PHYTOCHEMICAL ANALYSIS AND *IN VITRO*ANTIOXIDANT ACTIVITY OF HYDROALCOHOLIC EXTRACTOF*CLERODENDRUM INDICUM*

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Abstract

Free radicals, usually ROS and RNShave the potential to harm macromolecules including DNA, proteins, and lipids at high quantities. Plants may contain a wide variety of free radicalscavenging molecules, such as phenolic compounds. Which are rich in antioxidantactivity. *Clerodendrum indicum* L. is frequently used in traditional medicine in India for curing various diseases. Considering its benefit this study aims at investigating its phytochemical & antioxidant activity. The plant material was collected & subjected to extraction. Further qualitative & quantitative test along with antioxidants assays were also performed. Results revealed that plant contain flavonoid, diterpene, phenol, protein and saponin. Total phenol & flavonoid content was found to be 1.580 mg/100mg & 0.856 mg/100mg.The IC_{50} value for ascorbic acid was observed to be 27.82 while for extract it is noticed to be 150.53 indicating that the plant extract possess some antioxidant potential. Hence further studies are needed to found the actual mechanism involved in the antioxidant property and other pharmacological activities of *C. indicum.*

Keywords: Free radicals, Antioxidants, DPPH, Phytochemicals, Herbal medicine, *Clerodendrum indicum*

Introduction

Aerobic creatures require oxygen to survive. Humans and other animals' ability to utilise oxygen has made it possible for them to metabolise lipids, proteins, and carbohydrates to make energy, yet it has come at a price because, ironically, using oxygen speeds up human ageing and sickness. The regular cell

metabolism produces free radicals. Free radicals, usually ROS and RNS, are produced by the redox process when cells consume oxygen.Endogenous cellular ROS, which are mostly produced by mitochondria, have the

potential to harm macromolecules including DNA, proteins, and lipids at high quantities. Numerous clinical conditions, including cancer and inflammatory, pulmonary, cardiovascular, neurological, and digestive-tract diseases, have been linked to this damage (Jones, 2008; Albano, 2006).

By preventing the start or spread of an oxidative chain reaction, functioning as free radical scavengers, quenchers of singlet oxygen, and reducing agents, increasing the intake of exogenous antioxidants will mitigate the harm caused by oxidative stress (Sen and Chakraborty, 2011). The majority of exogenous antioxidants come from food and medicinal plants, including fruits, vegetables, cereals, mushrooms, drinks, flowers, spices, and traditional medicinal herbs. In addition, the sectors that process agricultural by-products provide additional potential sources of natural antioxidants (JadhavandBhutani, 2002).

These naturally occurring anti-oxidants are mostly composed of polyphenols (phenolic acids, flavonoids, anthocyanins, lignans, and stilbenes), carotenoids (xanthophylls and carotenes), and vitamins (vitamins E and C) derived from plant materials. These naturally occurring antioxidants, particularly polyphenols and carotenoids, often have a wide range of biological actions, including anti-inflammatory, antibacterial, antiviral (Barros*et al.*, 2010; Narayanaswamy and Balakrishnan, 2010).

Clerodendrum indicum L. is frequently used in traditional medicine in India and other countries to increase irritation in solidity and to cure conditions like asthma, hack, scrofulous affection, vermifuge, and antirheumatic. The herb is also used to treat fever, rot, cachexia, and malnutrition. These sources were examined for their regular science, common jobs, distinctive viewpoints, engineered components, and pharmaceutical relevance. The concentrated *Clerodendrum indicum* showed antinociceptive, antimicrobial, and antidiarrheal properties in in vitro tests (Bhujbal*et al*., 2009; Kar*et al*., 2014). Because of its immense tradition al use this study aims at phytochemical & antioxidant potential investigation of *C. indicum*.

Materials & Methods

Collection of plant

Botanical gardens typically contain a diverse collection of plants from around the world, including rare and endangered species. Fresh leaves of *Clerodendrum indicum* were gathered from vindhya herbals in Bhopal's small forest produce processing and research centre.

