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# PHYTOCHEMICAL ANALYSIS AND EVALUATION OF ANTIOXIDANT PROPERTY OF *ACACIA NILOTICA*

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## ABSTRACT

*Acacia nilotica* is a plant mainly used to treat various diseases; it acts as rich source of polyphenols therefore possess antioxidant potential.It used to treat diarrhoea and diabetes, also used as anti-fungal, anti-HCV and anti-plasmodial agents. Toothbrushes are made out of the tender twings. Chemically plant contains phenolic compounds, physterols, lipids, flavanoids, saponins and fixed oils, etc. Alkaloids and glycosides present in roots of *Acacia nilotica*. The plant offers several therapeutic values, considering this present study was planned to evaluatephytochemical and antioxidant properties of *Acacia nilotica*. Study reported various quality parameters and antioxidant potential of plant.

**Key-Words:** ***Botany, Plant, Acacia nilotica, Antioxidant***

## Introduction

*Acacia nilotica* is a traditional plant and its descriptions in classical texts available due to its medicinal value. The pods of plant used to reduce arterial blood pressure, treat diarrhoea and diabetes, etc. The presence of phyto-constituents like glycosides, phytosterols, carbohydrates,phenolic compounds, flavonoids and saponins, etc. contributed towards the therapeutic values of plantextract [1-5].

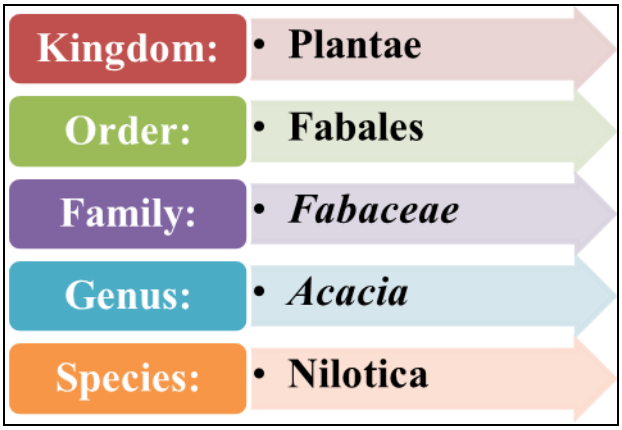
***The various parts of plant used for specific purposes as follows:***

- ✓ Inner bark contains tannin, which is used for tanning and colouring leather black.
- ✓ Twigs are used for tooth brushes.
- ✓ Young bark is used as fibre.
- ✓ The fruit exhibits algicidal activity against many species.

**Ayurvedic properties of *Acacia nilotica*:**

- ❖ *Rasa:* *Kashaya*
- ❖ *Guna:* *Guru* and *Ruksha*
- ❖ *Veerya:* *Sheeta*
- ❖ *Veepaka:* *Katu*

**Botanical Descriptions:**




*Acacia nilotica*, also known as *Vachellia nilotica*, it is a tree that grows to a height of 5 to 20 metres. It has a dense spherical crown, stems and branches that are often dark to black in colour, fissured and have a grey-pinkish slash. It also exudes a reddish gum [6-8]. It is an evergreen, medium-sized, crooked tree as depicted in **Figure 1**.

**Figure 1: *Acacia nilotica* Tree**

Considering therapeutic and traditional importance of plant, present study was planned to evaluate antioxidant properties of *Acacia nilotica*. The phytochemical and analytical standards also studied and reported to ensure quality parameters of plant extract.

**Material and Methods:**

**Collection, Identification and Authentication of Plant Material:**

The plant was collected from Indore districts and identified by Head department of Dravyaguna, Govt. Asthang Ayurveda College, Indore, (M.P.).

**EXTRACTION:**

The plantmaterial cleaned and ground to powder or cut into small pieces,that after soaked in petroleum ether first for defeating with occasional shaking atroom temperature for about 4-5 days.

**Successive solvent extraction:**

Soxhlet apparatus was used for the successive extraction of plant material; in this process powdered plant material wasextracted sequentially with petroleum ether, chloroform and methanol.

**Phytochemical Analysis of crude extracts:**

The presence of phyto-constituents was confirmed by different tests described for phyto-chemical analysis. Various tests were conducted to perform qualitative analysis for the presence of different phyto-constituents.

**CHROMATOGRAPHIC PURIFICATION:**

**Thin layer chromatography (TLC)**was performed for separating different constituents present in plant extract. The extract was concentrated by evaporating solvent and finally dried using vacuum drying technique. The sampling was done by dissolving sample into volatile solvent and let allowed solvent to get evaporated. Sample was applied to the plate using a thin capillary andfinallyeluted spots were detected in UV-chamber.

