International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.4, No.4, pp 1428-1431, Oct-Dec 2012

A Study On Phytochemical Screening And invitro Antioxidant Activity Of Calotropis gigantea L.

P.Elakkiya and G.Prasanna*

Department of Biochemistry, Sengamala Thayaar Educational Trust Women's College, Sundarakkottai, Mannargudi, Tiruvarur dt, Tamilnadu, India.

Corres. Author: prasannakeertana@yahoo.in

Abstract: The present investigation has been carried out to evaluate the phytochemicals and invitro antioxidant activity *of Calotropis gigantea*. Phytochemicals were analysed qualitatively and the result confirmed the presence of alkaloids, phenols, saponin, steroids. The invitro antioxidant activity of root was investigated by DPPH and FRAP method.. In both method , plant extract possess high antioxidant activity when compared with standard ascorbic acid due to presence of high content of various phytochemicals.
Key words: Antioxidant, *calotropis gigantea.*, Phytochemicals.

INTRODUCTION

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfer electrons from a substance to an oxidizing agent. oxidation reactions can produce free radicals which start chain reactions that damage cell. Antioxidant terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging a molecule commonly called "Free radicals". Free radicals are capable of attaching the healthy cells of the body, causing them to lose their structure and function (1). Free radicals are electrically charged molecules, i.e., they have an unpaired election, which causes them to seek out and capture electrons from other substance in order to neutralize themselves. Although the initial attach causes the free radical to become neutralized, another free radical is formed in the process, causing a chain reaction to occur, and until subsequent free radical are deactivated, thousands of free radical reactions can occur within seconds of the initial reaction. Antioxidant are capable of stablising, or deactivating free radicals before they attack cell. Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well- being (2).

Reactive oxygen species (ROS) is a term which encompasses all highly reactive oxygen containing molecules, including free radicals. Types of ROS include the hydroxyl radical, the super oxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, and various lipid peroxides **(**3). All are capable of reacting with membrane lipids, nucleic acids, proteins and enzymes, and other small molecules, resulting in cellular damage **(4**).

To protect the cells and organ systems of the body against reactive oxygen species, humans have evolved a highly sophisticated and complex antioxidant protection system. It involves a variety of compounds,both endogenous and exogenous in origin that function interactively and synergistically to neutralize free radicals *(4).*

These compounds include-

- Nutrient- derived antioxidants like ascorbic acid, vitamin-E, carotenoids .
- Antioxidant enzymes, e.g., superoxide dismutase, glutathione peroxidase, and glutathione reductase.
- Metal binding proteins, such as ferritin, lacto ferritin, albumin and ceruloplasmin (5).

In India, herbal medicines have been the basis of treatment and cure for various diseases. About 80% population of the developed countries use traditional medicines, derived from medicinal plants. In this study we analyed the phytochemicals present in *calotropis gigantea and* antioxidant activity by DPPH and FRAP method.

Calotropis gigantea L.belongs to Apocynaceae family (Tamil name:Erukku) is a tall shrub reaching 2.4-3m high (6). It cultivated throughout india in warm dry places from Punjab to western, central and southern India. It has one or a few stems, few branches, and relatively few leaves, mostly concentrated near the growing tip **(**7). In the traditional medicinal system, different parts of plants have been used for a variety of diseased conditions **(**8). The ethanolic extract of root exhibit anticancer activity while the methanolic extract show hypertensive effect (The wealth of India,1992). It contains cardinolides having digitalis like action (9). The root extract has been used by folks to kill guinea worms (10).

Theplant contain alkaloids, tannins, phenols and resins (11,12). The root bark contains amyrin, amyrin, taraxasterol and its isomar, taraxasteryl isovalerate, taraxasterol acetate, gigantin, giganterol, isogiganterol, sitosterol and a wax (13).

MATERIALS AND METHODS

Plant collection

The plant was collected from in and around madukkur, Tamilnadu. The collected samples were carefully kept in polythene bags. Then the plant samples were taken to the medicinal plants collection unit (MPCU), Indian Institute of Crop Processing Technology (IICPT), Thanjavur, Tamilnadu for further studies.

Preparation of Plant extracts

The disease free and fresh plant was selected for this investigation. About 1 gram of root was washed with tap and distilled water for three times. Again the plant materials was washed throughly with distilled water for three times**.** Methanolic extracts was prepared according to the methodology of Indian pharmacopoeia.The shady dried plant materials were subjected to pulverization to get coarse powder. The coarse powder material was subjected to suxhlet extraction separately and successively with methanol. The extract was cocentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40-50°c) and the methanolic extract put in airtight container and stored in refrigerator.

