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# ANTIOXIDATIVE ACTIVITY OF METHANOLIC EXTRACT OF *GLORIOSA SUPERBA* SEED, TUBER AND LEAVES

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***ABSTRACT***

*Medicinal plants play a key role in the human health care. About eighty percent of the world populations rely on the use of traditional medicine, which is predominantly based on plant materials. The present study was carried out to evaluate the antioxidative activity of methanolic extract of Gloriosa superba seed, tuber and leaves (MEGSSTL). The percentage radical scavenging of MEGSSTL on DPPH and the $IC_{50}$ (50% inhibitory concentration) value was found to be 38, 52 and 74 µg., hydrogen peroxide scavenging activity of MEGSSTL and the $IC_{50}$ value was found to be 50, 63 and 68 µg., nitric oxide scavenging activity of MEGSSTL and the $IC_{50}$ value was found to be the 61,71 and 84 µg., hydroxyl radical scavenging ability of MEGSSTL to 50 percent was found to be 70,76 and 85 µg ($IC_{50}$) and superoxide radical production in vitro in a dose dependent manner with an $IC_{50}$ of 58,70 and 75 µg. respectively. The dose dependent percentage scavenging activity of DPPH, hydrogen peroxide, nitric oxide, hydroxyl and superoxide radical by methanolic extracts indicated its antioxidative efficacy.*

***Keywords:-*** *Gloriosa superba, DPPH, hydrogen peroxide, nitric oxide, hydroxyl and superoxide radical*

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## INTRODUCTION

Oxidation is essential to many living organisms for the production of energy to fuel biological processes [8]. Oxidative stress is among the major causative factors in the induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, and neurodegenerative diseases [25]. Many human diseases are caused or negatively affected by free radicals. The most effective way to eliminate free radicals which cause the oxidative stress with the help of antioxidant [26]. Free radicals are generated as part of the body's normal metabolic process, and the free radical chain reactions are usually produced in the mitochondrial respiratory chain, liver mixed function oxidases, through xanthine oxidase activity, atmospheric pollutants and from transitional metal catalysts, drugs and xenobiotics [29]. Free radicals can initiate or propagate many diseases, such as inflammation, cancer, liver injury and cardiovascular diseases [2]. The most frequently encountered free radicals are the superoxide radical ($O_2^{\cdot -}$), nitric oxide radical ($NO^{\cdot}$), hydroxyl radical ($HO^{\cdot}$), hydrogen peroxide radical ($H_2O_2^{\cdot}$), and 1,1-diphenyl,2-picrylhydrazyl (DPPH). Free radicals being highly reactive atom oxygen is capable of becoming part of potentially damaging prooxidants [10].

Antioxidant is defined as any substance that delay or prevent the oxidation of cellular oxidizable substrate. They exert their effects by scavenging free radicals, activating a battery of detoxifying protein: or preventing the generation of free radicals [20] . Epidemiological studies have shown that the intake of natural antioxidant is associated with reduced risks of cancer, cardiovascular disease, diabetes and other diseases associated with aging [13]. Antioxidants can be of synthetic origin and a great number of secondary metabolites are isolated from plants, such as various phenolic compounds. Antioxidants work by donating an electron to a molecule that has been compromised by oxidation, bringing it back into a state of proper function [27]. *Gloriosa superba* is commonly known as Malabar glory and it is a perennial creeper in the family Liliaceae, native to Africa. Its stem is thin and grows at the rate of 20 feet per year. Leaves are ovate in shape about 6-8 inches long thread like at the apex that helps to climb on the trees. This plant contains 0.2-0.3percent colchicins and gloriosine alkaloids. This plant is used as an ayurvedic medicinal herb to cure diseases like arthritis, gout, ulcers, and bleeding [11].

## MATERIALS AND METHODS

### Collection of plant material

Fresh *Gloriosa superba* seed, tuber and leaves were collected from the outskirts of Thanjavur district, Tamilnadu. The collected leaves were washed thoroughly in tap water, shade dried and finely powdered.

### Preparation of MEGSSTL

Ten gram of powders of *Gloriosa superba* seed, tuber and leaves was filled in the thimble and extracted with 150 ml of methanol using a soxhlet extractor for 24 hours. The methanol extract was then distilled and evaporated to dryness. The concentrated extract was then accurately weighed and stored in small vials at -20$^0$ C for further use.

