ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 8.4
IJAR 2023; SP4: 12-15

Neha Shekhawat
Department of Zoology, DPG Degree College, Affiliated to Maharishi Dayanand University, Rohtak, Haryana, India

Correspondence Author;
Neha Shekhawat
Department of Zoology, DPG Degree College, Affiliated to Maharishi Dayanand University, Rohtak, Haryana, India

(*Special Issue*)
"National Conference on
Multidisciplinary research for sustainable development"

Antioxidant capacity of ethanolic extracts of three traditional medicinal plants

Neha Shekhawat

Abstract
The reactive oxygen species induces oxidation which can result in protein damage, mutations in DNA as well as disintegration of cell membrane. It can lead to induce development of various ailments like malignancy, cardiovascular and liver disorders, whereas antioxidants with free radical scavenging role, play a potential role in protecting damages by reactive oxygen species. In the current study, antioxidant activities of *Clitoria ternatea, Guazuma ulmifolia* and *Madhuca indica* were investigated. Their ethanolic extracts were determined based on their scavenging activity of the stable compound 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical scavenging assay. Crude ethanolic extract of *C. ternatea, G. ulmifolia* and *M. indica* were tested for their antioxidant activity by DPPH method spectrophotometrically and ascorbic acid was used as standard. Low absorbance is an indication of low concentration of free radicals and high antioxidant activity. The maximum antioxidant potential was seen in ethanolic extract of *C. ternatea* (84.00%) with the 500µg/ml concentration and minimal activity was observed in *G. ulmifolia* (62.67%). It is found that all the extracts have prominent antioxidant activities. The IC_{50}'s (inhibition concentration 50) of the ethanolic extracts are *Clitoria ternatea* (3.93 µg/ml), *Guazuma ulmifolia* (5.88 µg/ml) and *Madhuca indica* (4.53 µg/ml). The results obtained in the present study indicate that *Clitoria ternatea, Guazuma ulmifolia* and *Madhuca indica* can be expected source of antioxidant agents. Therefore, the investigation clearly demonstrates that these three plants are exceptionally advantageous for human wellbeing.

Keywords: Antioxidant activity, *Clitoria ternatea, Guazuma ulmifolia, Madhuca indica,* Ethanolic extracts and IC_{50}.

1. Introduction
Antioxidants are significant source of decreasing oxidative stress that is lead to cause harm to important organic biomolecules (Bektas *et al*, 2005) [1]. Antioxidant compounds in edibles play a pivotal role in protecting health and wellness. Various clinical evidences suggests that antioxidants lessen the risk for chronic diseases like cancer, heart and liver diseases. Preliminary sources of naturally existing antioxidant compounds are vegetables, whole grains and fruits. Numerous plant antioxidants like vitamin C, vitamin E, carotenes, phenolic acids and phytoestrogens have been investigated as potent source of altering the risk of critical ailments (Seyydnejad *et al*., 2010) [2]. The vast majority of the antioxidants in a regular diet is plant based and belongs to different classes of complex molecules with a wide variety of physiochemical activities.
A compound with at least one unpaired electrons in its external orbit, is known as free radical (Jesberger and Richardson, 1991) [15]. These additional unpaired electrons make these species so unstable and hence very receptive for different biomolecules because of the presence of unpaired electron(s) (Karlsson, 1997) [16], they try to pair their electron(s) as well as produce a strongly stable compound. Antioxidants are also called as radical scavengers which give defence against free radicals that may cause pathological conditions such as neurodegenertion, anaemia, arthritis, inflammation, Parkinson's diseases, asthma, mongolism, ageing process, ischemia, and many other diseases.

