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ARTICLE

# Determination of Antioxidant Activity of Acacia Nilotica Linn from different Regions of Baluchistan (Pakistan)

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Abstract
Free radicals are naturally occurring species with unpaired electrons that are formed during normal metabolic processes. Their formation in a high amount may cause oxidation of essential structural molecules of cell, the condition is known as oxidative stress which results in several health issues such as cancer, diabetes, heart diseases, inflammatory diseases, arthritis and a lot more. To overcome these effects, antioxidants, both synthetic and natural ones are consumed by people. Due to recent evidences about the long term harmful effects of synthetic antioxidants the interest towards the natural sources has increased. The aim of the current research is to comparatively analyze the antioxidant activity of acacia Nilotica Linn samples collected from different regions of Makran by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay on which no work has been done so far. The DPPH assay of the samples gave the following results with antioxidant activity values as 18.51851%, 4.47761%, and 8.25958% for the samples of acacia Nilotica Linn from Shahrak, acacia from Turbat, acacia from Pedrak. The results revealed that acacia Nilotica Linn from Shahrak exhibits maximum free radical scavenging activity. More work on chemical composition of the tested samples has to be done.

**Keywords:** Acacia Nilotica Linn, free radical, antioxidants, DPPH assay

## 1. INTRODUCTION

Antioxidants poly a significant role for the protection of health. According to scientific confirmation antioxidants decrease the risk for many chronic diseases which include heart and cancer diseases [1]. Naturally occurring major sources of antioxidants are vegetables, fruits, leaves etc [2]. The antioxidant compounds are phenol and polyphenols, flavonoids, carotenoids, steroids and thiol these are natural supplements [3]. The benefits of these antioxidants is that they provide antioxidant defense against oxidative stress and show low chances of causing diseases. The antioxidants of the body are insufficient to protect the body thus supply of external antioxidants is also necessary [4]. Potential approaches for natural antioxidants have been examined in different plant materials. A number of diseases affected by (ROS) which are formed during metabolic processes of cell [5]. In normal conditions for immunity of the biological system since it protects the body from being infected by bacterial and viruses. But their excessive production can harm the tissues [6]. These free radicals cause lipid peroxidation in membrane which is considered to be the starting step towards the cell-injury [7].These free radicals are stabilized by antioxidants by reacting with them before they oxidized any cellular component and in this way they protect the cell from

oxidative injury [8].

Pakistan, which is an agricultural country, rich in aromatic and medicinal plants which are used as traditional medicine for health care [9]. Acacia nilotica is a plant which has known for its useful sources of bioactive properties. Acacia nilotica which gives number of bioactive components that shows anti-hypertensive, anti-platelet, aggregatory, anti-inflammatory, spasmogenic, antispasmodic and vasoconstrictor properties [10]. An inexpensive and a fast simple method for the measurement of antioxidant activity involves the use of ( DPPH) which is being used to test the ability of compounds which act as hydrogen donor or free radical scavengers to determine antioxidant activity [11]. The technique of (DPPH) test is based on the (DPPH) reduction which is a stable free radical. The unpaired electrons of free radical (DPPH) shows extreme absorption at 517 nm with (purple color) [12]. As the antioxidant and DPPH react with each other which was the stable radical it becomes paired off because due to the existence of a H-donor and it will became DPPH-H and as concerns the absorptions reduced from the DPPH[13]. The DPPH-H is form, which results in decolonization (yellow color) with respect to total taken electrons [14]. This plant has been testified to own antioxidant properties. So this work has been carry out to assess Acacia nilotica plant for their possible potential to antioxidant action by DPPH Scavenging method [15].

## 2. EXPERIMENTAL WORK

### 2.1 Materials and Methods

### 2.2 Sample collection

Leaves of acacia Nilotica commonly known as desi-kiker were collected from three regions of Makran division of Baluchistan (Pakistan). Each sample were given a number as sample 1, sample 2, sample 3. Sample 1, collected from Shahrak in 25th October 2020, sample 2, collected from Turbat in 30th October 2020, sample 3, collected from Pedrak in 3rd November 2020.

### 2.3 Chemicals and reagents

All the chemicals and reagents used were of analytical evaluation. Ethanol from sigma Aldrich (www.sigma-aldrich.com), distilled water, and DPPH (2, 2-reagent 95%) from Alfa Aesar, united states.

### 2.4 Extraction

Air-dried leaves of this plant were ground into powered. 1 gram of each sample was dissolved individually in of 100ml 70% ethanol (absolute). After stirring each sample solution for 5 minutes they were stirred and kept for 5 days at room temperature [11]. After the given period of interval each solution was stirred by magnetic stirrer for 30 minutes and then these were filtered by wattman filter paper to remove the solid residues.

### 2.5 DPPH radical scavenging assay

Free radical scavenging activities of different leaves extract were measured by (DPPH). In brief, 0.5ml sample solution, 3ml ethanol absolute and 0.3ml of freshly prepared solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution were mixed to get four tests of each sample. Four blanks for each sample were prepare by mixing 3.3 ethanol absolute and 0.5mal sample solution. A control was prepared by adding 0.3mal (DPPH) solution in 3.5ml ethanol and all these were kept in dark for 2hours at room temperature. After that absorbance of control, blacks and tests were measured at 517nm (UV-visible Spectrophotometer (UV 752 (D), China). Ascorbic acid which is a synthetic antioxidant was used as a standard. A 25ppm ethanolic solution of ascorbic acid as standard. The blank and test of ascorbic acid solution were prepared by following the same protocol as was used for the sample and absorbance was measured [12, 13].

