EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 10
Issue: 10
263-266
Year: 2023

DETERMINATION OF ANTIOXIDANT POTENTIAL OF *ACACIA NILOTICA* LEAF EXTRACT USING FENTON REACTION

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Article Received on 02/08/2023 **Article Revised on 23/08/2023** **Article Accepted on 13/09/2023**

ABSTRACT
From ancient times, plants and plant-derived products have been used as folkloric medicines for a variety of health disorders owing to their tremendous therapeutic potential. The leaves of *Acacia nilotica* are widely used in the Indians traditional medicine to treat various types of diseases. The present study aimed to determine the antioxidant efficacy of crude *Acacia nilotica* leaf extract in the In vitro test system as Fenton reaction. The dried leaves of Acacia nilotica was extracted with methanol using a Soxhlet extractor. The total phenolics content of leaf as determined by Fenton reaction and was found to be good antioxidant activity as dose depended manner. The antioxidant activity of plant extract was carried out with ascorbic acid as a standard reducing agent. All the analysis was made with the use of UV-Visible Spectrophotometer. In this plant Acacia nilotica leaf extract there was a remarkable concentration dependent free radical scavenging and reducing power was exhibited. In conclusion the present study indicates that Acacia nilotica leaf extract may be a potential source of natural antioxidant. The results suggested that the studied leaves have strong antioxidant potential. Further studies are being carried out in order to provide complete data of the antioxidant activity and characterization of the principle antioxidant agents, which can be used to treat various oxidative stress-related diseases.

KEYWORDS: Antioxidant activity, Fenton Reaction, Hydroxyl radical, ascorbic acid, Acacia nilotica, TBARS.

1. INTRODUCTION
Natural products from dietary components such as Indian spices and medicinal plants are known to possess antioxidant activity (Devasagayam, 2004). Acacia nilotica (L.) Del. is a medicinal plant belonging to the family Fabaceae. The plant is widely distributed in tropical and subtropical regions. A. nilotica is rich in bioactive compounds and used for prevention and treatment of various ailments and infectious diseases. In ayurvedic medicine practice, it is believed that leaves, bark and pods of A. nilotica can be used against cancer, diarrhea, fever and menstrual problems (Ambasta, 1994). The plant is rich in polyphenolic compounds, in which catechins are hypothesized to possess antioxidant and anti-inflammatory activities (Maldini, et al., 2011). A. nilotica has been reported to have inhibitory effect against hepatitis C virus protease (Hussein, et. al., 2000) and multidrug resistant bacteria pathogens (Sadiq, et al., 2017). In aerial parts of the plant, a variety of phenolic compounds were identified with a wide range of biological activities (Singh, et al., 2008). In recent years, researchers have tried to isolate strong, nontoxic antioxidants from edible plants to prevent autoxidation and lipid peroxidation with the aim to replace synthetic antioxidants (Tamuly, et al., 2015). Plant extracts containing high amounts of bioactive compounds especially antioxidants, have the potential of being used in food, agriculture, nutraceuticals, cosmetics and pharmaceutical products (Tuncel, et al., 2015).

Free radicals (Reactive oxygen species and Reactive nitrogen species) are responsible for DNA damage which leads to inflammatory diseases and progression to cancer (Wiseman and Halliwell, 1996). In the past several years, unprecedented progress has been made in the recognition and understanding of roles of reactive oxygen species in many diseases. These include atherosclerosis, vasospasms, cancers, trauma, stroke, asthma, hyperoxia, arthritis, heart attack, age pigments, dermatitis, cataractogenesis, retinal damage, hepatitis, liver injury, and periodontis, which are age-related. The body protects itself from the potential damages of reactive oxygen species. Its first line of defense is superoxide dismutases, glutathione peroxidases, and catalase. Scientists have indicated that antioxidant nutraceuticals supplied from daily diets quench the reactive oxygen species or are required as cofactors for antioxidant enzymes (Lee, et. al., 2004).