Extensive literature survey revealed that flavonoids are far stretched secondary metabolites along with antioxidant and antiradical activities (Makari, *et al.*, 2008; Augustin, *et al.*, 2005; Aviram, 2000; Polterait, 1997; Prior, 2003; Trease and Evans, 1989; Lu and Foo, 2000; Koleva, *et al.*, 2002) [3, 4, 5, 6, 7, 9, 10]. Over the past few years, one of the areas which attracted a great deal of attention to the scientists, is the considerable remedial potential of plant based antioxidants in controlling deteriorating diseases which are associated with distinct oxidative damage. Numerous plant extracts as well as various classes of phytochemicals have been found to have quite prominent antioxidant property (Vani, *et al.*, 1997; Larson, 1988 and Tripathi, 1996) [11, 12, 13]. In the current study, we investigated the antioxidant potential of the crude extracts of three medicinally important plants. The majority of the active antioxidant compounds are possibly flavonoids, flavones, anthocyanins, coumarins, lignans, catechins and isocatechins.

Materials and Methods

Plant material and Preparation of extract

All the aerial parts of selected medicinal plants, *Clitoria ternatea, Guazuma ulmifolia and Madhuca indica* were collected freshly from the various field areas and get identified by the herbarium incharge of Botany Department, University of Rajasthan, where the research has been carried out as well as specimens voucher were deposited there. By using the Soxhlet Method of extraction, ethanolic extracts of these plants had been produced, 50 gm powdered dry plant material was extracted with 200 ml ethanol for 24 hours by soxhlet equipment. Then, the extracts were filtered by filter paper and concentrated under vaccum sounding apparatus for 30 min. All the ethanolic extracts were maintained and stored at 4 °C.

DPPH Radical Scavenging Activity

The antioxidant potential of the crude ethanolic extracts was investigated on the basis of their scavenging property of the stable compound 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical. DPPH is a strongly stable free radical possesses an odd additional electron in its structure and generally utilized for the recognition of radical scavenging reaction through chemical analysis. 1ml of each aliquot of all make-up concentrations (1-500 µg/ml) of the extracts was added to 3 ml of 0.004% ethanolic DPPH free radical solution. Then, after 30 minutes of span, the absorbance of the all the concentrations was taken at 517 nm by a UV spectrophotometer which was compared with the corresponding absorbance of standard ascorbic acid concentrations (1-500 µg/ml). Hatano *et al.*, described this method to measure the absorbance with slight modifications. At last, the % inhibition activity was calculated by the below formula:

$$\% \text{ Radical scavenging activity} = \frac{\text{Absorbance of bank} - \text{Absorbance of sample}}{\text{Absorbance of bank}} \text{ x } 100$$

Table 1: Free Radical scavenging activity of ethanolic extracts of *C. ternatea*, *G. ulmifolia* and *M. indica*.

Samples	Observations	Concentrations					
		1µg/ml	5 µg/ml	10 µg/ml	50 µg/ml	100 µg/ml	500 µg/ml
Standard	Absorbance	0.70	0.66	0.58	0.35	0.15	0.09
	% inhibition	6.66	12.00	22.67	53.33	80.00	88.00
C. ternatea	Absorbance	0.78	0.70	0.62	0.37	0.18	0.12
	% inhibition	4.00	6.67	17.33	50.67	76.00	84.00
G. ulmifolia	Absorbance	0.86	0.83	0.75	0.58	0.34	0.28
	% inhibition	1.33	4.00	8.00	22.67	54.67	62.67
M. indica	Absorbance	0.81	0.78	0.69	0.47	0.23	0.18
	% inhibition	2.67	4.00	13.33	37.33	69.33	76.00

Results

DPPH provides the free radicals that are basically utilized for evaluating primary radical scavenging activity of an individual secondary metabolite or a plant's specific extract. The current results suggest that all the investigated ethanolic plant extracts have moderate to potent antioxidant activity. The antioxidant potentials of the particular compound, present in the extracts may depend on structural parameters, likewise the number of different functional groups such as phenolic hydroxyl group, keto groups, free carboxylic groups and other structural characters (Patt and Hudson, 1990) [14]. *Clitoria ternatea* shows remarkable activity as compared to other experimental plants.