### Assessment of *in vitro* antioxidative activity of MEGSSTL

DPPH radical scavenging activity was measured according to the method of [18]. Hydrogen peroxide scavenging activity of MEGSSTL was determined according to the method described by [24]. Nitric oxide radical scavenging activity of MEGSSTL was determined by the method described by [7]. The hydroxyl radical scavenging activity was analyzed according to the method of [6]. Superoxide radical scavenging activity of MEGSSTL was determined by the method of [17].

## RESULT

### *In vitro* antioxidant assays

### Effect of MEGSSTL on antioxidative activity.

The *in vitro* antioxidant activity of MEGSSTL was assessed by studying its ability to scavenge DPPH, hydrogen peroxide, nitric oxide, hydroxyl and superoxide radicals.

### DPPH radical scavenging activity of MEGSSTL

The MEGSSTL showed a dose dependent DPPH scavenging activity. Figure 1 shows the dose dependent DPPH radical scavenging activity of MEGSSTL and the $IC_{50}$ value was found to be 38,52 and 74 µg.

### Hydrogen peroxide scavenging activity of MEGSSTL

The MEGSSTL showed a dose dependent hydrogen peroxide scavenging activity. Figure 2 shows the dose dependent Hydrogen peroxide scavenging activity of MEGSSTL and the $IC_{50}$ value was found to be 50,63 and 68 µg.

### Nitric oxide radical scavenging activity of MEGSSTL

The MEGSSTL showed increasing activity of Nitric oxide as shown in Figure 3 with increasing concentration and the $IC_{50}$ value was found to be 61, 71 and 84 µg.

### Hydroxyl radical scavenging activity of MEGSSTL

The hydroxyl radical scavenging activity of MEGSSTL was found to be dose dependent as shown in Figure 4. The ability of MEGSSTL to scavenge 50 percent of hydroxyl radical was found to be 70,76 and 85 µg ($IC_{50}$).

**Superoxide radical scavenging activity of MEGSSTL**

The effect of MEGSSTL on *in vitro* scavenging of superoxide radical is shown in Figure 5. The MEGSSTL inhibited superoxide radical production *in vitro* in a dose dependent manner with an $IC_{50}$ of 58, 70 and 75 µg.

