Antioxidant Activity Of Centella asiatica Leaves By DPPH Radical Scavenging Method

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Abstract:
The ***DPPH (1, 1diphenyl-2-picryl hydrazyl)*** radical scavenging technique was used in this work to measure the antioxidant activity of several ***Centella asiatica*** ethanolic leaves extracts. Plant species that shown that, when compared to the reference standard ascorbic acid, an alcoholic ***(prepared in 70% ethanolic solution)*** extract of the plant's leaves at higher concentrations had more antioxidant capacity. They showed significant ***DPPH*** radical scavenging antioxidant activity. Ascorbic acid and alcoholic leaves extract were found to have absorbances for reducing power of ***0.0350*** and ***0.0580***, respectively. Because ***flavonoids*** and ***phenols*** are present, ethanol extract may have the greatest antioxidant action.

Keywords: 1***, 1diphenyl-2-picryl hydrazyl***, ***Centella asiatica,*** antioxidant, phenols etc.

INTRODUCTION:

With the help of substances like ascorbic acid, tocopherol, and glutathione as well as enzymes like ***superoxide dismutase (SOD)*** and ***catalase***, all human cells are able to defend themselves against the harm caused by free radicals.[1] Antioxidant supplements are essential to prevent oxidative damage since various disease processes might occasionally compromise these defence systems. The creation of ethnomedicines with potent antioxidant activities but minimal cytotoxicity has recently received a lot of interest.[2] The majority of the antioxidant chemicals found in a regular diet are sourced from plant sources and belong to several groups of substances with a wide range of physical and chemical characteristics.[3] The use of the free radical ***1, 1-Diphenyl-2-picrylhydrazyl (DPPH)***, which is frequently used to test a compound's ability to act as a free radical scavenger or hydrogen donor and to evaluate antioxidant activity, is a quick, easy, and affordable way to measure the antioxidant capacity of food.[4] The reduction of ***DPPH***, a stable free radical, is the foundation of the ***DPPH*** test technique.[5] The highest absorption of the free radical ***DPPH*** with an odd electron occurs at ***517 nm (purple colour)***. In the presence of a hydrogen donor, the stable free radical ***DPPH***, which is reactive with antioxidants, pairs off and is reduced to the ***DPPHH*** (When compared to the ***DPPH-H*** form, radicals cause decolorization from ***purple*** to ***yellow colour***), depending on how many electrons are caught, which has a lower absorbance than ***DPPH.***[6] A ***70%*** ethanolic leaves extract of ***Centella asiatica*** was used in our experiment to assess the antioxidant capacity or the free radical scavenging activities.

MATERIALS AND METHODS:

Plant Material-

Centella asiatica leaves were purchased as plant material from the Agartala battala market, and Professor Badal Kumar Datta of the Tripura University's Taxonomy department confirmed its legitimacy.

Extraction-

Centella asiatica's leaves were pulverized after cleaned with fresh water being dried for seven days at room temperature. Using a magnetic stirrer, the powder ***(100 g)*** was combined with ***500 ml*** of a ***7:3 ethanol:water*** solution for ***15 hours***. Following the centrifugation at ***2850 g***, the supernatant was decanted. The complete extraction procedure was carried out once again after adding the pellet to ***500 ml*** of ethanol-water. The two phases' supernatants were combined in a flask with a flat bottom before being concentrated under low pressure in a rotary evaporator. Following concentration, the extract was lyophilized. The leftover material was stored at ***- 20°C*** for later use.

ASSESMENT OF ANTIOXIDANT ACTIVITY USING THE DPPH RADICAL SCAVENGING TECHNIQUE:

Centella asiatica leaves extract's capacity to scavenge free radicals was evaluated using the ***DPPH (1, 1-diphenyl-2-picrylhydrazyl)*** method. ***0.1 mM DPPH*** solution in ethanol was made, to put it briefly. Three millilitres ***(3ml)*** of extract in ethanol were mixed with one millilitre ***(1)*** of this solution at various concentrations- ***5 µg/ml, 10 µg/ml, 15 µg/ml, 20***

µg/ml, 25 µg/ml, 30 µg/ml, 35 µg/ml, 40 µg/ml, 45 µg/ml, 50 µg/ml. The mixture was briskly shaken before and let it to stand at room temperature for next ***30 minutes***. Then, a spectrophotometer was used to detect the absorbance at ***517 nm.***[7] Ascorbic acid was utilized as the reference standard chemical, and three copies of the experiment were performed. Higher levels of free radical activity were indicated by the reaction mixture's lower absorbance.[8]

***DPPH* scavenging effect *(%)* or Percent inhibition = [{(A_0 - A 1) ÷ A_0} × 100]**

Here,

A_0 **= The absorbance of control reaction**

A1 **= The absorbance in presence of test or standard sample.**

RESULTS:

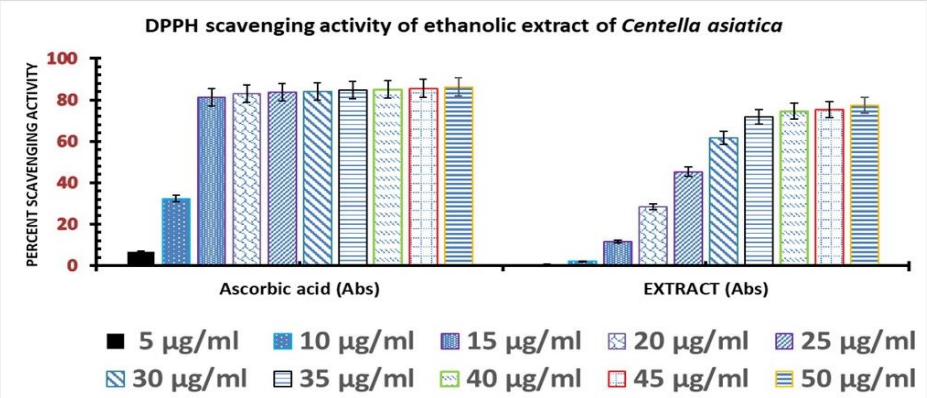
By using the ***DPPH*** scavenging assay technique, the ethanolic extract of this plant's leaves demonstrated significantly good antioxidant capacity like conventional ascorbic acid. According to a UV visible spectrophotometer, standard ascorbic acid and alcoholic extract had absorbances at ***517 nm*** of ***0.0350*** and ***0.0580***, respectively. It indicates that a plant's ethanolic leaves extract at a greater concentration trapped more free radicals produced by ***DPPH,*** which decreased absorbance.

Table 1. Absorbance of ethanolic leaves extract of ***Centella asiatica*** with standard ascorbic acid at ***517nm*** by UV visible spectrophotometer *(**DPPH** scavenging assay method)*

SL NO.	CONCENTRATION OF DRUG (µg/ml)	*Ascorbic acid (Absorbance in nm)*	*Leaves extract (Absorbance in nm)*
1	5	0.2382	0.254
2	10	0.173	0.25
3	15	0.0479	0.226
4	20	0.0435	0.1829
5	25	0.0415	0.14
6	30	0.041	0.098
7	35	0.039	0.072
8	40	0.0382	0.065
9	45	0.0368	0.063
10	50	0.035	0.058

Table 2. Percentage (%) of inhibition of ethanolic leaves extract of ***Centella asiatica*** with ascorbic acid *(**DPPH** scavenging assay method)*

SL No.	CONCENTRATION OF DRUG (µg/ml)	ASCORBIC ACID (% INHIBITION)	*LEAVES EXTRACT* (% INHIBITION)
1	5	6.771037182	0.587084
2	10	32.28962818	2.152642
3	15	81.25244618	11.54599
4	20	82.97455969	28.41487
5	25	83.75733855	45.20548
6	30	83.95303327	61.64384
7	35	84.73581213	71.81996
8	40	85.04892368	74.55969
9	45	85.59686888	75.34247
10	50	86.30136986	77.29941



Graph 1. Percentage of inhibition in various concentration of ***Centella asiatica leaves*** extract.

DISCUSSION:

Free radicals are produced continuously inside the biological systems due to cellular metabolism and also for so many reasons which can seriously harm tissues and biomolecules, resulting in a variety of disease states, including degenerative illnesses, and severe lysis.[9] Although many synthetic medications offer protection from oxidative damage, they can have unfavourable side effects. Consuming natural antioxidants from dietary supplements and conventional medications is an additional approach to the issue.[10,11] Numerous natural antioxidants have recently been extracted from various plant components .[12] various studies used concentrated leaves extract to assess the antioxidant and polyphenol levels of this plant. This study of the ***DPPH*** radical scavenging technique found that ethanolic extract of ***Centella asiatica*** species leaves has also a good antioxidant capacity like the ascorbic acid.

CONCLUSION:

According to the findings of the current investigation, a ***70%*** ethanolic leaves extract of the ***Centella asiatica***, which may include significant levels of plant's secondary metabolites like ***flavonoids*** and ***phenolic*** compounds, demonstrates strong antioxidant and free radical scavenging properties. These in-vitro tests show that this plant leaves extract is a large source of naturally occurring antioxidants, which may be useful in halting the progression of various oxidative stressors to maintain our normal physiological rhythmicity. However, it is still unknown that actually which elements are responsible to show this antioxidative action. To isolate and identify the antioxidant components in the plant extract, more research is thus required. Before using this extract in a therapeutic setting, it is also necessary to evaluate its in-vivo antioxidant activity by following the ethical guidelines of an animal research.

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