**High-performance thin layer chromatography (HPTLC):**

Several mobile phases were used for the HPTLC analysis, and the final mobile phase was chosen based on trial-and-error analysis. The sample was applied using a micro-injector, and the mixture included in the plant extract was separated using the gradient elution technique.

**Infrared spectroscopy:**

In order to determine the functional groups present in plant extract, IR spectroscopy was used. In this case, the KBr pellet technique was used to apply the sample, and structure elucidation was done using spectra that were obtained after certain functional groups present in the plant sample absorbed IR radiation.

***IN-VITRO* ANTI-OXIDANT ACTIVITY:**

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) techniquewas used to perform anti-oxidant activity of the fractions obtainedfrom columnchromatography.

**Preparation of standard solution:**

Methanol was used to dissolve the necessary amount of ascorbic acid to create concentrations of 50, 100, 250, 500, and 750µg/ml.

**Preparation of test sample:**

To create sample stock solutions, 10 mg of dried methanolic extract were dissolved in 10 ml of methanol to achieve a concentration of 1 mg/ml.

**Preparation of DPPH solution:**

In order to protect it from light, the test tubes were covered with aluminium foil containing 4.3 mg DPPH dissolved in 3.3 ml of methanol.

**Protocol for estimation of DPPH scavenging activity:**

The absorbance was measured at 516 nm right away after 50 ml of DPPH stock solution and 3 ml of methanol were added for the control reading. The test material was screened at various concentrations (50, 100, 250, 500, and 750 µg/ml), and each dose was made with methanol as the solvent. DPPH solution were transferred to the test tube,after 20 minutes of incubation, absorbance at 516 nm was measured using methanol as a blank in a UV-Visible spectrophotometer (Systronics). The % decrease and $IC_{50}$ values were calculated using the following formulas [9, 10].

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

**Results and Discussion**

Analyses of the phytochemistry and pharmacology of particular plantwas part of the current investigation. Chloroform, petroleum ether, and methanol were successively extracted from the dried plant bark material using Soxhlet apparatus. The appearance of different extractsis depicted in **Table 1**.

**Table 1: Physical appearance of different extracts of *Acacia nilotica*:**

| S. No. | Extract | Physical Appearance |
|---|---|---|
| 1 | Methanol Extract | Deep Green and Sticky |
| 2 | Petroleum Ether | Dark Brown and Oily |
| 3 | Chloroform Extract | Green and Sticky |
| 4 | Ethyl acetate | Dark Brown and Gooey |

**Phyto-chemical analysis:**

The various tests were performed for the phyto-chemical analysis of constituents present in plant extract.Petroleum ether extract contains alkaloid and flavonoid. Methanol extract contains alkaloid, flavonoid, saponins and tannins. Chloroform extract showed presence of flavonoid. Methanol extract showed presence of maximum constitutes of therapeutic importance.Chloroform extract showed presence of only flavonoid. The presence of alkaloid, flavonoid, saponins and tannins attributed for the medicinal value of plant.

**Thin layer chromatography (TLC)**

The result of TLCanalysis showed different fractions obtained from the extract of *Acacia nilotica*. The $R_f$ value in

Chloroform: methanol (90:09), Chloroform: methanol (81:15), Chloroform: methanol (45:46) and Chloroform: methanol (00:98) were found to be 0.08, 0.16, 0.73 and 0.88 respectively, while in other eluting solvents no residue was observed as depicted in **Figure 2.**

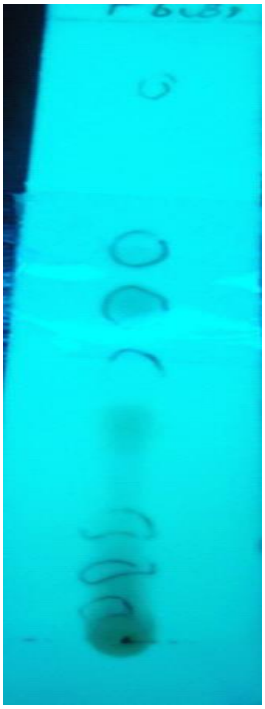


**Figure2: TLC Plate of fraction of *Acacia nilotica***

**High-performance thin layer chromatograph (HPTLC)**

The HPTLCanalysis showed different retention time for various constituents present in plant extract (**Figure 3**). The retention time for various constituent present in *Acacia nilotica* were observed as follows:

| *Phyto-constituents* | *Retention Time* |
|---|---|
| ✓ Alkaloids | 11.806 |
| ✓ Flavanoids | 48.628 |
| ✓ Phenol | 28.050 |
| ✓ Terpenoids | 30.017 |
| ✓ Steroids | 31.785 |
| ✓ Saponins | 48.517 |