Phyhytochemical analysis

Chemical tests were carried out on the methanolic root extract using standard procedures to identify the phytoconstituents.

Antioxidant activity

The total amount of antioxidant chemical present in the *calotropis gigantea* was measured by DPPH method (14**)** and FRAP method (15).

DPPH Method

In DPPH method , the 2,2' diphenyl 1-picrylhydryl radical was the oxidizing radical to be reduced by the antioxidant (AH) present in the given sample. The whole reaction was indicated as

DPPH+AH……>DPPH-H-A^0

The disappearance of the DPPH radical absorption at 517 nm by the action of antioxidants was measured spectrophotometrically in a solution until the absorbance remains constant.

FRAP Method

At low P^H , reduction of ferric tripyridyl triazine (Fe^{3+}–TpTz) complex to ferrous (which has intensively blue colour) can be monitored by measuring the change in absorption at 593nm. The reaction was non specific, in that any half reaction that has lower redox potential under reaction condition, then that of ferrous (Fe^{3+}) half reaction, will derive the ferric (Fe^{2+}) ion formation. The change in absorbance was denoting antioxidant present in the plant extract.

RESULTS AND DISCUSSION

Qualitative phytochemical screening

Phytochemical compounds were screened in *calotropis gigantea* through qualitative method.The results indicated the Presence of alkaloids, saponins , phenols, sterols, flavonoids and absence of terpenoids and tannins, which was shown on Table 1.

Phytochemical screening revealed the presence of phenols and flavonoids which could be responsible for its anti mutagenic, antioxidant and anti inflammatory activity. The importance of these substance on the plant as antimicrobial agent against the pathogen has been exphasized by several workers (16).

Table 1: Phytochemical screening of *Calotropis gigantea*

S.NO	Phytochemical screening	Root extract
1.	Tannins	_
2.	Phlobatannins	_
3.	Saponin	+
4.	Flavonoids	+
5.	Steroids	+
6.	Terpenoids	_
7	Cardiac glycosides	_
8	Alkaloids	+
9.	Flavonoids	+

+presence:-absence

In vitro antioxidant studies

Free radical and reactive oxygen species are well known inducers of cellular and tissue pathogenisis leading to several human diseases such as cancer, inflammatory disorder, and diabetrs mellitus as well as in aging process (17). Many plant species with antioxidant activities act as protective agent against these diseases (18).

In the present study, the potent antioxidant activity of methanolic root extract of *calotropis gigantea* was assayed by DPPH method. The DPPH method provide information on the reactivity of test compounds with a stable free radical. Because of its odd electron 2,2' Diphenyl Picryl Hydrazyl Radical (DPPH) gives strong absorption band at 517nm in visible spectroscopy (19). The methanolic extract of*calotropis gigantea* had comparable DPPH radical scavenging activity with reference to standargd ascorbic acid and it was shown on Table 2.The percentage of inhibition was measured at 30 minutes interval 82.6%,86.2%,90.5% and the standard ascorbic acid inhibition percentage were 51.4%,54.6%, 61.6%. Free radical scavenging capacity of *calotropis gigantea* may be due to the flavonoids, which are typical phenolic compounds,act as metal chelators and Free radical scavengers (*20).*

Table 2 : Antioxidant activity of *calotropis gigantea* by DPPH method

Concentration (µg/ml)	Antioxidant activity in %	
	Root extract	Ascorbic acid
100	82.6	51.4
200	86.2	54.6
300	90.5	61.6

FRAP assay measures the reducing ability of antioxidants against oxidative effects of reactive oxygen species. Electron donating antioxidants can be described as redox reaction. Total antioxidant power may be referred analogously to total reducing power (21).In the present study,the antioxidant activity by FRAP method was performed, the percentage inhibition of root were 72.4%,76.3%, 79.6%. and it were shown on Table 3.