**Figure 1 Percentage DPPH radical scavenging activity of MEGSSTL**

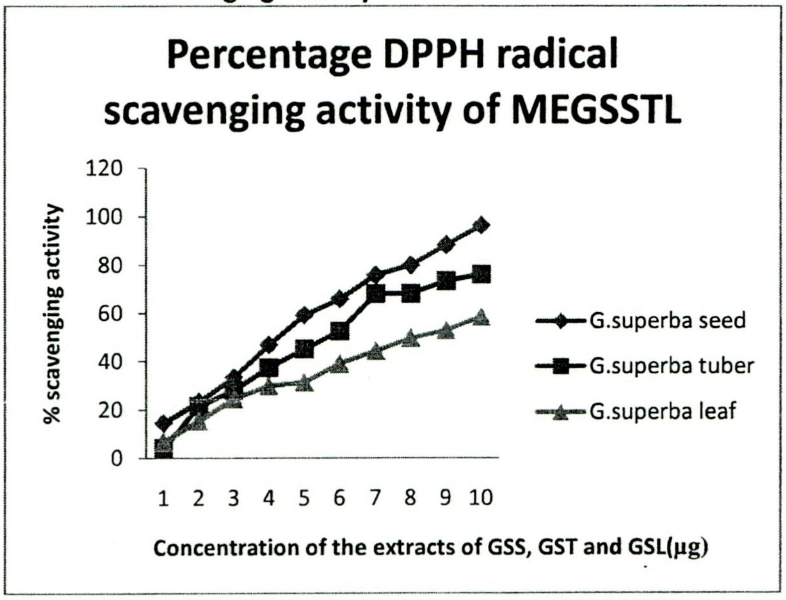



**Figure 2 Percentage hydrogen peroxide radical scavenging activity of MEGSSTL**

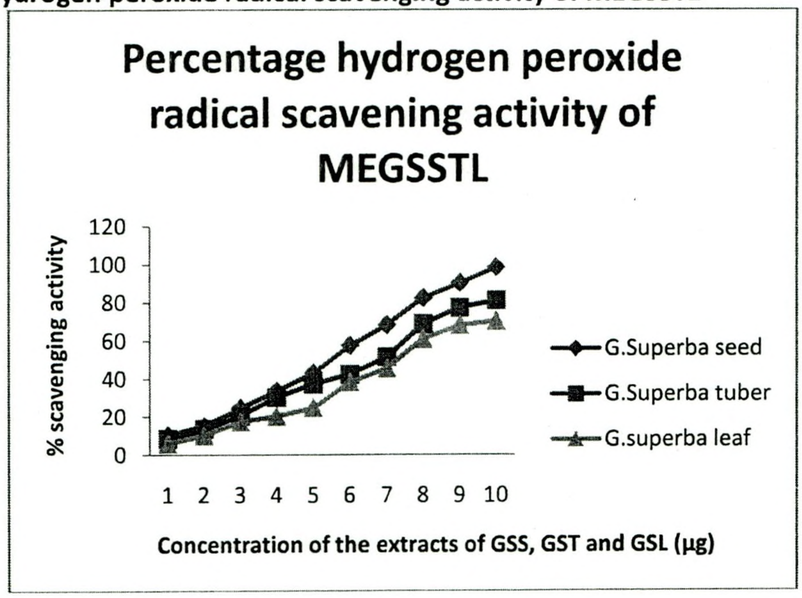


**Figure 3 Percentage nitric oxide radical scavenging activity of MEGSSTL**

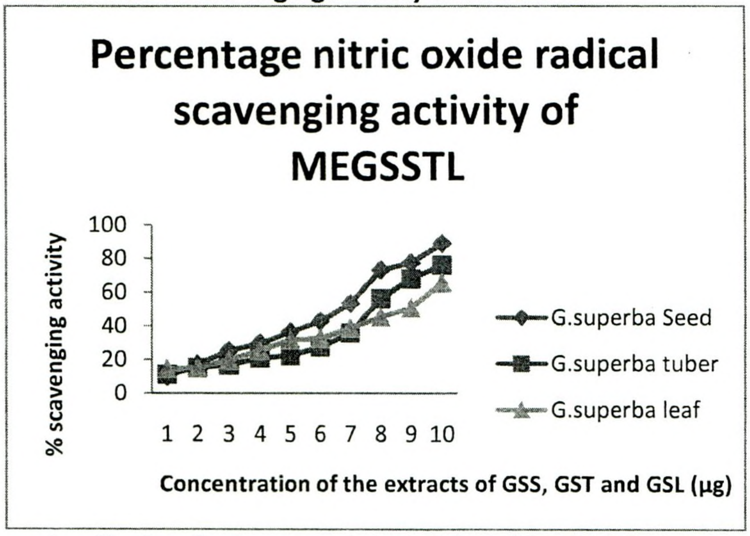



**Figure 4 Percentage hydroxyl radical radical scavenging activity of MEGSSTL**

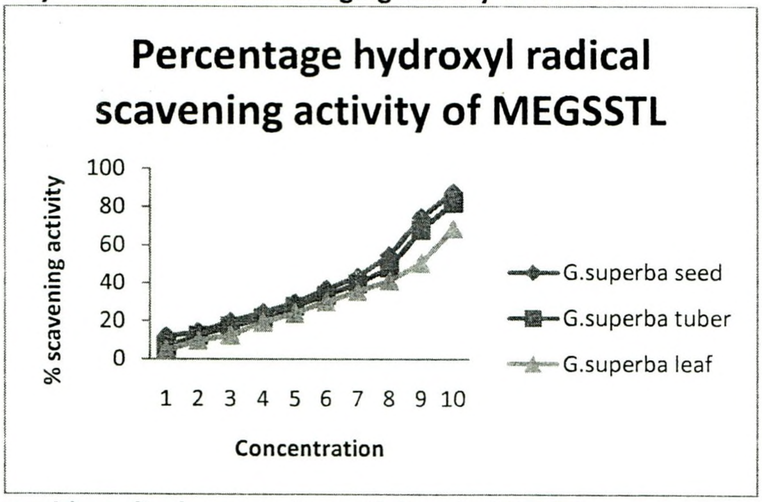
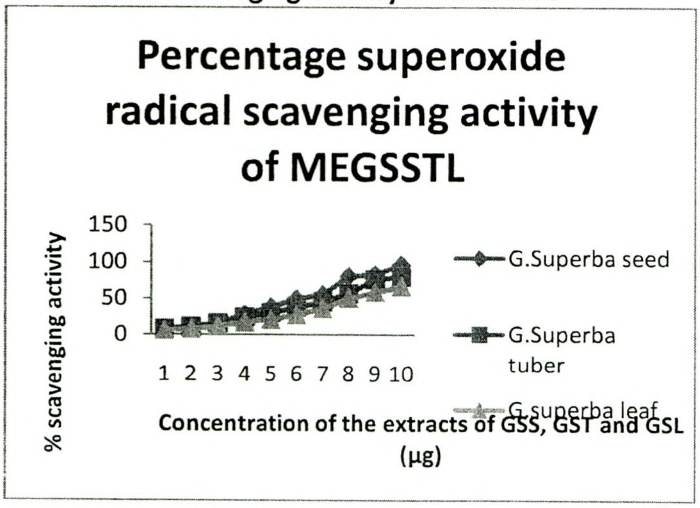



**Figure 5 Percentage superoxide radical scavenging activity of MEGSSTL**



**DISCUSSION**

DPPH (2,2-diphenyl-1-picryl-hydrazyl) is a stable free radial with red color (absorbed at 517nm). The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples. DPPH is a stable nitrogen-centered free radical the color of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron- donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers [15]. DPPH radical scavenging activity of MEGSSTL and the $IC_{50}$ value was found to be 38, 52 and 74 µg. The extracts of the *Abutilon indicum* stem showed promising free radical scavenging effect of DPPH in a concentration dependant manner [9]. *S. leptocarpa, A. Mexicana* and *S. afzelii* extracts exhibited good DPPH activity [14]. The methanolic extracts of leaves and flowers of *Lippia alba* exhibited very significant DPPH radical scavenging activity compared to the standard antioxidant ascorbic acid [1]. The methanolic extracts of the bark of *Ficus glomerata* exhibited remarkable DPPH free radical scavenging activity [5].

The ability of $H_2O_2$ to initiate lipid peroxidation is dependent on its ability to generate hydroxyl radical through the Fenton reaction. Harber Weiss reaction explains that both $H_2O_2$ and superoxide radical are required in the presence of metal catalyst for the formation of hydroxyl radical, the oxygen