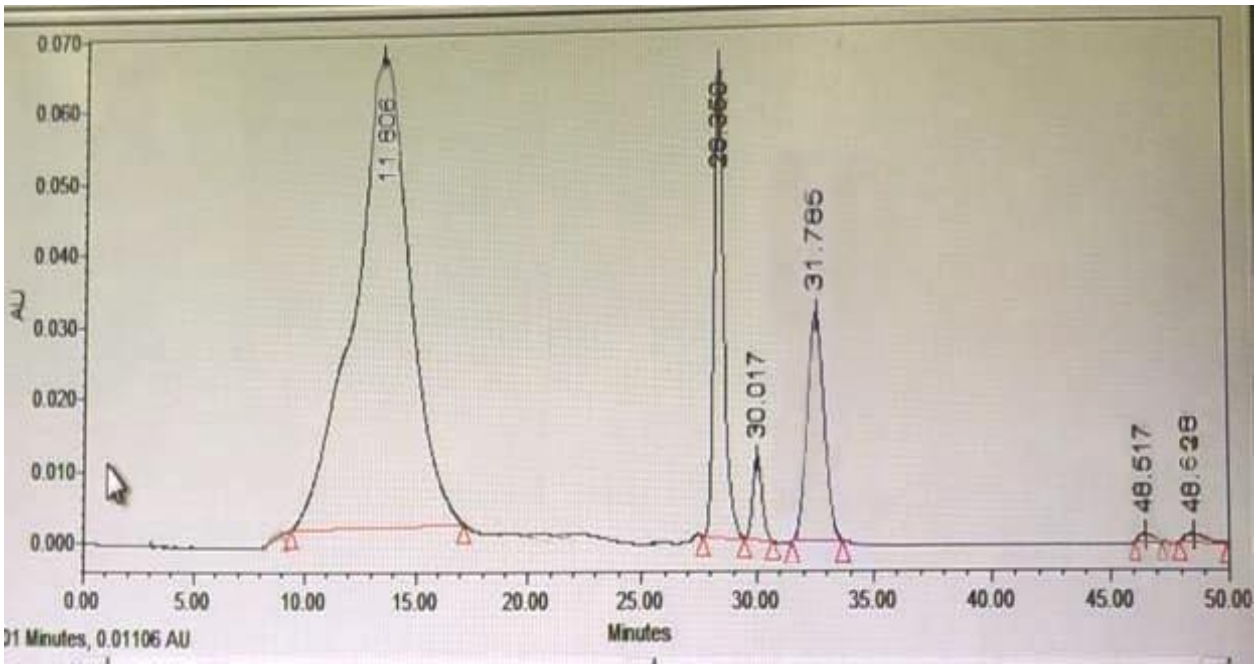



**Figure 3: HPTLC of *Acacia nilotica***

**IR-Spectroscopy:**

The IR analysis showed presence of -OH bonded intermolecular Hydrogen bond at 3382.08 $cm^{-1}$, C-H stretch at 1662.33$cm^{-1}$,-C=C- conjugated polyene peak at 1611.37$cm^{-1}$,C=O stretch at 1612.71$cm^{-1}$, C-O- ester peak at 1095.18$cm^{-1}$and-C-O-CO ether peak at 1003.38$cm^{-1}$(**Figure 4**).

