) and ascorbic acid (34.67%) were obtained using the linear regression equation.[16]In conclusion, and the present study demonstrates that the alcoholic extract of *C pareira* possesses potent free radical scavenging, antioxidant, and gastroprotective activities. The extract can protect against oxidative damage to lipids and proteins and maintain the levels of antioxidant molecules and enzymes in vivo. The preliminary studies in the present investigation depicted CPE to contain a huge amount of polyphenolics, to which its antioxidant activity may be ascribed. Further investigations on isolation and characterization of the active compounds responsible for the antioxidant capacity of *C pareira* roots are underway in our laboratory.[17]The per cent DPPH free radical scavenging potential of ethanolic leaf and stem extract of *C. pareira* was evaluated, and results obtained were compared with BHT as a standard. The ethanolic extract of the stem of *C. pareira* showed the highest antioxidant potential of 1.03±0.19 µg/ml to neutralize DPPH radicals. At the same time, minimum capacity was observed for the ethanolic extract of leaves, i.e. 0.95±0.17 µg/ml.[12]The antioxidant assays were carried out from the methanolic extracts of leaf, stem, root and callus. The IC_{50} value of DPPH (32.5±0.62 µg/ml) and ABTS (55.0±0.17 µg/ml) radical activity was found to be maximum in the methanolic extract of stem compared to other extracts.[18] Percentage radical scavenging activity with IC_{50} value of different samples in ethanolic and aqueous solvent. Among the ethanolic extract of different plant samples, the DPPH radical

scavenging activity of *Fraxinus Floribunda* (IC_{50}: 5.4 µg/ml), *Viscum album* (IC_{50}:6.47µg/ml) and *Periploca calophylla* (IC_{50}: 11.69µg/ml) were found to be significant whereas *Abelmoschus esculentus*(IC_{50} value >100 µg/ml) possessed deficient DPPH radical scavenging activity concerning ascorbic acid standard (IC_{50}: 4.06 µg/ml).the current study, *C. pareira*, showed good antioxidant activity compared to ascorbic acid and ferric thocayante. The aqueous extract of *C. pareira* leaf showed significant antioxidant activity in the following order: Hydrogen peroxide scavenging (H_2O_2) assay, thiobarbituric acid, and DPPH assay (42.3±0.04, 35.3±0.03, and 28.7±0.05%).The plant extracts of *C. pareira* exhibited strong Hydrogen peroxide scavenging (H_2O_2) activity compared to the standard acarbose.

6. CONCLUSION

Although *Cissampelos pareira* belongs to the Menispermaceae family known for its use in folk medicine, there are few reports in the literature regarding the species. This study suggested the presence of secondary metabolites, which is probably responsible for the observed antioxidant activity of polar extracts. The current study reveals that *C. pareira* aqueous, acetone ethanol and methanolic extracts have potential antioxidants of free radical-scavenging activities (H_2O_2) assay compared to the other method by *invitro* tests showed that plant extracts. Most of the therapeutic characteristics of *C. pareira* have been supported by rigorous laboratory experiments and scientific studies, but many of these uses are traditional and anecdotal. Sources of naturally occurring antioxidants that may perform as a defence against oxidative stress. Traditional benefits of plant species for a variety of illnesses may point to a high amount of antioxidant activity. In antiquity, it was used to treat many diseases, including ulcers, wounds, rheumatism, fever, asthma, cholera, diarrhoea, inflammation, snakebites, malaria and rabies. It was also recommended for blood purification. Pre-clinical and clinical experiments used to study the pharmacology of *C. pareira* need to receive more attention in future research. Furthermore, before therapeutic usage, scientific confirmation of the traditional knowledge of *C. pareira* is essential to guaranteeing its safety, efficacy and mechanism of action.

7. ACKNOWLEDGEMENTS

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8. AUTHORS CONTRIBUTION STATEMENT

Elavarasan, P conceived of the presented idea. Muruganantham, A. developed the theory and performed the computations. Ambikapathy, V and Panneerselvam, manuscript with support written by authors. Babu, S. verified the analytical methods. Kanmani, A and Prakash, P supervise the project work. All authors discussed the results and contributed to the final manuscript.

9. CONFLICT OF INTEREST

Conflict of interest declared none.

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