species largely responsible for the damage of macromolecules [3]. Hydrogen peroxide scavenging activity of MEGSSTL and the $IC_{50}$ value was found to be 50,63 and 68 µg. Organic fractions of *Garcinia kola* and *Njavara rice bran* showed the dose dependent Hydrogen peroxide scavenging activity respectively[21].

NO is an important chemical mediator generated by endothelial cells, macrophages, neurons and involved in the regulation of various physiological processes. Overproduction of NO can mediate toxic effects, *e.g.* DNA fragmentation, cell damage and neuronal cell death. NO does not interact with the bioorganic macromolecules such as the DNA or proteins directly[19]. During infections and inflammations, formation of NO is elevated and may bring about some undesired deleterious effects like renal dysfunction, tumor growth, etc. The peroxynitrite produced during the reaction of NO with $O^{2-}$ is probably responsible for genetic damage [23]. The MEGSSTL showed increasing activity of Nitric oxide as shown in Figure 3 with increasing concentration and the $IC_{50}$ value was found to be 61, 71 and 84 µg. The scavenging of nitric oxide by methanolic extract of *Pisonia grandis* (MEPG), ethylacetate fraction of *Pisonia grandis* (EAFPG) and ethanolic fraction of *Pisomia grandis* (EFPG) and Ascorbic acid standard were concentration dependent [12].

Hydroxyl radical is a highly reactive radical formed in biological systems and capable of damaging almost every molecule found in living cells. This radical has the capacity to induce carcinogenesis, mutagenesis and rapidly initiates lipid peroxidation [16]. The ability of MEGSSTL to scavenge 50 percent of hydroxyl radical was found to be 70,76 and 85 µg ($IC_{50}$). Free radical scavenging activity of fruit extract *Cucumis trigonus* was estimated using DPPH, Superoxide, Nitric oxide, Hydroxyl radical and Reducing power assay.