Table 3 : Antioxidant activity of *calotropis gigantea* by FRAP method

Concentration (µg/ml)	Antioxidant activity in %	
	Root extract	Ascorbic acid
100	72.4	62.7
200	76.3	65.3
300	79.6	67.9

The radical scavenging and inhibition of lipid peroxidation by the extracts was due to the

quenching free radicals or reduction of Fe3+ to Fe2+, which can be attributed to the presence of number of polyphenolics such as flavonoids, anthocyanins etc., In our study, we concluded both DPPH and FRAP method , there was a highest antioxidant activity of root when compared with standard. The plant extract enriched with flavonoids can be used in routine life to treat various diseases which are due to free radicas generation in our body (22).

REFERENCES

1. Sies,H., Stahl,W., Sevanian,A., Nutritional , dietary and postprandial Oxidative stress, *J. Nutr,* 135 (2005),969-972.
2. Halliwall,B., Gutteridge,J.M., Grootveld,M., Methods for the measurements of hydroxyl radicals in biomedical systems; Deoxyribose degradation and aromatic hydroxylation, *Methods of Biochem Anal*, 33(1988),59-90.
3. Stephens,J.W., Brain, S.C., Khanolkar, M.P., Atherosclerosis, 202 (2009), 321-329.
4 .Zhang, J., Dai, G., Yan, L.,Xuq., Zhang,P., Zhon., *Euro Journal of Pharmacol,* 7(1) (2008), 19-22.
5. Jacob,V., Michael,A.,Nutritional antioxidants: Mechanism of action, analysis of activities and medicinal applications,Nutrition, 49(1999),1-7
6. Kirtikar A.R. AND Basu B.D., *Indian Medicinal plants*, 111 (1935), 1606-1610.
7. Sharma P.C., Yelne, M.B., Dennis, T.JLodhra (symplocos racemosa*)*. Data base on medicinal plants used in ayueveda. *Central council for research in ayurveda and siddha*,New Delhi, 5(2000e), 164-170
8 Nandkarni A.K.,*Indian Materia Medica*,Popular Book Depot, Bombay, 1(1976),246.
9. Asolkar L V.,Kakkar K K and Chakre O J., Second supplement to Glossary of Indian Medicinal plants with active principles, part I, NISC(CSIR), New Delhi ,(1992),157-159.
10. Jain S K., Sinha B K and Saklani A, *Ethnobotany*, 1(1989),92.
11. Handa, S S.,and Kappoor, V.K .,*Pharmacognosy*, Vallabh prakashan, New Delhi, 2nd edn, (1989), 283.
12. Evans N.C., *Pharmacognosy*,w.b. Saunders, New yark,15th edn.,2002:471.
13. Chopra, R.N., Chopra,I.C., Handa,K.L., Kapur, L.D, Indigenous drugs of india. (1958), Academic publishers, Calcutta, India.
14. Sadasivam,S and Manikam,M.,Biochemical methods, New age international (p) limited publishers, New Delhi,(2005), 256.
15. Benzie I.F.F and Strain J J., The ferric reducing availability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay.*Anal Biochem*,239 (1996),70-76.
16. Mahadevan,A.,Recent studies in the disease resistence of plants.In:Agarwal,G.P AND Bilgrami, K.S.(eds) physiology of parasitism symposium. Today and tomorrows printers and publishers,(1979),78.
17. Aviram, M., Review of human studies on oxidative damage and antioxidant protection related to cardiovascular disease, *Free Rad.Res*, 33(2000), 585-87.
18. Wargovich, M.J., Woods, C., Hollis DM.,Zander ME., Herbals, Cancer prevention and health. *J. Nutr*, 131 (2001),3034S-3036S.
19. Wang, H., Bloom,O., Zhang,M.,Vishnubhakat J.M., Ombrellino,M ., Che, J. HMG-1 as late mediater of endotoxin lethality in mice, *Science*, 285(1999), 248-51.
20. Kandaswami, C., Middleton, E., Free radical scavenging and antioxidant activity of plant flavonoids in free radicals in diagnostic medicine, plenum press, New yark, (1994), 351-376.
21. Alluri, v.k.s.,Tayl, V.N.R.,Dodda, S., Mulabagal, V., Hsin sheng,T., Gottumukkala, V.S., Assess ement of bioactivity of Indian plants using brain shrimp (*Artemia salina*) lethality assay. *Intl.J.Applied sci.Engineering*, 3(2)(2005),125-134.
22. Aviject jain,Akhil chincholikar., Evaluation of antioxidant activity of flavonoid from *Tephrosia purpurea* pers.,*Adv. Pharmacol.Toxicol*,7(1) (2006),346-350.

*****