### 2.6 Statistical analysis

Reduction in absorbance is a degree of better potential of substance is scavenging activity. The antioxidant activity is determined and calculated by using the equation 1.

DPPH Scavenging effect (%) or percent inhibition = A°-

$A^1 / A^\circ * 100$ (equation. 1)

Where $A^\circ$ was the absorbance of control reaction and $A^1$ was the absorbance in presence of test or standard sample.

## 3. RESULTS AND DISCUSSION

The acacia nilotica leaves showed greater antioxidant potential when these are compare with the standard ascorbic acid by DPPH method [14]. The absorbance is measured at 517nm the analysis involves UV-visible spectrophotometer which shows maximum absorbance of sample 1 (18.51851%) and 8.25958%, 4.47761% shown by the sample of Turbat and Pedrak. For standard ascorbic acid value were obtained as 22.28571%.

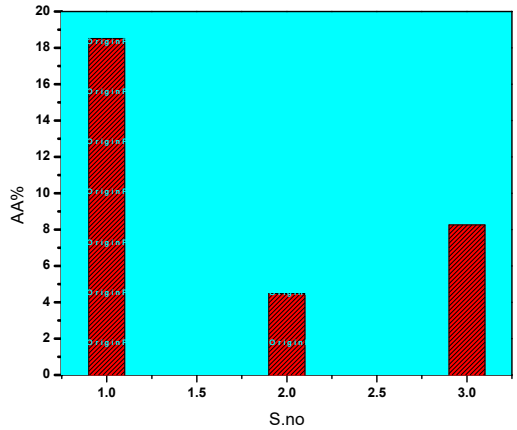


FIGURE 1. Absorbance of different extract of acacia nilotica at 517nm by uv-visible spectrophotometer by DPPH Scavenging assay method.

It means that plant species gives better antioxidant activity by free radical scavenging method as they are compared with standard. So, we can say that acacia nilotica has potential antioxidant activity and it can get a way towards the pharmaceutical uses. The values of control and blank are given in Table 1.Determination of AA% in real samples of acacia Nilotica Linn is given Table 2. AA% of standard (Ascorbic acid) is given Table 3. Absorbance of different extract of acacia nilotica at 517nm by uv-visible spectrophotometer by DPPH Scavenging assay method is given Figure 1.

TABLE 1. Values of control and blanks.

| Solution | Absorbance | Mean± SD | Co variance |
|---|---|---|---|
| Blank 1 | 0.200 | 0.201 | 0.00082 |
| | 0.201 | | |
| | 0.202 | | |
| Blank 2 | 0.152 | 0.153 | 0.00082 |
| | 0.153 | | |
| | 0.154 | | |
| Blank 3 | 0.148 | 0.1493 | 0.00129 |
| | 0.149 | | |
| | 0.151 | | |
| Control | 0.270 | 0.271 | 0.00082 |
| | 0.271 | | |
| | 0.272 | | |

TABLE 2. Determination of AA% in real samples of acacia Nilotica Linn.

| S. No | Sample type | Absorbance | Mean± SD | Co variance | AA% |
|---|---|---|---|---|---|
| 1 | Acacia from Shahrak | 0.420<br>0.421<br>0.422 | 0.421 | 0.00082 | 18.51851% |
| 2 | Acacia from Turbat | 0.344<br>0.345<br>0.346 | 0.345 | 0.00082 | 4.47761% |
| 3 | Acacia from Pedrak | 0.459<br>0.460<br>0.461 | 0.46 | 0.00082 | 8.25958% |

TABLE 3. AA% of standard (Ascorbic acid).

| Absorbance | Meann± SD | Co variance | AA% |
|---|---|---|---|
| 0.220<br>0.221<br>0.222 | 0.221 | 0.00082 | 22.28571% |

## 4. CONCLUSION

Antioxidants are one of the major requirements of our body in today's world where there are higher risks of disease due to increasing environmental pollution. Since the pollution and several other factors contribute as exogenous source of free radical formation resulting in oxidative stress that results in a number of chronic health issues. For a long period of time the attention of health care institutions and product manufacturers are seeking for the best natural source of antioxidants that are of high value due to their safety assurance. The long term harmful effects of synthetic antioxidants have grabbed the attention of researchers to seek for the safe natural and low cost antioxidant sources. Plants and the derivatives of plants have been proven as the best source of antioxidants. This work

reveals new essential natural sources of antioxidants that are regional acacia samples from three different regions of Makran. The results indicate that all these natural products are significant antioxidants that need to be further analyzed for their chemical composition.

**Authors Contribution**

M.H supervised research work and has the main idea. ZAA did practical work and wrote the manuscript. FH, AZ, MH, SM, NU and MJ revised the manuscript and provided suggestions. FK is coworker in research. AH coworker in research.

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**Conflict of Interest**

The authors declare no conflict of interest. All the authors approved the submission of the manuscript.

**Data Availability statement**

The data presented in this study are available on request from the corresponding author.

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