An hypothesis for the role of free radicals in cancer was elaborated by D. Harman in 1962 who suggested that it might be possible to reduce the extent of damage caused

by free radicals through three dietary changes: (i) caloric reduction, i.e., lowering the level of free radical reactions arising in the course of normal metabolism; (ii) minimize dietary components that tend to increase the level of free radical reactions (e.g., polyunsaturated fats); and (iii) supplement the diet with one or more free radical reaction inhibitors (anti-oxidants). With respect to (ii) and (iii), lipid peroxidation exemplifies the type of chain reaction initiated by free radicals, with unsaturated fatty acids being the primary center of free radical attack. Anti-oxidants act as free radical scavengers and are able to terminate these reactions (Black, 2002). Still no work is performed on Acacia nilotica leaf as antioxidant. Thus main objective of this study to explore the antioxidant activity of methanolic seed extracts.

2. MATERIALS AND METHODS

Plant material – Acacia nilotica leaf was collected from Local Herbal Garden, Raipur (Chhattisgarh), India.

Chemicals and Reagent samples – The reagents used were of highest purity (>99.95%) and were purchased from Sigma Chemical Co. (Germany) and other. Sample absorbances were read using a Lambda 532 nm, UV Spectrometer made by Varian.

Preparation of extract - Dried powdered of Acacia nilotica leaf (10 g) were extracted by continuous mixing in 100 ml 50% methanol, 24 h at room temperature. After the filtration process, methanol was evaporated until only water remained through evaporation on water bath at 60-70 0c temperature. The final extract was kept in air tied box.

Fenton Reaction to assess OH -radical scavenging activity

The OH- radical scavenging activity of Acacia nilotica leaf extract (10–100 ug/ml) was determined according to the deoxyribose method reported of Halliwell, *et al*., (1987). In the protocol the presence of 100 lM EDTA. $FeCl_3$, H_2O and ascorbic acid were prepared in degassed H_2O prior to use. The reaction tube contained (final concentrations) 3.6 mM deoxyribose, 100 lM EDTA, 1 mM H2O2, 100 lM L- ascorbic acid, 100 lM $FeCl_3$, H_2O in 25 mM phosphate buffer, pH 7.4 in 1.0 ml total volume. Samples was kept in incubation at 38º C, 1 hrs, 1.0 ml 1.0% TBA in 0.05 M NaOH and 1.0 ml 10% TCA were added to the reaction mixture after that samples was heated in a boiling water bath for 15 min. After the samples were cooled, the absorbances were read at 532 nm. The IC50 value of the plant extract was compared with that of ascorbic acid, which was used as the standard. The lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The percentage of inhibition of hydroxyl radical was calculated as follows:

$$\% \text{ Inhibition} = \text{Abs: } \frac{532 \text{ nm Control Abs.} - 532 \text{ nm sample Abs.} \times 100}{532 \text{ nm Control Abs}}$$

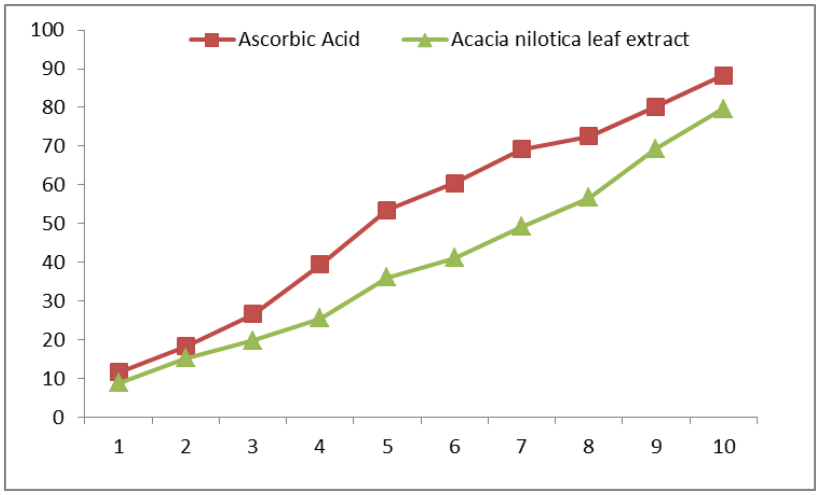
Antioxidant capacity of test compounds was expressed as IC_{50}, the concentration necessary for 50% inhibition concentration of TBARS.