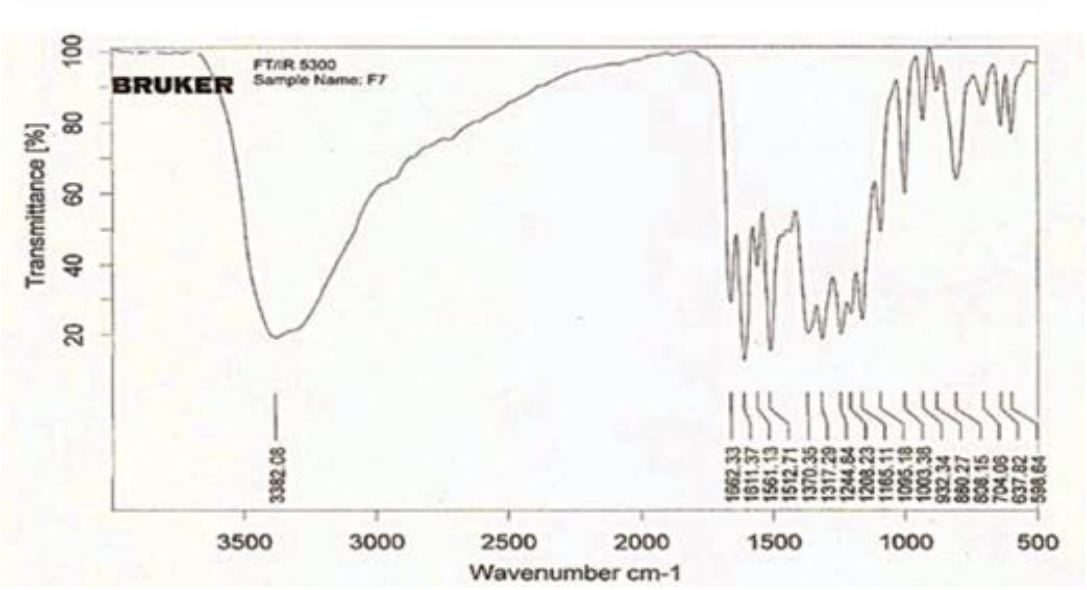



**Figure 4: IR spectrum of isolated compound of *Acacia nilotica***

***INVITRO* ANTIOXIDANT ACTIVITY**

Ascorbicacid was used as standard during DPPH assay which was performed to estimate radical scavenging capacity of plant extract. The non linear regression analysis was used to determine $IC_{50}$ value of isolated fraction. The $IC_{50}$ value of isolated fraction of *Acacia nilotica* was found to be507.33µg/ml.

The high contents of flavonoids in the methanol extract of *Acacia nilotica* showed antioxidant potential or high radical scavenging activity, than other extract. The *Acacia nilotica* extract showed presence of natural constituents like; flavonoids, saponins, and terpenoids, which contributed towards the radical scavenging potential of plant extract. The radical scavenging activity was observed in dose dependent manner as depicted in **Figure 5**.

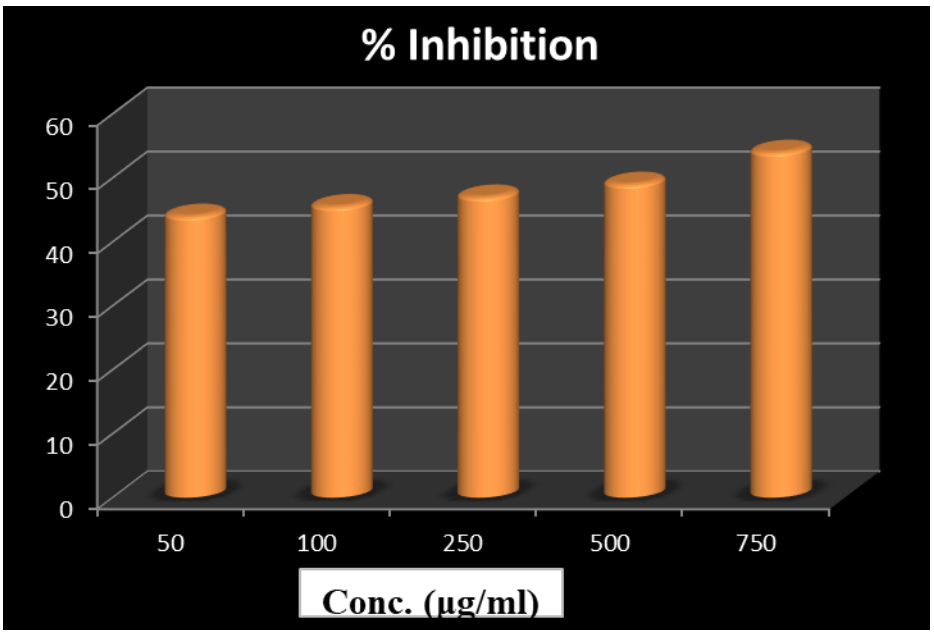



**Figure 5: DPPH inhibition curve of extract of *Acacia nilotica***

**Discussion**

The literature survey revealed that bark of *Acacia nilotica* is used in cough, diarrhea and in skin disease. The constituents present in *Acacia nilotica*are flavonoids, alkaloids and saponins, which could be used as antioxidant, antibacterial and anti-inflammatory agents. Traditionally bark, leaves, pods and flowers recommended for cancer, congestion, fever, diarrhoea, hemorrhoid, tuberculosis and bleeding piles, etc.

The analytical study revealed presence of functional groupslike ether, ester, conjugated polyene, carbonyl group and hydroxyl groups, etc. The presence of high contents of flavonoids in extract of *Acacia nilotica*can be attributed to its antioxidant potential or high radical scavenging activity [11, 12].

**Conclusion**

The flavonoid content was highest in *Acacia nilotica* which contributed towards

the antioxidant property of plant extract.Hence the plant extract can be used as potential antioxidant agent without any side effect.

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