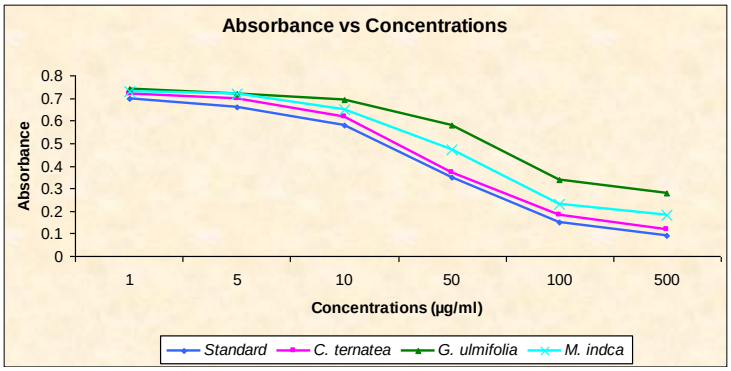

Fig 1: DPPH scavenging Assay of the ethanolic extracts of *Clitoria ternatea*, *Guazuma ulmifolia*, and *Madhuca indica* compared with standard ascorbic acid

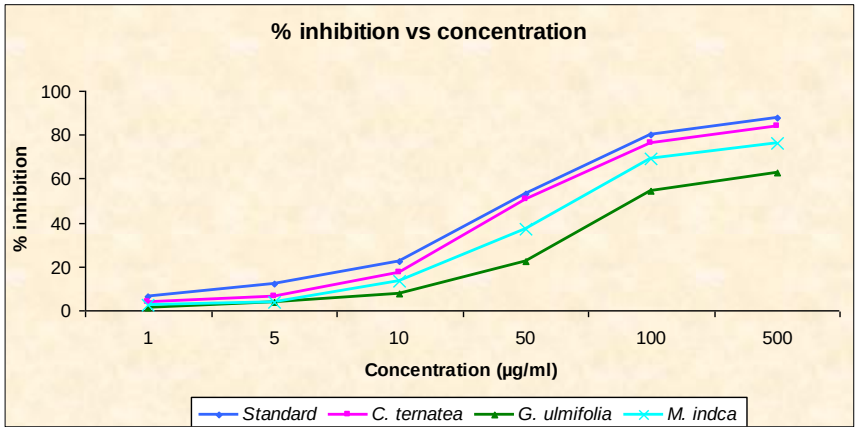

Fig 2: Evaluation of IC_{50} 's of the ethanolic extracts of *Clitoria ternatea, Guazuma ulmifolia, Madhuca indica* and standard ascorbic acid

From the Fig. 1 and 2, it is found that IC_{50}'s (inhibition concentration 50) of the ethanolic extracts are *Clitoria ternatea* (3.93 µg/ml), *Guazuma ulmifolia,* (5.88 µg/ml), and *Madhuca indica* (4.53 µg/ml), which indicates the significant antioxidant activity of the crude extracts.

Conclusions

In recent years, antioxidants have acquired importance because of their capability of neutralizing free radicals. They are commonly referred as micronutrients and scavengers, which play a pivotal role in human health as the natural biological defense mechanisms cannot operate under extreme oxygen stress. It is regularly acknowledged that, in circumstances of oxidative stress, responsive oxygen species, like superoxide (O_2), hydroxyl (OH) and peroxyl (OOH, ROO) radicals are produced. The reactive oxygen species play an important role related to the degenerative or pathological processes of various serious diseases such as cancer, coronary heart disease, cataracts, Alzheimer's disease, aging, inflammation and atherosclerosis (Kumpulainen and Salonen, 1999) [19].

As the worldwide situation is presently changing towards the utilization of nontoxic plant items having customary therapeutic use, these plants ought to be underlined for the control of different sicknesses. So, there is a developing interest of natural products in human diet, both due to the possible negative effects of synthetic food additives on human health and to the increased consumer perception of this problem in recent years. Various investigations exhibit that a vast number of medicinal important plants, aromatic herbs, fruits and leaves of few berry plant species biosynthesize certain phytochemicals which possess antioxidant properties and may be utilized as a native source of free radical scavenging bioactive compound (Javanmardi, *et al.*, 2002; Miliauskas, *et al.*, 2004; Sacchetti *et al*., 2005; Wang & Lin, 2000; Yu, *et al.*, 2005) [18, 20, 21, 22, 23]. The plants are preclinically evaluated to some extent; if these claims are scientifically evaluated clinically, then it can help mankind to provide remedies of numerous ailments.

