



TINOSPORA CARDIFOLIA: SOURCE OF ANTIOXIDANT

Singh Pallavi

Research scholar, Department of Botant, Ranchi University

Prof Dr. H.P. Sharma

University Professor, Department of Botant and Former PVC of SKMU, Dumka

ABSTRACT Large numbers of medicinal plants is very well known for their antioxidant property and are taken in raw form or their chemical composition is present in the market. The present study aims to determine the qualitative and quantitative estimation of antioxidant present in *Tinospora cordifolia*. For the purpose of investigation methanolic extract was used.

KEYWORDS : Antioxidant, *Tinospora cordifolia*, CUPRAC Assay, Flavonoid.

INTRODUCTION

The use of medicinal plants is not just a custom of the distant past. It has been used for centuries before the advent of orthodox medicine. Leaves, flowers, stems, roots, seeds, fruit, and bark can all be constituents of herbal medicines. Herbal medications have been used for relief of symptoms of disease since long. (Maqsood S *et al.*, 2010). Despite the great advances observed in modern medicine in recent decades, plants still make an important contribution to health care. Much interest, in medicinal plants however, emanates from their long use in folk medicines as well as their prophylactic properties, especially in developing countries. The medicinal values of plants lie in their component phytochemicals, which produce definite physiological actions on the human body. Recently there has been growing interest in research into the role of plant-derived antioxidants in food and human health. Large number of medicinal plants has been investigated for their antioxidant properties. Natural antioxidants either in the form of raw extracts or their chemical constituents are very effective to prevent the destructive processes caused by oxidative stress (Zengin G *et al.*, 2011). Although the toxicity profile of most medicinal plants have not been thoroughly evaluated, it is generally accepted that medicines derived from plant products are safer than their synthetic counterparts (Vongtau HO *et al.*, 2005 and Oluyemi KA, 2007). Plants and plant based medications have been man's prime therapeutic tools due to the presence of various complex substances that show a striking structural diversity. These biochemical products which are found as secondary metabolites in plants are extractable and act as a vast source of natural antioxidant which can help to prevent the onset and counteract progression of oxidative stress (M. A. Hossain *et al.*, 2013). Furthermore, natural antioxidants are perceived as efficient, safe, cost effective and affordable in comparison with synthetic antioxidants that might serve as leads for the development of novel drugs and in food industry to prolong the shelf life of foods, especially those rich in polyunsaturated fats (Sannigrahi, U. K. *et al.*, 2010). Traditionally used natural antioxidants are already exploited but, there is still a demand to explore more information concerning the antioxidant potential of plant species. One such plant is *Tinospora cordifolia*.

Tinospora cordifolia commonly known as Guduchi, Amrita, Gurach, is a large, glabrous deciduous climbing shrub and belongs to family Menispermaceae. Leaves are simple, alternate, exstipulate, long petiolate, chordate in shape showing multicoated reticulate venation and the stems are rather succulent with long filiform fleshy aerial roots from the branches. Flowers are small and unisexual where male flowers are in clusters, female flowers are solitary. Chemical constituent of the plant are alkaloids, glycosides, steroids, sesquiterpenoid, aliphatic compound, essential oils, mixture of fatty acids and polysaccharides.

The alkaloids include berberine, bitter gilonin, non-glycoside gilonin giloterol. The major phytoconstituent in *Tinospora cordifolia* include tinosporine, tinosporide, tinosporaside, cordifolide, cordifol, heptacosanol, clerodane furano diterpene, diterpenoid furanolactone tinosporidine, columbin and b-sitosterol. Berberine, Palmatine, Tembertarine, Magniflorine, Choline, and Tinosporin are reported from its stem (S.S. Singh, *et al.*, 2003)

MATERIAL METHOD

Collection of plant sample

Tinospora cordifolia was gathered from the medicinal garden of Ranchi University campus and was shade dried. Grinding of the shade dried material into fine powder was done.

Preparation of methanolic extract

Plant powder was soaked with methanol (1:10) in a flask covered with aluminum foil to avoid evaporation. This flask was kept for 48 hours in shaker incubator. Using Whatman filter paper no. 1 this mixture was filtered after 48 hours and the filtrate was collected in a beaker. Then the filtrate was kept in incubator at 37°C to evaporate the solvent and the extract obtained was stored at 4°C for further use.

Phytochemical Screening

The phytochemical screening of the powdered specimen aqueous extract was carried using standard procedures to identify the constituents viz. Tannin, Phlobetannin, Terpenoid, Saponin, Flavonoid, Cardiac glycoside, Phenol, Steroid, Alkaloid, Anthraquinone and Carbohydrate (Jain *et al.*, 2014)

CUPRAC

This assay is based on reduction of Cu(II)–neocuprine complex to highly colored Cu(I)-neocuprine complex, which is measured at 450nm absorbance. Neocuproine, an aromatic heterocyclic compound which is used for the spectroscopic determination of copper. Neocuproine, a methylated phenanthroline derivative which chelates with the copper from cuprous chloride and forms a chromogenic redox reagent bis(neocuproine) copper(II) chloride which is a novel reagent for the CUPRAC antioxidant capacity assay and calculated by

$$C = A/\epsilon l$$

Where, A is absorbance, C is capacity, ϵ is Molar coefficient and l is path length.

CuCl₂, Neocuprine, ammonium acetate 1ml each was pipette in test tubes of different concentration and to this solution plant extract was added. This was left at room temperature for 30 minutes and absorbance was recorded.

Phytochemical estimation

Flavonoid Estimation

By using colorimetric method and AlCl₃ total Flavonol is determined. The principal involve, AlCl₃ forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. And also Acid labile complexes with the ortho dihydroxyl group in the ring A or B of flavonoid is formed by AlCl₃. And Gallic Acid Equivalent was calculated by $y = mx + c$ Where y is O.D value, m is slope value, x is amount if the test component and c is extinction coefficient.

RESULTS

Phytochemical screening done by using the dry powder of whole plant demonstrate the presence of Tannin, Phlobetannin, Terpenoid, Saponin, Flavonoid, Cardiac glycoside, Phenol, Steroid, Alkaloid, Anthraquinone and Carbohydrate. The result has been shown in table 1.

Serial no.	Phytochemical	Test	Present/ Absent
1	Tannin	Ferric chloride	present
2	Protein and amino acid	Ninhydrin test	present
3	Carbohydrate	Benedict's test	present
4	Saponin	Froth test	present
5	Flavonoid	Alkaline reagent lead acetate zinc HCl test	absent
6	Phenol	Ferric chloride	present
7	Alkaloid	Mayer's test	present
8	Resins	Acetone-water	present

ANTIOXIDANT ASSAY

CUPRAC Assay

In this methodology results are reported in Trolox Equivalent. The antioxidant capacity from concentration 10µg-1000µg was found to increase from 0.0277 TE (mol) to 0.0839 TE (mmol).

Phytochemical Estimation

Flavonoid Estimation

Total Flavonoid content was determined by colorimetric method and reported as Gallic Acid Equivalent by reference to standard curve ($y=9.5455x+16.364$, $R^2=0.8047$). The optical density of Total flavonoid recorded at 10 µg and 1000 µg was 0.053 and 0.125.

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