Superoxide anion is an oxygen-centered radical with selective reactivity. This species is produced by a number of enzyme systems in auto-oxidation reactions and by non enzymatic electron transfers that univalently reduce molecular oxygen. Overproduction of superoxide anion radical, contributes to redox imbalance and are associated with harmful physiological consequences [22]. MEGSSTL inhibited superoxide radical production *in vitro* in a dose dependent manner with an $IC_{50}$ of 58, 70 and 75 µg. Thus, MEGSSTL could maintain redox homeostasis in body by inhibiting superoxide anion production. The extracts of *Hygrophilla auriculata*, had good DPPH radical scavenging activity, but low Superoxide anion scavenging activity [28].

## CONCLUSION

The results of free radical scavenging showed that *MEGSSTL* has significant antioxidant activity. Further studies which aimed at the isolation and structure elucidation of antioxidant active constituents from the plant and *in vivo* studies are needed for better understanding the mechanism of action as an antioxidant.

## REFERENCES


1) Ara N, Nur H, In vitro antioxidant activity of methanolic leaves and flowers extracts of *Lippa alba*, Resarch Journal of Medicine and Medical science, 2009, 4(1), 107-110.
2) Arokiyaraj S, Martin S, Perinbam K, Arokianathan P M, Beatrice V, Free radical scavenging activity and HPTLC finger print of Pterocarpus sentalinus L. an *in vitro* study, Indian Journal of Science and Technology, 2008, 1(7), 1-3.
3) Ashwini SK , Kiran R , Soumya KV , Sudharshan SJ , Prashith Kekuda TR , Vinayaka KS , Raghavendra HL, Insecticidal and *in vitro* antioxidant potency of extracts of *Cryptolepis buchanani* roem. & schult, International Journal of Pharmaceutical Sciences, 2010, 2(1), 418-425.
4) Balakrishnan A, Kokilavani R, *In vitro* Free Radical Scavenging Activity of Ethanolic Extract of *Cucumis trigonus* Roxburxii fruit, International Journal of Pharmaceutical & Biological Archives, 2011, 2(5), 1439-1443.
5) Dildar Ahmed, Sharafat Ali Tariq, *In vitro* study of antimicrobial and antioxidant activities of methanolic extracts of leaves, fruits and bark of *Ficus glomerata,* Int. J. Med. Arom. Plants, 2012, 2(1), 30-33.
6) Elizabeth K, Rao M W A, Oxygen radical scavenging activity of Curcumin, Int J Pharmaceu., 1990, 58, 237-240.
7) Green L C, Wagner D A, Glouwski J, Skipper P L , Wishnok J S, Tannenbam S R, (), Analysis of nitrate and nitrite (15N) nitrate in biological fluids, Anal. Biochem., 1982, 126, 131-136.
8) Gulcin I, Bursal E, Sehitoglu H M, Bilsel M, Goren A C, Polyphenol contents and antioxidant activity of lyophilized aqueous extract of propolis from Erzurum, Turkey, *Food Chem. Toxicol.,* 2010, 48, 2227-2238.
9) Guno sindhu chakraborthy, Prashant m ghorpade, Free radical scavenging activity of Abutilon Indicum (LINN) sweet stem extracts, International Journal of Chem Tech Research, 2010, 2(1), 526-531.
10) Hasan S M R., Hossain Md M., Akter R., Jamila M., Mazumder Md E H, Rahman S, (), DPPH free radical scavenging activity of some Bangladeshi medicinal plants, Journal of Medicinal Plants Reasearch, 2009, 3(11), 875-879.
11) Jana S, Shekhawat G S, *Gloriosa superba*, 2010, 1, 1-2.
12) Jayakumari S, Arthanareswaran Vijayalakshmi A , Malarkodi Velraj, Ravichandran V, Free Radical Scavenging Activity of *Pisonia grandis* R.Br Leaves, Ind J Pharm Edu Res, 2012, 46(1), 1199-1202.
13) Kratchanova M, Dener P, Ciz M, Lojek A, Mihailor A, Evaluation of antioxidant activity of medicinal plants containing polyphenol compounds comparison of two extraction systems, ABP Biochimica Polonica Acta, 2010, 57(2), 229-234.
14) Lagnika L, Anago E, Sanni A, Screening for antibacterial, antioxidant activity and toxicity of some medicinal plants used in Benin folkloric medicine, Journal of Medicinal Plants, 2011, 5(5), 773-777.
15) Lavanya R, Uma Maheshwari S, Harish G, Bharath Raj J, Kamali S, Hemamalani D, Bharath Varma J, Umamaheswara Reddy C, *In-vitro* Antioxidant Activity of Methanolic Extract in Leaves of *Anisomeles malabarica Linn,* Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2010, 1(4), 738.
16) Manian R, Anusuya N, Siddhuraju P, Manian S, The antioxidant activity and free radical scavenging potential of two different solvent extracts of *Camellia sinensis* (L.) O. Kuntz, *Ficus bengalensis* L. and *Ficus racemosa* L., Food Chem., 2008, 107, 1000-1007.