Extraction by maceration method

40 gram ofleavesof *Clerodendrum indicum*was extracted with petroleum ether utilising maceration at room temperature. The extraction process was continued until the material had been sufficiently

defatted.

Defatted powdered leavesof *Clerodendrum indicum* were macerated in hydroalcoholic solvent (ethanol: water; 75:25). Over their boiling temperatures, the extract was evaporated. Lastly, calculate the dried extracts % yield.

Phytochemical test

The screening of phytochemical compounds was done as per standard protocol (Kokate, 1994).

Total phenolic content

The Folin-Ciocalteu test was used to calculate the total phenolics content. Various aliquot of extracts or standard solutions of gallic acid (10 to 50µg/ml) was added. Also, 10 mg of plant extract was dissolved in 10ml of methanol to make plant extract stock. The mixture was combined with 1 ml of the Folin-Ciocalteu phenol reagent and agitated. 1 ml of a Na_2CO_3 solution were added to the mixture after 5 minutes. The volume was then adjusted to the proper level. UV-Visible spectrophotometer was used to measure the absorbance at 765 nm against the reagent blank following a 10 minute incubation period at room temperature. The amount of total phenolics was measured in mg Gallic acid Equivalents (GAE) (Parkhe&Bharti, 2019).

Total flavonoid content

The aluminium chloride colorimetric test was used to determine the amount of total flavonoid (Parkhe&Bharti, 2019). Different aliquot of standard solutions of quercetin (5, 10, 15, 20, and 25µg/ml) were prepared. Also, 10 mg of plant extract was dissolved in 10ml of methanol to make plant extract stock. Three ml of this extract was used for estimation of flavonoids. 1ml of 2% AlCl3 was added to each standard along with extract. Reaction was allowed to run for 15 min at room temperature. At 420 nm, the mixture's absorbance was measured in comparison to a blank. The amount of flavonoid in total was measured in mg of quercetin equivalents.

DPPH scavenging

The spectrophotometer was used to measure the DPPH scavenging activity with a little modification (Jain and Parkhe, 2018). 1.5 ml of the stock solution (6 mg in 100 ml methanol) was made so that it produced an initial absorbance when combined with 1.5 ml of methanol. After 15 minutes, a decrease in absorbance was seen when sample extract at various concentrations (10-100 g/ml) was present. After diluting 1.5 ml of the DPPH solution with methanol to make 3 ml, the absorbance was measured right away at 517 nm for the control reading. In a series of volumetric flasks, 1.5 ml of DPPH and 1.5 ml of the test sample at various concentrations were added. Each concentration was measured using absorbance at zero time. After 15 minutes, at 517 nm, DPPH absorbance finally decreased with the sample at a varied concentration (Parkhe & Jain, 2018).

The percentage inhibition of free radical DPPH was calculated from the following equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%.

Results & Discussion

The result of phytochemical study revealed the presence of flavonoid, diterpene, phenol, protein, saponin. Total phenol & flavonoid content was found to be 1.580 mg/100mg &0.856 mg/100mg. DPPH assay is considered as a valid method to evaluatescavenging activity of antioxidants, since the radical compoundis very stable and do not have to generate as in other radical.When DPPH radicals interact with the proper reducing agents, electrons pair off and the solution loses colour stoichiometrically as a function of the quantity of electrons absorbed. Such reactivity has frequently been used to evaluate a plant extract's potential as a free radical scavenger. Alcoholic extract's DPPH assay revealed a dose-dependent rise in the percentage of free radical inhibition. The DPPH activity was performed using ascorbic acid as standard. The IC_{50} value for ascorbic acid was observed to be 27.82 while for extract it is noticed to be 150.53.