3. RESULT

The result of the facts of the examined *Acacia nilotica* leaf extracts as well as control solution on OH^- radical production. They show that all extract of Acacia nilotica leaf extract and control solution as an Ascorbic acid inhibited the production of OH^- radicals. The % of free radical scavenging activity of the hydro-ethanolic extract of *Acacia nilotica* presented in table 1 have reducing power, the free radical OH^- scavenging activity of the extract increases with increasing the concentration.

Table 1: Antioxidant Activity of *Acacia nilotica* leaf extract using Fenton reaction.

Concentration (in µl)	% of inhibition	
	Ascorbic acid (Mean ± SE)	Acacia nilotica leaf extract (Mean ± SE)
10	11.60±0.88	08.74±0.45
20	18.23±1.12	15.16±0.87
30	26.67±0.45	19.72±0.80
40	39.48±0.27	25.44±1.27
50	53.52±1.52	36.14±1.00
60	60.46±0.45	41.10±0.55
70	69.24±0.94	49.11±0.84
80	72.55±0.42	56.72±0.96
90	80.24±0.40	69.32±0.52
100	88.29±0.25	79.71±0.23
Blank - 0.31O		



Graph 1: Show the antioxidant Activity of Ascorbic acid and *Acacia nilotica leaf extract* extract using Fenton reaction.

4. DISCUSSION AND CONCLUSION

Acacia nilotica Linn commonly known as Babul is a multipurpose tree. As the world is turning back towards the herbal drug, it is the need of the hour to re-evaluate the knowledge of traditional medicine through vast review. In the Unani traditional system of medicine, all parts of the plant have been used as a remedy for various diseases and are imputed for their medicinal properties.

Medicinal plants have a long history of use for the benefit of mankind. According to the report of the World Health Organization (W. H. O), about 80% of the world's population relies chiefly on traditional therapies (Tyagi, et al., 2016). Acacia nilotica Linn commonly known as Babul and Kikar has been used in Unani and other Indian System of Medicine for hundreds of years for the prevention and treatment of various health ailments. It was first described by Linnaeus in 1773 (Bashir, et al., 2014). A. nilotica L belongs to the kingdom Plantae and family Fabaceae (Rather, et al., 2015). It is the second-largest genus of the family Fabaceae, with about 1350 species. It is distributed throughout tropical and warm temperate areas of the world like Asia, Australia, Africa and America (Sharma, et al., 2014; Rajvaidhya, et al., 2012). A. nilotica has various complex phytoconstituents including alkaloids, volatile essential oils, phenols, phenolic glycosides, and terpenes. These types of phytoconstituents play a role in the therapeutic actions of A. nilotica. Earlier traditional description confirmed that A. nilotica has a rich amount of nutrients and contains a high therapeutic value which is capable of prevention, mitigation, and treatment of various infectious diseases and deleterious conditions (Sadiq, et al., 2015). The studies based on the animal model established that A. nilotica and its chief phytoconstituents play a pivotal role in anti-bacterial, anti-inflammatory, anti-diabetic, anti-cancer, and anti-hypertensive management. It is considered a safe medicinal plant and modulates the numerous therapeutic actions without any adverse effect.

In our study the antioxidant activity of Acacia nilotica leaf extracts as well as positive control on OH^- radical production. They show that all concentration of seed extract of *Acacia nilotica* extract and control solution as an Ascorbic acid inhibited the production of OH^- radicals. The % of free radical scavenging activity of the hydro-ethanolic extract of Acacia nilotica presented in table1 have reducing power, the free radical OH^- scavenging activity of the extract increases with increasing the concentration.

The extracts showing free radical scavenging activity and total Antioxidant activity contain phenolic compound, suggesting that the antioxidant activity may be due to a great extent to the polyphenol content present in extract.

Above in vitro studies shows that ethanolic extract of *Acacia nilotica* leaves possess promising antioxidant, it may be due to polyphenol and other chemical constituent present in extract. Identification of all chemical constituent in seed extract that are responsible for antioxidant activity requires further investigation, The crude methalonic extract merits further experiments in vivo. However, present study showed new natural antioxidant that can replace the synthetic ones to be used in foods and cosmetics.

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