17) McCord J M, Fridovich I, The reduction of Cytochrome C by Milk Xanthine Oxidase, J. Biol.Chem,, 1968, 43, 5753-5760.
18) Mensor LT, Menezes FS, Leitao GC, Reis AS, Dos Santos T, Coube CS, Leitao SG, Screening of Brazillian plant extracts for antioxidant activity by the use of DPPH free radical method, Phytotherapy Research, 2001, 15, 127-130.
19) Moni Rani Saha, Rumana Jahangir Md, Mynol Islam Vhuiyan, Israt Jahan Biva, In Vitro Nitric Oxide Scavenging Activity of Ethanol Leaf Extracts of Four Bangladeshi Medicinal Plants, S. J. Pharm. Sci., 2008, 1(1&2), 57-62.
20) Neveu V, Jimenez, Vos F, Crespy V, Chaffayt LD, Mennen L, Knox C, Eisner R, Cruz J, Wishart D, Scalber A, Phenol explorer; an online comphrensive database on polyphenol contents in food, Database, 2010, 1-9.
21) Okoko T, Chromatographic characterization, *in vitro* antioxidant and free radical scavenging activities of *Grarcinia kola* seeds, African Journal of Biotechnology, 2009, 8(24), 7133-7137.
22) Pervaiz S, Clement M, Superoxide anion: oncogenic reactive oxygen species, Int. J. Biochem. Cell Biol., 2007, 39, 1297–1304.
23) Roberfroid M, Calderon PB, Free radicals and oxidation phenomena in biological systems. Dekker, New York., 2008, 81-263.
24) Ruch RJ, Chang SJ, Klaunig JE, Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea, carcinogenesis, 1989, 10, 1003-1008.
25) Serbetci Tohma H, Gulcin I, Antioxidant and radical scavenging activity of aerial parts and roots of Turkish liquorice (*Glycyrrhiza glabra* L), *Int. J. Food Propert.,* 2010, 13, 657-671.
26) Sharma RK, Chatterji S, Raj DK, Menta R., Rai DK, Menta R, Rai RK, Singh RK, Watal G, Antioxidant activities and phenolic contents of the aqueous extracts of some Indian medicinal plants, Journal of Medicinal Plants Research, 2009, 3(11), 944-948.
27) Stankovic MS, Topuzovic M, Solujic S, Mihailovic V, Antioxidant activity and concentration of phenols and flavanoids in the whole plant and plant parts of *Teucrium chamaerdys* L. Var. *Glanduliferum Haussk*, Journal of Medicinal Plants Research, 2010, 4(20), 2092-2098.
28) Subhendu S, Mishra, Patel KK, Navdeep Raghuwanshi , Ashish Pathak , Prangya Parimita Panda, Kundlik Girhepunje, Niranjan Patro Ch, Screening of ten indian medicinal plant extracts for antioxidant activity, Annals of Biological Research, 2011, 2 (1), 162-170.
29) Zengin G, Aktumsek A, Guler G O, Cakmak Y S and Yildiztugay E, Antioxidant properties of methanolic extract and fatty acid composition of *Centaurea urvillei* DC. Subsp. *Hapekiana Wagenitz, Rec. Nat. Prod.,* 2011, 5, 123-132.

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