Table 1: Result of phytochemical screening of *Clerodendrum indicum*

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids** Hager's Test:	 -Ve
2.	**Glycosides** Legal's Test:	 -Ve
3.	**Flavonoids** Lead acetate Test: Alkaline test:	 +Ve +Ve
4.	**Diterpenes** Copper acetate Test:	 +Ve
5.	**Phenol** Ferric Chloride Test:	 +Ve
6.	**Proteins** Xanthoproteic Test:	 +Ve
7.	**Carbohydrate** Fehling's Test:	 -Ve
8.	**Saponins** Froth Test:	 +Ve
9.	**Tannins** Gelatin test:	 -Ve

Table 2: Results of total phenol content

S. No.	Extract	Total phenol content mg/100mg
1.	Hydroalcoholic	1.580

Table 3: Results of total flavonoids content

S. No.	Extract	Total flavonoids content mg/100mg
1.	Hydroalcoholic	0.856

Table 4: % Inhibition of ascorbic acid and extract of *Clerodendrum indicum* using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Hydroalcoholic extract
1	10	41.52	3.6
2	20	47.70	4.95
3	40	52.92	18.01
4	60	67.43	19.81
5	80	75.89	27.47
6	100	89.63	32.43
IC_{50} value		**27.82**	**150.53**

Conclusion

Natural products and their derivatives are becoming increasingly popular in illness treatment and prevention due to their low toxicity. Since ancient times, people have employed *C. indicum* and its components, especially in the Indian Subcontinent, for therapeutic purposes. Clinical investigations have proven that neem is essential in the prevention of several ailments. Through the modification of several cell signalling pathways, the active components' role as chemo preventive impact has been shown in distinct cancer types. To determine the precise mechanism of action in the treatment of diseases, a thorough investigation using animals should be conducted. The presence of antioxidative activity of the extract of *C. indicum* provided a new therapeutic path against the various diseases and its complications. Further studies are needed to found the actual mechanism involved in the antioxidant

property and other pharmacological activities of *C. indicum*.

References

- Jones DP. Radical-free biology of oxidative stress. American Journal of Physiology-Cell Physiology. 2008 Oct; 295(4):C849-68.
- Albano E. Alcohol, oxidative stress and free radical damage. Proceedings of the nutrition society. 2006 Aug; 65(3):278-90.
- Sen S, Chakraborty R. The role of antioxidants in human health. InOxidative stress: diagnostics, prevention, and therapy 2011 (pp. 1-37). American Chemical Society.
- Jadhav HR, Bhutani KK. Antioxidant properties of Indian medicinal plants. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 2002 Dec;16(8):771-3.
- Barros L, Oliveira S, Carvalho AM, Ferreira IC. In vitro antioxidant properties and characterization in nutrients and phytochemicals of six medicinal plants from the Portuguese folk medicine. Industrial Crops and Products. 2010 Nov 1;32(3):572-9.
- Narayanaswamy N, Balakrishnan KP. Evaluation of some medicinal plants for their antioxidant properties. Int J Pharm Tech Res. 2011 Jan;3(1):381-5.
- Bhujbal SS, Kewatkar SM, More LS, Patil MJ. Antioxidant effects of roots of Clerodendrumserratum Linn. Pharmacognosy Research. 2009;1(5).
- Kar P, Goyal AK, Das AP, Sen A. Antioxidant and pharmaceutical potential of Clerodendrum L.: An overview. International Journal of Green Pharmacy (IJGP). 2014;8(4).
- Kokate CK. Ed. Practical Pharmacognosy, 4thEdn.,*VallabhPrakashan*: 1994; 112:120.
- GeetaParkhe, Deepak Bharti. Phytochemical investigation and determination of total phenols and flavonoid concentration in leaves extract of *Vitextrifolia* Linn. Journal of Drug Delivery & Therapeutics. 2019; 9(4-A):705-707.
- ParkheG, JainP. Study of antioxidantpotential of hydroalcoholic extract of *Anethumgraveolens*. Career.IntJSci Technol.2018;1(2):39-45.