References

1. Bektas T, Sokmen M, Akpulat HA, Sokmen A. *In vitro* antioxidant activities of the methanol extracts of five *allium species* from turkey. Food chem. 2005;92(1):89-92.
2. Seyydnejad SM, Niknejad M, Darabpoor I, Motamedi H. Antibacterial activity of hydroalcoholic extract of *Callistemon citrinus* and *Albizia lebbeck*. Am. J. Appl. Sci. 2010;7:13-16.
3. Makari HK, Haraprasad N, Patil HS, Ravikumar. *In Vitro* Antioxidant Activity of The Hexane And Methanolic Extracts Of Cordia Wallichii And Celastrus Paniculata. The Internet J. Aesthetic and Antiaging Medicine. 2008;1:1-10.
4. Augustin S, Claudine M, Christine M, Christian R. Dietarypolyphenols and the prevention of diseases. Crit. Rev.Food Sciences. 2005;45:287-306.
5. Aviram M. Review of human studies on oxidative damage andantioxidant protection related to cardiovascular diseases. Free Rad. Res. 2000;33:S85-S87.
6. Polterait O. Antioxidants and free radical Scavengers of Natural origin Current Org. Chem. 1997;1:415-440.
7. Prior RL. Fruit and vegetables in the prevention of cellular oxidative damage. American Journal of Clinical Nutrition. 2003;78:570S-578S.
8. Trease GE, Evans WC. Trease and Evans Pharmacognosy: A Physicians's Guide to Herbal Medicine. 13th Edition. Bailliere, Tindall. London; c1987.
9. Lu Y, Foo Y. Antioxidant activities of polyphenols from sage (*Salvia officinalis*.) Food Chem. 2000;75:197-202.
10. Koleva II, Van Beek TA, Linssen JPH, De Groot A, Evstatieva LN. Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. Phytochemical Analysis. 2002;13:8-17.
11. Vani T, Rajani M, Sarkar S, Shishoo CJ. Antioxidant properties of the Ayurvedic formulation Triphala and its constituents. Int. J. Pharmacognosy. 1997;35(5):313-317.
12. Larson RA. The antioxidants of higher plants. Phytochem. 1988;27(4):969-978.
13. Tripathi KD. Essentials of Med. Pharmacol. 1996;3:432.
14. Patt DE, Hudson BJF. Natural antioxidants not exploited commercially. In: food antioxidants. Hudson B.J.F., Ed: Elsevior Applied Science: London, U. K.; c1990. p. 171-191.
15. Jesberger JA, Richardson JS. Oxygen free radicals in brain dysfunction. Intl. J. Neurosci. 1991;57:1-17.
16. Karlsson J. Introduction to Nutraology and Radical Formation. In: Antioxidants and Exercise. Illinois: Human Kinetics Press; c1997. p. 1-143.

17. Hatano T, Kagawa H, Yasuhara T, Okuda T. Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. Chem. Pharm. Bull. 1988;36:1090-2097.
18. Javanmardi J, Khalighi A, Kashi A, Bais HP, Vivanco JM. Chemical characterization of basil (*Ocimum basilicum* L.) found in local accessions and used in traditional medicines in Iran. Journal of Agricultural and Food Chemistry. 2002;50(21):5878–5883.
19. Kumpulainen JT, Salonen JT. Natural Antioxidants and Anticarcinogens in Nutrition, Health and Disease, The Royal Society of Chemistry, UK; c1999. p. 178-187.
20. Miliauskas G, Venskutonis PR, Van Beek TA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chemistry. 2004;85(2):231-237.
21. Sacchetti G, Maietti S, Muzzoli M, Scaglianti M, Manfredini S, Radice M. *et al*. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chemistry. 2005;91(4):621-632.
22. Wang SY, Lin HS. Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. Journal of Agricultural and Food Chemistry. 2000;48(2):140-146.
23. Yu LL, Zhou KK, Parry J. Antioxidant properties of cold pressed black caraway, carrot, cranberry, and hemp seed oils. Food Chemistry. 2005;91(4):723-729.